INSPIRATORY MUSCLE TRAINING ATTENUATES THE HUMAN RESPIRATORY MUSCLE METABOREFLEX

by

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ABSTRACT

PURPOSE: High-resistance inspiratory muscle work results in inspiratory muscle fatigue and sympathetically mediated increases in heart rate (HR) and mean arterial pressure (MAP). We hypothesized that 5 wks of inspiratory muscle training (IMT) would attenuate such a response. **METHODS:** An experimental group (EXP, n = 8) performed IMT 6 days/wk for 5-wks at 50% of maximal inspiratory pressure (MIP), while a mock training group (SHAM, n = 8) performed IMT at 10% MIP. Pre- and post- training, subjects underwent a eucapnic resistive breathing protocol (RBT) at a resistance of approx. 50% MIP (breathing frequency = 15 breaths/min, duty cycle = 0.70) while HR and MAP were continuously monitored. RBT duration was matched within each subject pre and post. MIP was assessed weekly. **RESULTS:** MIP increased significantly (p < 0.05) in the EXP group (-125.0 ± 28.4 to -145.5 ± 33.3 cm H_2O, mean ± SD) but not in the SHAM group (-141.2 ± 31.8 to −147.5 ± 32.0 cm H_2O). Mean RBT duration was significantly shorter in the SHAM group (392 ± 93.7 sec vs. 677 ± 197 sec). Prior to IMT, the RBT resulted in significant, rises in HR (SHAM: 58.7 ± 5.2 to 82.9 ± 11.6 beats/min; EXP: 61.7 ± 9.3 to 82.7 ± 12.1 beats/min) and MAP (SHAM: 88.1 ± 4.6 to 106.2 ± 7.3 mmHg; EXP: 84.5 ± 4.0 to 99.2 ± 8.1 mmHg) in both groups. The SHAM group responded similarly to the RBT post-training (HR: 56.9 ± 6.2 to 76.8 ± 8.5 beats/min; MAP: 89.3 ± 9.0 to 102.5 ± 8.0 mmHg). Following IMT in the EXP group, the RBT failed to increase HR and MAP to the same extent as before training (HR: 58.6 ± 8.7 to 73.8 ± 7.1 beats/min; MAP: 84.5 ± 3.3 to 88.7 ± 6.1 mmHg). MIP measured before and after the RBT did not change in either group pre or post training. **CONCLUSIONS:** IMT reduces the HR and MAP response to resistive inspiratory muscle work. This may indicate a reduction in sympatho-excitation due to either reduced accumulation of, or reduced responsiveness to, metabolites within the inspiratory muscles.

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INTRODUCTION

Muscle fatigue is defined as a temporary loss in the capacity of a muscle to develop force and/or velocity as a result of loading of the muscle (2). In humans, the occurrence of skeletal muscle fatigue is a well-documented phenomenon and is generally believed to occur in any skeletal muscle exposed to prolonged or repeated contractions (126). Fatigue can occur in response to a failure at any one step along the muscle contraction pathway, from the brain to the peripheral contractile machinery (brain, spinal cord, peripheral nerve, neuromuscular junction, muscle cell membrane, transverse tubular system, Ca^{2+} release, actin-myosin activation, cross-bridge formation) (91). As a result, the study of muscle fatigue is extremely complex with the sites and mechanisms of skeletal muscle fatigue remaining unclear despite over a century of formal study.

In general, there are three different forms of skeletal muscle fatigue: central fatigue, low-frequency peripheral fatigue, and high-frequency peripheral fatigue. Central fatigue refers to a reduced capacity of the muscle to develop force as a result of a reduced motor output from the central nervous system. Central fatigue typically occurs in response to sustained or intermittent maximal voluntary contractions (6, 23, 39, 63) or sustained isometric contractions (26). The two forms of peripheral muscle fatigue represent failures at the neuromuscular junction or the contractile apparatus of the muscle, and are differentiated by the stimulation frequency at which contractile force is found to be below control values; commonly identified with the use of a force-frequency curve (56). High-frequency fatigue, occurs in response to high-frequency muscle stimulation and is characterized by a recovery within minutes, whereas low-frequency fatigue may occur in response to a variety of stimuli and is characterized by a recovery period that can last for over 24 hours (56).

The inspiratory muscles consist of the diaphragm, the external intercostal muscles, and the accessory muscles (scalene and sternocledomastoid muscles) and are the only skeletal muscles in the body whose functioning is necessary to sustain human life. As such, their ability to remain fatigue resistant is of great importance. Despite this, research has shown that in humans, the inspiratory muscles are susceptible to all three types of fatigue following high intensity exercise (10, 12, 53, 60, 68, 119) or resistive breathing protocols (7, 15, 65, 71, 75, 76, 117).

Recent findings have shown that respiratory muscle work has cardiovascular consequences. For example, acute changes in leg blood flow (Q_{leg}) and muscle sympathetic activity occur in response to fatiguing levels of inspiratory muscle work. Some of the initial evidence for a link between Q_{leg} and sympathetic activity was provided from work by Harms and colleagues (47), in which the work of breathing during heavy cycling exercise was manipulated with the use of proportional assist ventilators and threshold resistive breathing devices to unload and load the inspiratory muscles respectively. The work from this group showed that at maximal exercise intensity there was a significant correlation between the work of breathing and Q_{leg} and leg oxygen uptake. In addition, they found that the change in leg vascular resistance (LVR) that occurred during exercise was related to the change in norepinephrine spillover; a proxy for muscle sympathetic activity. Further research has shown that these relationships that are observed between the work of breathing and LVR, norepinephrine spillover, and Q_{leg} are only present during maximal exercise, with no relationship being present at submaximal exercise intensities of up to 75% of VO_{2max} (129). These works have implicated a role of blood flow competition by the inspiratory muscles in inducing these physiological changes. Follow-up studies have employed the use of the microneurography technique to take direct recordings of muscle sympathetic nerve activity

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(MSNA) at the peroneal nerve of the leg, and doppler ultrasound measurements to assess femoral artery blood flow during inspiratory muscle fatigue (97, 108). What has been shown is that fatigue of the inspiratory muscles is associated with increases in sympathetic activity and reductions in blood flow to the peripheral musculature that are not observed when subjects perform similar, yet non-fatiguing, breathing tasks that match the breathing frequencies (f_b), tidal volumes (V_t), duty cycles (time on inspiration divided by the sum of the time on inspiration and time on expiration, T_I/T_{TOT}) or inspiratory pressures of the fatiguing trials (97). This suggests that high levels of central respiratory motor output do not by themselves cause the observed vascular and sympathetic changes and that inspiratory muscle fatigue plays a vital role in these responses.

What has been proposed is that the chemosensitive afferent type III and IV fibres that innervate the diaphragm are stimulated in response to the accumulation of the metabolic by-products associated with diaphragm fatigue. Group III and IV afferents serve as sensory fibres that also exist in other skeletal muscles throughout the body. They are believed to fire in response to stimuli such as metabolic by-products (104), mechanical deformation (122), temperature (82), and vascular distension during muscular contraction (46). It is suspected that the activation of these afferent fibres occurs under conditions when blood supply to the inspiratory muscles is insufficient and metabolite accumulation occurs, resulting in sympathetically mediated excitation to most systemic vascular beds. Presumably, the end result is a vasoconstriction in the splanchnic, renal, and mesenteric vasculatures as well as the vascular beds of inactive skeletal muscles (31, 62). This is believed by some to be an adaptive response to allow blood flow to be diverted towards the fatiguing inspiratory muscles at a cost to the other muscles and organs of the body (31, 78, 94).

Indirect evidence supporting the inspiratory metaboreflex hypothesis has been made available by the study of clinical populations. In some patients with severe chronic obstructive pulmonary disease (COPD), a plateau in leg oxygen uptake occurs during cycling exercise of increasing intensity (100). According to the respiratory metaboreflex hypothesis, such a response would be expected. The inspiratory muscles of these subjects must demand greater blood flow to compensate for the increased work that they perform, theoretically resulting in a "stealing" of blood away from the exercising leg muscles. Similarly, with the sustained hyperventilatory response seen in patients suffering from chronic heart failure, the inspiratory muscle metaboreflex is likely a common phenomenon during exercise in this population. In favour of this notion, it has been shown that by unloading the inspiratory muscles of individuals suffering from chronic heart failure, endurance exercise performance dramatically improves (77).

Direct support for the inspiratory metaboreflex hypothesis has come from the study of healthy humans and animals during exercise. Rodman et al. (86) showed that following transient lactic acid infusion into the phrenic artery of resting or exercising canines, reductions in hindlimb blood flow and increases in MAP occur. This response was shown to be absent with the administration of a sympathetic blockade. Recently in humans, McConnell and Lomax (70) showed that high resistance inspiratory muscle work not only reduces Q_{leg} but contributes to the onset of peripheral muscle fatigue during lower body exercise. As a whole, the evidence from the above research strongly indicates the existence of this inspiratory muscle metaboreflex in response to fatiguing levels of work and metabolite accumulation within the respiratory muscles.

A similar metaboreflex is known to occur in response to fatiguing work performed by other skeletal muscles innverated with type III and IV afferents. For example, Seals (95)

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has demonstrated that in response to intense handgrip (HG) exercise, lower leg MSNA is increased. It was further shown by this researcher that the increase in MSNA is associated with a reduced blood flow to the calf and an increased HR, MAP and calf vascular resistance.

Of interest, is whether or not the observed inspiratory metaboreflex can be attenuated in humans following an inspiratory muscle training (IMT) protocol. IMT has consistently been shown capable of improving maximal inspiratory pressure (MIP) values (44, 106, 114) and inspiratory muscle endurance (28, 51, 73). Other studies have shown IMT to be associated with whole-body exercise endurance improvements in both healthy populations (28, 107, 112) and COPD patient populations (19, 85, 93). Additionally, research in rats has shown endurance training (treadmill running) to be capable of increasing the oxidative characteristics of the diaphragm via increases in type I and decreases in type II muscle fibre composition and increases in the activity of the oxidative enzyme citrate synthase (124). Furthermore, four weeks of HG training has been shown to increase forearm exercise endurance time and partially inhibit the increase in mean arterial pressure (MAP) normally associated with the so-called peripheral muscle metaboreflex (74). The primary question asked in this thesis is whether IMT may be capable of attenuating the inspiratory muscle metaboreflex in a similar manner.

Purpose

The purpose of this study was to determine if five weeks of an inspiratory muscle training (IMT) intervention is capable of altering the cardiovascular response to resistive work of the inspiratory muscles.

Hypotheses

It was hypothesized that in comparison to pre-training values and values from a mock-training control group, five weeks of IMT would result in:

1) An increased inspiratory muscle strength.

2) An attenuated inspiratory muscle metaboreflex.

METHODS

General Procedures

Healthy normotensive male volunteer subjects (n = 16, age range, 21-34 years) free of cardiovascular, neurological or pulmonary disease were recruited from the general student body at the University of British Columbia, Vancouver, Canada. All testing was performed in the Health and Integrative Physiology Lab at the university campus. This study was approved for ethics by the Clinical Research Ethics Board at the University of British Columbia and conformed to the Declaration of Helsinki.

After verbal and written explanation of the research study, written informed consent was obtained from all subjects. Subjects were assigned to an experimental (EXP) or a mock (SHAM) IMT group. The EXP group trained at 50% of MIP, six days a week, for five weeks. The SHAM group performed mock IMT at 10% of MIP, six days a week, for five weeks. Subjects were not made aware of which training group they had been assigned to. Prior to the commencement of the training program, subjects underwent familiarization testing and pre-training testing. After exactly five weeks of training, subjects underwent post-training testing. During familiarization, pre, and post testing, MIP, maximal expiratory pressure (MEP), and HG strength were all assessed. Measurements of MEP were made to ensure that no training of the expiratory muscles was occurring. As has been done by other

authors (37, 79), HG measurements were used as an index of subject effort and monitored to ensure that changes in MIP were not strictly due to altered effort over time. Following the MIP, MEP and HG tests on each of the three testing days, subjects also underwent a resistive breathing task (RBT). Prior to the pre and post testing, subjects refrained from caffeine, exercise, alcohol and food for 12 hours.

Spirometry was assessed on all subjects during the familiarization visit and was used to characterize the subjects as having normal lung function. All other measurements made during familiarization testing were excluded from analysis and were performed solely in an attempt to minimize the learning effect that may have otherwise strongly influenced the results.

Anthropometric measurements

Height, weight, and age of all subjects was documented for descriptive purposes. Height was measured at the end of a deep inspiration.

Spirometry

Tests of forced vital capacity (FVC), and forced expiratory volume in one second ($FEV_{1.0}$) were performed in all subjects using dedicated equipment (Spirolab II, Medical International Research, Vancouver, BC) and performed to the specifications of the American Thoracic Society guidelines (3). In order to exclude subjects with obstructive respiratory problems, all subjects were required to have an $FEV_{1.0}$/FVC of at least 80% of predicted values (based on age, height, weight and ethnicity).

Respiratory Muscle and Handgrip Strength Testing

MIP and MEP tests required subjects to wear nose clips and perform a maximal volitional inspiratory or expiratory effort through a mouthpiece attached to an occluded three way stop cock (2100 series, Hans Rudolph, Kansas City, MO). A small pinhole within the

stop cock allowed for the passage of air and served to prevent glottic closure and minimize the recruitement of the buccal muscles. Mouth pressure (P_m) was measured with a calibrated pressure transducer (Model MP45-36-871, Validyne, Northridge CA) connected via polyethylene tubing to an additional outlet on the device. The MIP and MEP tests were performed in a seated position and conformed to American Thoracic Society and European Respiratory Society Guidelines (1). All MIP procedures were initiated from residual volume and all MEP manoeuvers were initiated from total lung capacity.

HG measurements were conducted with subjects in a standing position, with their right hand gripping a comfortably adjusted hand dynamometer (Model 76618, Lafayette Instrument Company, Lafayette Indiana). Subjects were instructed to perform a maximal grip with their right arms at their side but not braced against their body.

During all strength testing the MIP, MEP and HG measurements were taken in series until three values within 15% of one another had been obtained for each test. For each variable the three acceptable measurements were then averaged and this mean value was taken as the test value.

Resistive Breathing Task

All RBTs took place with subjects sitting in a semi-recumbant position. Prior to the RBT, subjects underwent 8-minutes of non-resistive resting eupnoea in order to ensure stable baseline cardiorespiratory parameters. Following this rest period, subjects attempted to inspire to a target P_m corresponding to 60% of their MIP while breathing through a custom-made acrylic y-valve. In order to create an optimal level of inspiratory resistance without introducing any expiratory resistance, the size of the inlet on the inspired side of the valve was capable of being reduced and was able to accommodate for each subject's individual P_m demands. The valve was supported on the subject with a head support device

(Series 2726, Hans Rudolph, Kansas City, MO) and the inspired side of the valve was connected, via a breathing tube, to a pneumotachograph (Model 3813, Hans Rudolph, Kansas City, MO) in order to allow for the measurement of flow. Subjects were instructed to breathe to the timing of auditory tones played from a computer. These tones were used to maintain subjects at a T_I/T_{TOT} of 0.70 and f_b of 15 breaths per minute. This combination of T_I/T_{TOT} values and inspiratory resistance values has previously been shown to reduce diaphragmatic blood flow in anaesthetized dogs (18) and predict the onset of diaphragm fatigue in humans (16, 17). P_m was measured from a small port on the mouthpiece which was connected to the pressure transducer via polyethylene tubing. Feedback for the subjects regarding the P_m they were generating was provided by a computer monitor displaying for them the maximal P_m of each breath. Aside from the breath timing and P_m demands, subjects were also instructed to attempt to isolate the diaphragm during inspiration and minimize the recruitment of accessory breathing muscles. Subjects were verbally encouraged to maintain proper timing, breathing technique and P_m as needed.

CO$_2$ gas sensors and analyzers (CD-3A, AEI Technologies Applied Electrochemistry, Pittsburgh Pennsylvania) were used to sample expired %CO$_2$ from an outlet located in the y-valve. The partial pressure of alveolar CO$_2$ was obtained from the %CO$_2$ signal. End-tidal CO$_2$ (PET_{CO_2}) was calculated from the peaks of this continuous partial pressure CO$_2$ trace. As a result of the unnatural ventilations imposed by the RBT, PET_{CO_2} was expected to deviate from eupnoeic values. To compensate for this, exogenous 100% CO$_2$ gas was carefully titrated as needed into the inspirate throughout the RBT in an attempt to maintain the PET_{CO_2} at the level observed during the 8-minute rest period for each subject on the given testing day. The %CO$_2$, P_m and inspiratory flow signals were sampled

at 100 Hz using an analog-to-digital converter (PowerLab/16SP model ML795, ADI, Colorado Springs, CO) and stored on a computer for subsequent analyses. Integration and cyclic analysis of the flow signal was performed online using commercially available software (Chart V5.4.2, ADInstruments, Colorado Springs, CO) and allowed for the instantaneous calculation of f_b, V_t and minute ventilation (V_I). T_I and T_{TOT} were calculated using a threshold analysis of the inspiratory volume signal coupled with a macro used to measure the timing of each component of the breathing cycle for each breath. Inspiratory flow rate was calculated as V_t/T_I. Offline integration of the P_m signal allowed for the calculation of inspiratory muscle force development (f_b x $\int P_m$).

Termination of the familiarization and baseline RBTs was based upon the presence of a plateau in the rise in MAP. The RBT_{POST} was performed for the same duration as that of the RBT_{PRE}. Every RBT was followed by 8-minutes of non-resistive recovery.

Prior to the resting period, and following the recovery period, MIP, MEP and HG tests were performed in all subjects while in the semi-recumbent position. These tests were implemented in order to provide an index as to whether respiratory muscle fatigue or changes in motivation were occurring over the course of the RBT. Additionally, the semi-recumbent MIP values obtained prior to the RBT were used to prescribe the 60% MIP target for the familiarization and pre-training RBTs. During the RBT_{POST}, an attempt was made to have subjects generate P_m values similar to that observed during baseline testing. To this end, during the RBT_{POST}, subjects were prescribed three different P_m targets corresponding to the highest minute average P_m values from each third of the RBT_{PRE}.

Femoral Artery Blood Flow

A Doppler Ultrasound and an 11-3L ultraband linear transducer (Sonos 5500, Philips Electronics, Andover MA) with ultrasound gel (Aquasonic 100 Ultrasound Transmission

Gel, Parker Laboratories Inc., Orange, NJ) were used to assess Q_{leg} at the right femoral artery at a landmark location 2-3 cm distal to the inguinal ligament. This site of insonation was chosen because it is believed to minimize turbulence and because it represents an optimal vantage point from which to clearly view the femoral artery (81, 99). All ultrasound and doppler measurements were recorded to VHS (T-120, Maxell Canada, Concord, Ont) to allow for subsequent offline analyses. Peripheral vascular and lower limb pre-settings, and Colour and B-Mode functions were used to locate the femoral artery and measure cross sectional areas. Pulse Wave mode was used to obtain single-beat blood velocity values. Based on the time-averaged velocity mean (TAVM) per beat, mean Q_{leg} per minute was calculated using the following formula: Flow = Velocity x Cross Sectional Area. To perform this calculation, the cross sectional area of the femoral artery was calculated using diameter measurements of the artery taken offline once every minute. For the calculation of each minute of flow, a mean of the TAVM values obtained for that minute was multiplied by the mean of the cross sectional areas obtained at the start and end of that minute.

Other Cardiovascular Parameters

Throughout the eupnoeic periods and RBTs, arterial pressure, and HR were monitored continuously at the finger on a beat-by-beat basis with the use of a finger arterial plethysmograph (Finometer, FMS, Finapres Medical Systems BV, Arnhem, The Netherlands). As was the case for the ventilatory data, these recordings were sampled at 100 Hz using an analog-to-digital converter and stored on a computer for subsequent analyses. Cyclic peak and nadir detection of the arterial pressure trace allowed for the calculation of systolic and diastolic blood pressure respectively. MAP was calculated as 1/3 pulse pressure + diastolic pressure. In addition, LVR was calculated as MAP/Q_{leg}. Systolic and diastolic blood pressures were also collected every minute with the use of an automated blood

pressure cuff (BPM-100, VSM MedTech Ltd., Vancouver, Canada). On the pre and post test days the average systolic and diastolic blood pressure values obtained from the automatic cuff over the resting eupnoeic period were used to correct the values from the arterial plethysmograph for each subject. This correction factor was then applied to the plethysmograph values obtained during the rest, recovery and RBT periods.

Inspiratory Muscle Training

Following the pre test, subjects reported to the lab and were assigned a respiratory muscle trainer (Powerlung "Sport", Vacumed, Ventura, CA) set at a resistance corresponding to 50% or 10% of their seated baseline MIP. Subjects in the EXP group were encouraged to inspire briskly whereas subjects in the SHAM group were told to inspire normally. To ensure the subjects did not experience expiratory resistance, all subjects were instructed to remove the device for every expiration. In addition, during each training breath, all subjects were encouraged to practice slow and relaxed expirations; each lasting for approximately 4 to 6 seconds. Subjects were instructed to perform IMT sessions once a day, 6 days a week, for five weeks. Each IMT session involved 3 sets of 75 breaths with a five-minute rest between sets. The training protocol adopted by the EXP group in this study was based closely upon an IMT regimen previously shown to elicit significant improvements in MIP (44). Subjects were asked to perform five training sessions a week at home and reported to the laboratory for supervised training sessions once a week. During these laboratory visits all subjects had their seated MIP, MEP, and HG re-assessed and had the resistance on their trainer adjusted to maintain a progressive training workload. The first training session was supervised, at which time correct technique was demonstrated by all subjects. Subjects were expected to keep a daily log of their IMT activity indicating the date and time of their training sessions. These logs were reviewed each week during the lab

visits, at which time subjects were encouraged to adhere to the training program. Compliance to the training program was based upon this self-report data and only fully completed training sessions were included in this analysis.

Data Analysis and Statistics

Independent t-tests were used to assess for differences between SHAM and EXP groups for spirometry values, descriptive characteristics, and baseline MIP, MEP and HG values. An independent t-test was also used to assess for differences between groups in the duration of the RBT_{PRE}. A dependent t-test was used to assess for possible differences in f_b x $\int P_m$ between the RBT_{PRE} and RBT_{POST} of either training group. MIP, MEP and HG values for each group were compared from baseline to follow-up using one-tailed paired t-tests. One-tailed paired t-tests were also used to compare the percent change in the semi-recumbent MIP, MEP, and HG values that occurred following the RBT_{PRE} and RBT_{POST}. For the analysis of the RBT data, mean values for Q_{leg}, MAP, HR, and LVR were calculated for each minute of the RBTs of each subject. A single mean value was calculated for both the entire 8-minute resting period and the first minute of recovery. Due to variation in the duration of RBTs between subjects, select points (eupnoea, minute 1, minute 2, minute 3, final minute, and first minute of recovery) were used for statistical comparisons. For each RBT, repeated measures analysis of variance (ANOVA) were performed to detect significant changes from eupnoeic values in those variables measured. In instances when the repeated measures ANOVA was found to be significant, Tukey's post hoc test was used to determine where the differences lay. To compare the HR and MAP data pre to post for each time point in each group, one-tailed dependent t-tests were performed with Bonferroni corrections made to adjust the type I error rate per comparison. An alpha level of 0.05 was used for all tests of significance. Unless otherwise noted, all data are expressed as means ± S.D. All

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statistics were performed with the use of commercial statistical software (STATISTICA, Version 6.1, Statsoft Inc., Tulsa, OK).

RESULTS

The spirometry and physical characteristics of the two training groups are displayed in Table 1. Both training groups exhibited healthy lung function, with $FEV_{1.0}/FVC$ values exceeding 87% of predicted values for all subjects. No significant differences between SHAM and EXP groups were found for any of the lung function measures or physical characteristics ($p > 0.05$).

Table 1. Physical characteristics and spirometric data for the SHAM and EXP groups. Values are means ± S.D. No significant differences existed between the two training groups ($p > 0.05$). Definitions of abbreviations: BMI = body mass index; FVC = forced vital capacity; $FEV_{1.0}$ = forced expiratory volume in 1 second.

	SHAM (n = 8)	EXP (n = 8)
Age (yrs)	26.6 ± 3.6	25.0 ± 2.6
Height (cm)	179.5 ± 8.2	178.6 ± 9.4
Mass (kg)	80.2 ± 12.4	73.5 ± 6.8
BMI (kg/m^2)	24.8 ± 2.5	23.1 ± 1.8
FVC (L)	5.3 ± 0.5	5.5 ± 1.4
$FEV_{1.0}$ (L)	4.5 ± 0.4	4.4 ± 1.0
$FEV_{1.0}/FVC$ (%)	85.1 ± 4.2	81.9 ± 4.2
$FEV_{1.0}/FVC$ (% Predicted)	101.4 ± 5.2	97.1 ± 5.2

Baseline MIP and HG strength were similar between the two training groups prior to the start of training ($p > 0.05$) however MEP values were significantly greater in the SHAM group ($p < 0.01$). Based on the self-report training logs, subjects in the SHAM group maintained a mean training compliance of 88% over the course of the five-week training program. This was less than the reported 97% compliance in the EXP group, however not statistically so. The trend for a lower training compliance in the SHAM group was driven primarily by one subject (Subject 9) who exhibited a compliance of only 40%. With data from this subject removed, the training compliance of the SHAM group improved to over 95%.

The MIP, MEP and HG values pre and post training for both groups are displayed in Table 2. A significant training response was only observed in the MIP of the EXP group. All subjects in the EXP group displayed some rise in MIP after five weeks of training, with a group mean improvement of 17% (range: 1-32%). Although there was a trend for improvements in MIP in the SHAM group, this finding was neither consistent across all subjects, nor significant. The trend for improvements in MIP in the SHAM group was driven primarily by one anomalous subject (Subject 15) who displayed a MIP increase of 48%; the greatest MIP improvement of all subjects in the study.

Table 2. Seated MIP, MEP and HG values pre and post training for the SHAM and EXP groups. Values are means ± S.D. Definitions of abbreviations: MIP = maximal inspiratory pressure; MEP = maximal expiratory pressure; HG = handgrip strength. * Significantly different from pre training value (p <0.05).

	SHAM (n = 8)		EXP (n = 8)	
	Pre	Post	Pre	Post
MIP (cm H_2O)	-141.2 ± 31.8	-147.5 ± 32.0	-125.0 ± 28.4	-145.5 ± 33.3*
MEP (cm H_2O)	158.9 ± 20.9	156.7 ± 21.1	127.2 ± 17.5	130.2 ± 30.2
HG (kg)	50.3 ± 8.0	51.9 ± 9.0	48.2 ± 6.9	49.2 ± 6.3

Variability of Q_{leg}, LVR, HR and MAP

Due to technical difficulties with the Doppler measurements, the average within-subject coefficients of variation for the resting measurements of Q_{leg} were greater than ± 20% and ± 14% in the SHAM and EXP groups respectively. These coefficients of variation are nearly three fold greater than previously published values and approach the level of change expected to occur as a result of the RBTs (97). Additionally, the high degree of variability in the Q_{leg} recordings resulted in similarly high variability in the LVR values. The excessive variation in both Q_{leg} and LVR precluded the detection of any trends in these variables and, as a result, these data were omitted from all further analyses.

Unlike the Q_{leg} and LVR values, the within-subject coefficients of variation for HR and MAP were within the range of previously reported values (97). The average resting coefficients of variation for HR over the two days (Pre and Post) was ± 4.1% and ± 2.4% for the SHAM and EXP groups respectively. The variability in MAP for the SHAM and EXP

groups was similarly low, with average coefficient of variation values of $\pm$ 2.2% and $\pm$ 1.9% respectively.

RBT Pre and Post Characteristics

The RBT_{PRE} of the SHAM group was an average of 392 $\pm$ 93.7 sec in duration (range: 266 sec – 560 sec). This was significantly shorter (p <0.005) than the average duration of 677 $\pm$ 197 sec witnessed by the EXP group (range: 443 sec – 996 sec). As described in the methods, to allow for equal comparisons within training groups, RBT_{POST} durations for each subject were matched to those of the RBT_{PRE}.

Over the course of the RBT_{PRE}, subjects in both groups had great difficulty achieving the target 60% MIP level, with the SHAM and EXP groups achieving mean peak P_m values corresponding to 47.3 $\pm$ 4.3% of MIP and 44.3 $\pm$ 8.4% of MIP respectively. Despite this, subjects did generate a substantial amount of force with mean f_b x $\int P_m$ values over 200 times eupnoeic levels for both groups. During the RBT_{POST}, subjects in both groups tended to inspire slightly more forcefully but were once again well below target levels, with mean peak P_m values corresponding to 50.2 $\pm$ 5.1% of MIP in the SHAM group and 48.9 $\pm$ 10.5% of MIP in the EXP group. Only in the EXP group were these greater P_m values during the RBT_{POST} associated with significantly greater mean f_b x $\int P_m$ values (RBT_{PRE} = -1561 $\pm$ 457; RBT_{POST} = -1711 $\pm$ 441).

During all RBTs, the electronic tones were found to be capable of maintaining subjects' f_b values at or near 15 breaths per minute, with T_I/T_{TOT} values in the range of 0.70. For both groups V_t/T_I was relatively consistent throughout all RBTs with mean values near 0.38 l/sec; less than the mean of 0.55 l/sec observed during eupnoea. As expected, the prescribed breathing protocol did result in some degree of hyperventilation for all subjects during all RBTs, with V_I values significantly above those found during rest. This

hyperventilation resulted in significant drops in PET_{CO_2} during the first minutes of the RBTs but was adequately compensated for with the administration of supplemental CO_2 during subsequent minutes. Mean values from the RBT_{PRE} of the SHAM and EXP groups are displayed in Tables 3 and 4 respectively. Mean values from the RBT_{POST} of the SHAM and EXP groups are displayed in Tables 5 and 6 respectively.

Table 3. Mean data for the RBT_{PRE} of the SHAM group. Values are means $\pm$ S.D. Definitions of abbreviations: MAP = mean arterial pressure; HR = heart rate; V_t = tidal volume; f_b = breathing frequency; V_I = minute ventilation; T_I/T_{TOT} = duty cycle; Peak P_m = peak mouth pressure; f_b x $\int P_m$ = product of breathing frequency and integrated mouth pressure; V_t/T_I = mean inspiratory flow rate; PET_{CO_2} = end-tidal P_{CO_2}; Min = minutes of RBT; End = final minute of RBT; Rec 1 = first minute of recovery. * Significantly different from resting eupnoea ($p < 0.05$).

	MAP (mmHg)	HR (beats min^{-1})	V_t (l)	f_b (breaths min^{-1})	V_I (l min^{-1})	T_I/T_{TOT}	Peak P_m (cm H$_2$O)	f_b x $\int P_m$	V_t/T_I (l s^{-1})	PET_{CO2} (mmHg)
Eupnoea	88.1	58.7	0.768	14.1	9.2	0.34	-0.9	-9	0.54	37.3
	$\pm$ 4.6	$\pm$ 5.2	$\pm$ 0.209	$\pm$ 3.3	$\pm$ 1.7	$\pm$ 0.04	$\pm$ 0.6	$\pm$ 4	$\pm$ 0.15	$\pm$ 2.5
Min 1	90.6	80.8*	1.19*	15	16.0*	0.74*	-63.2*	-2022*	0.41	33.8*
	$\pm$ 9.3	$\pm$ 9.4	$\pm$ 0.195	$\pm$ 0.1	$\pm$ 2.6	$\pm$ 0.01	$\pm$ 18.3	$\pm$ 652	$\pm$ 0.06	$\pm$ 1.8
Min 2	102.3*	78.3*	1.163*	15	15.7*	0.74*	-62.0*	-1938*	0.39	38.1
	$\pm$ 7.9	$\pm$ 9.4	$\pm$ 0.175	$\pm$ 0.0	$\pm$ 2.4	$\pm$ 0.01	$\pm$ 12.8	$\pm$ 541	$\pm$ 0.06	$\pm$ 1.8
Min 3	101.3*	78.9*	1.129*	15.2	15.4*	0.74*	-60.3*	-1839*	0.39	38.4
	$\pm$ 9.3	$\pm$ 10.5	$\pm$ 0.165	$\pm$ 0.4	$\pm$ 2.4	$\pm$ 0.02	$\pm$ 15.2	$\pm$ 532	$\pm$ 0.07	$\pm$ 2.9
End	106.2*	82.9*	1.157*	15	15.6*	0.74*	-68.6*	-1954*	0.39	38
	$\pm$7.3	$\pm$ 11.6	$\pm$ 0.215	$\pm$ 0.1	$\pm$ 2.9	$\pm$ 0.02	$\pm$ 17.6	$\pm$ 680	$\pm$ 0.07	$\pm$ 2.3
Rec 1	96.9*	65	1.164*	16.7	16.0*	0.37	-0.8	-10	0.93*	37.3
	$\pm$ 6.8	$\pm$ 10.7	$\pm$ 0.344	$\pm$ 4.4	$\pm$ 3.8	$\pm$ 0.06	$\pm$ 0.2	$\pm$ 3	$\pm$ 0.35	$\pm$ 5.5

Table 4. Mean data for the RBT_{PRE} of the EXP group. Values are means ± S.D. Definitions of abbreviations: MAP = mean arterial pressure; HR = heart rate; V_t = tidal volume; f_b = breathing frequency; V_I = minute ventilation; T_I/T_{TOT} = duty cycle; Peak P_m = peak mouth pressure; f_b x $\int P_m$ = product of breathing frequency and integrated mouth pressure; V_t/T_I = mean inspiratory flow rate; PET_{CO_2} = end-tidal P_{CO_2}; Min = minutes of RBT; End = final minute of RBT; Rec 1 = first minute of recovery. * Significantly different from resting eupnoea ($p < 0.05$).

	MAP (mmHg)	HR (beats min⁻¹)	V_t (l)	f_b (breaths min⁻¹)	V_I (l min⁻¹)	T_I/T_{TOT}	Peak P_m (cm H_2O)	f_b x $\int P_m$	V_t/T_I (l s⁻¹)	PET_{CO2} (mmHg)
Eupnoea	84.5	61.7	0.781	12.9	8.0	0.3	-1	-8	0.54	39.6
	± 4.0	± 9.3	± 0.294	± 4.7	± 1.5	± 0.05	± 0.4	± 3	± 0.14	± 3.1
Min 1	81.3	81.3*	1.095*	15.0	14.9*	0.72*	-56.8*	-1762*	0.38*	35.1*
	± 8.8	± 8.4	± 0.164	± 0.1	± 2.2	± 0.04	± 19.3	± 531	± 0.05	± 3
Min 2	90.1	78.1*	1.068*	15	14.5*	0.72*	-55.6*	-1690*	0.37*	36.6
	± 6.7	± 7.9	± 0.160	± 0.1	± 2.2	± 0.05	± 16.6	± 482	± 0.05	± 4.2
Min 3	93.7*	79.2*	1.055	15.1	14.4*	0.71*	-59.4*	-1692*	0.37*	38.5
	± 12.6	± 9.6	± 0.179	± 0.3	± 2.5	± 0.05	± 17.5	± 529	± 0.05	± 2.5
End	99.2*	82.7*	1.007	14.8	13.6*	0.72*	-51.0*	-1471*	0.35*	37.7
	± 8.1	± 12.1	± 0.187	± 0.4	± 2.7	± 0.04	± 16.0	± 491	± 0.05	± 2.3
Rec 1	92.1*	65.8	0.942	17.3*	13.7*	0.35	-0.9	-10	0.80*	38.3
	± 4.9	± 10.0	± 0.366	± 4.6	± 3.1	± 0.03	± 0.2	± 2	± 0.20	± 6.2

Table 5. Mean data for the RBT_{POST} of the SHAM group. Values are means ± S.D. Definitions of abbreviations: MAP = mean arterial pressure; HR = heart rate; V_t = tidal volume; f_b = breathing frequency; V_I = minute ventilation; T_I/T_{TOT} = duty cycle; Peak P_m = peak mouth pressure; f_b x $\int P_m$ = product of breathing frequency and integrated mouth pressure; V_t/T_I = mean inspiratory flow rate; PET_{CO_2} = end-tidal P_{CO_2}; Min = minutes of RBT; End = final minute of RBT; Rec 1 = first minute of recovery. * Significantly different from resting eupnoea ($p < 0.05$).

	MAP (mmHg)	HR (beats min⁻¹)	V_t (l)	f_b (breaths min⁻¹)	V_I (l min⁻¹)	T_I/T_{TOT}	Peak P_m (cm H_2O)	f_b x $\int P_m$	V_t/T_I (l s⁻¹)	PET_{CO2} (mmHg)
Eupnoea	89.3	56.9	0.994	10.7	9.1	0.33	-0.5	-7	0.58	35.0
	± 9.0	± 6.2	± 0.380	± 1.6	± 3.3	± 0.06	± 0.3	± 2	± 0.25	± 5.0
Min 1	89.8	79.1*	1.157	14.9*	15.6*	0.73*	-68.3*	-2122*	0.40	32.7
	± 9.0	± 6.0	± 0.230	± 0.1	± 3.1	± 0.02	± 19.3	± 756	± 0.07	± 4.7
Min 2	100.4*	76.1*	1.115	15.0*	15.1*	0.74*	-66.8*	-2010*	0.38	35.0
	± 9.0	± 6.9	± 0.221	± 0.0	± 3.1	± 0.02	± 16.9	± 715	± 0.07	± 5.0
Min 3	101.3*	76.2*	1.093	15.0*	14.8*	0.72*	-65.2*	-1895*	0.38	36.6
	± 9.3	± 8.6	± 0.202	± 0.0	± 2.9	± 0.03	± 15.7	± 606	± 0.07	± 4.5
End	102.5*	76.8*	1.080	15.1*	14.7*	0.70*	-71.6*	-1891*	0.39	36.2
	± 8.0	± 8.5	± 0.180	± 0.1	± 2.6	± 0.03	± 19.4	± 669	± 0.06	± 4.7
Rec 1	95.6	63.6*	1.097	16.8*	16.0*	0.36	-0.8	-9	0.93*	34.7
	± 7.9	± 4.7	± 0.242	± 3.9	± 4.6	± 0.06	± 0.2	± 2	± 0.38	± 5.1

Table 6. Mean data for the RBT_{POST} of the EXP group. Values are means ± S.D. Definitions of abbreviations: MAP = mean arterial pressure; HR = heart rate; V_t = tidal volume; f_b = breathing frequency; V_I = minute ventilation; T_I/T_{TOT} = duty cycle; Peak P_m = peak mouth pressure; f_b x $\int P_m$ = product of breathing frequency and integrated mouth pressure; V_t/T_I = mean inspiratory flow rate; PET_{CO_2} = end-tidal P_{CO_2}; Min = minutes of RBT; End = final minute of RBT; Rec 1 = first minute of recovery. * Significantly different from resting eupnoea ($p < 0.05$).

	MAP (mmHg)	HR (beats min⁻¹)	V_t (l)	f_b (breaths min⁻¹)	V_I (l min⁻¹)	T_I/T_{TOT}	Peak P_m (cm H_2O)	f_b x $\int P_m$	V_t/T_I (l s⁻¹)	PET_{CO2} (mmHg)
Eupnoea	84.5	58.6	0.940	11.0	8.5	0.30	-0.8	-7	0.56	36.8
	± 3.3	± 8.7	± 0.331	± 2.9	± 2.4	± 0.04	± 0.4	± 3	± 0.13	± 4.6
Min 1	78.8*	76.5*	1.079	14.9	14.4*	0.69*	-61.8*	-1700*	0.39*	31.6*
	± 8.7	± 8.2	± 0.064	± 0.3	± 0.81	± 0.05	± 14.7	± 551	± 0.02	± 3.9
Min 2	86.2	75.1*	1.080	15.0	14.6*	0.71*	-64.6*	-1749*	0.38*	34.9
	± 5.6	± 6.6	± 0.095	± 0.1	± 1.2	± 0.04	± 17.7	± 590	± 0.03	± 4.4
Min 3	89.5*	73.6*	1.015	15.0	13.7*	0.69*	-63.4*	-1602*	0.37*	36.9
	± 6.1	± 6.3	± 0.107	± 0.1	± 1.4	± 0.05	± 20.2	± 549	± 0.04	± 3.6
End	88.7	73.8*	0.985	15.0	13.3*	0.69*	-56.4*	-1588*	0.36*	36.9
	± 6.1	± 7.1	± 0.138	± 0.1	± 1.8	± 0.04	± 17.7	± 612	± 0.05	± 5.2
Rec 1	87.7	61.1	1.080	16.0*	13.2*	0.34	-0.8	-9	0.68	34.2
	± 3.3	± 9.0	± 0.504	± 6.3	± 4.1	± 0.05	± 0.3	± 4	± 0.20	± 4.8

MIP, MEP, HG Fatigueability

The percent change values for MIP, MEP and HG before and after the RBTs in both pre and post tests for the SHAM and EXP groups are displayed in Table 3. For both SHAM and EXP groups, there were no significant differences in the drops in MIP following the RBT_{PRE} compared to the drops in MIP following the RBT_{POST}. In the SHAM group, the drop in MEP values was significantly less following the RBT_{POST} compared to that seen during the RBT_{PRE}. In the EXP group there was a significantly greater drop in HG values following the RBT_{POST}.

Table 7. Mean changes in strength following the RBTs of the SHAM and EXP groups. Data are represented as percent change from values prior to the RBT. Values are means ± S.D. Definitions of abbreviations: MIP = maximal inspiratory pressure; MEP = maximal expiratory pressure; HG = handgrip strength. * Significantly different from RBT_{PRE} ($p < 0.05$).

	SHAM (n = 8)		EXP (n = 8)	
	Pre	Post	Pre	Post
MIP (%)	-3.1 ± 7.4	-0.5 ± 5.8	-3.8 ± 5.5	-5.6 ± 5.8
MEP (%)	-6.0 ± 5.3	0.4 ± 6.3*	-6.6 ± 6.7	-2.5 ± 5.6
HG (%)	-7.0 ± 5.0	-5.8 ± 5.1	-0.1 ± 5.5	-5.6 ± 5.3*

RBT$_{PRE}$: HR and MAP

In the RBT$_{PRE}$ of both training groups, HR was significantly elevated within the first minute of resistive breathing and sustained this level of elevation until the end of the RBT$_{PRE}$. Over the course of the RBT$_{PRE}$, HR rose an average of 41.4% in the SHAM group and 34.7% in the EXP group. Following the RBT$_{PRE}$ in both training groups, HR quickly normalized, returning to near resting values within the first minute of recovery. MAP showed a slightly more gradual response with significant elevations witnessed by minutes 2 and 3 of the RBT$_{PRE}$ in the SHAM and EXP groups respectively. Compared to values at eupnoea, MAP values at the end of the RBT$_{PRE}$ were elevated an average of 20.7% in the SHAM group and 17.3% in the EXP group. MAP did not normalize as quickly as the HR response and remained elevated beyond the first minute of recovery. The individual MAP and HR responses observed during the RBT$_{PRE}$ for the SHAM and EXP groups are displayed in Figures 1A and 2A respectively.

Figure 1. Data for individual SHAM subjects during A) RBT$_{PRE}$ and B) RBT$_{POST}$. Definitions of abbreviations: MAP = mean arterial pressure; HR = heart rate. The dotted lines denote the start and end points of the resistive breathing tasks.
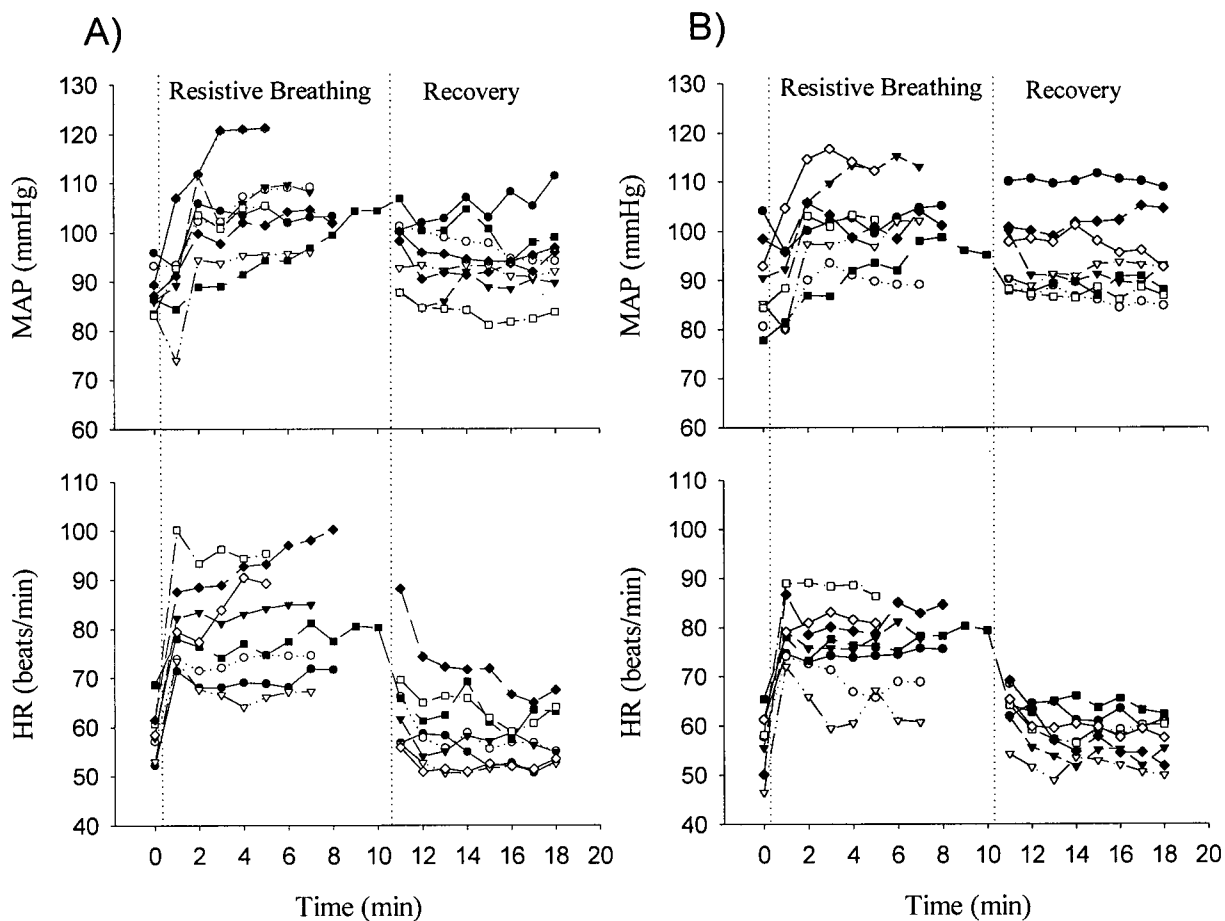
Figure 2. Data for individual EXP subjects during A) RBT$_{PRE}$ and B) RBT$_{POST}$. Definitions of abbreviations: MAP = mean arterial pressure; HR = heart rate. The dotted lines denote the start and end points of the resistive breathing task.
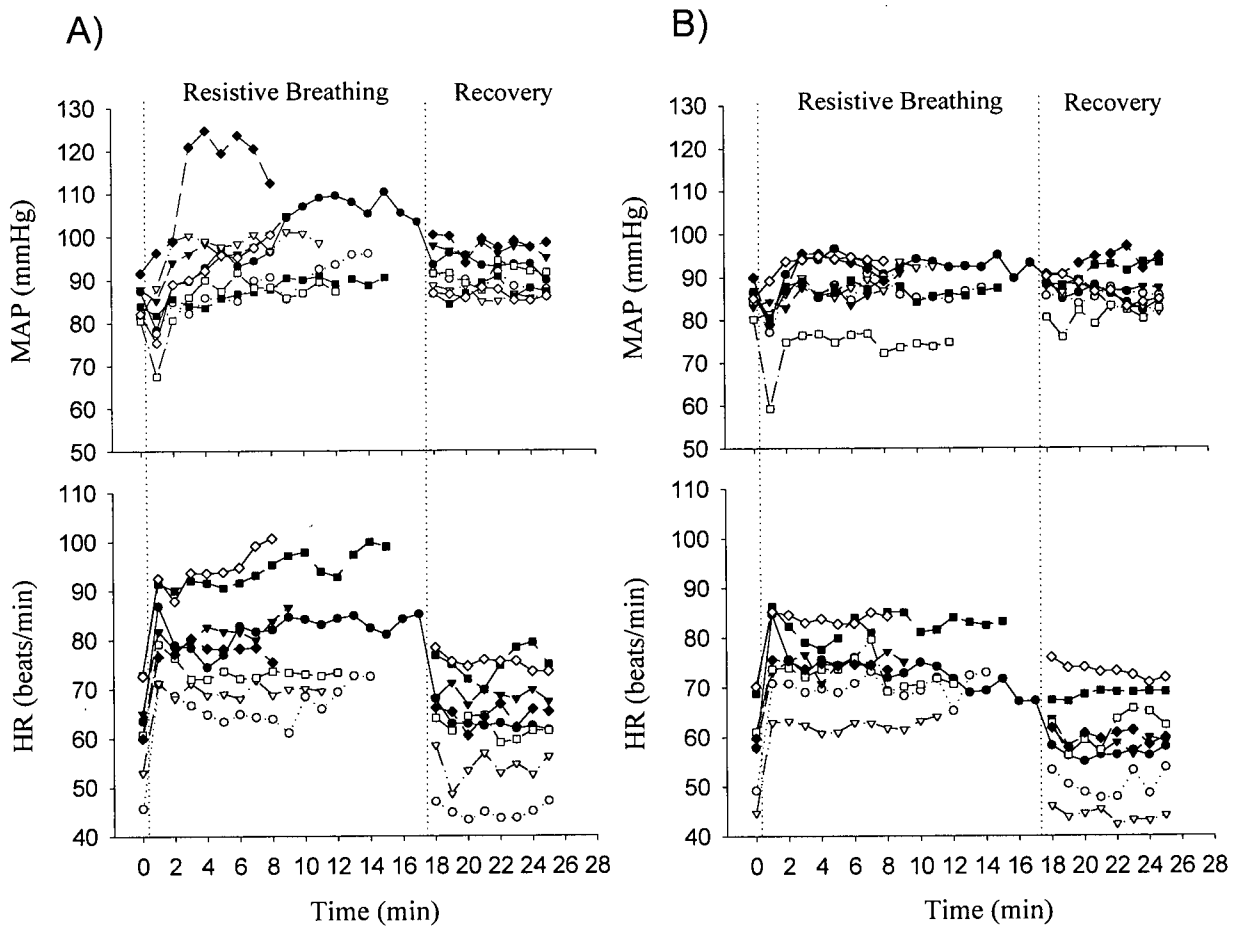
RBT$_{POST}$: HR and MAP

The mean HR and MAP responses of the RBT$_{PRE}$ and RBT$_{POST}$ for both the SHAM and EXP groups are displayed in Figure 3. In response to the RBT$_{POST}$, HR and MAP in the SHAM group increased by an average of 35.6% and 15.4% respectively. These increases in HR and MAP were less than those witnessed during the RBT$_{PRE}$, but not significantly so.

Figure 3. Heart rate and mean arterial pressure changes during RBT$_{PRE}$ and RBT$_{POST}$ in the SHAM and EXP groups. Data are represented as percent change from eupnoea. Values are means ± S.D. Definition of abbreviations: HR = heart rate; MAP = mean arterial pressure; Eup = eupnoea; Min = minute; End = final minute of RBT; Rec1 = first minute of recovery from RBT. * Significantly different from RBT$_{POST}$ ($p < 0.05$).
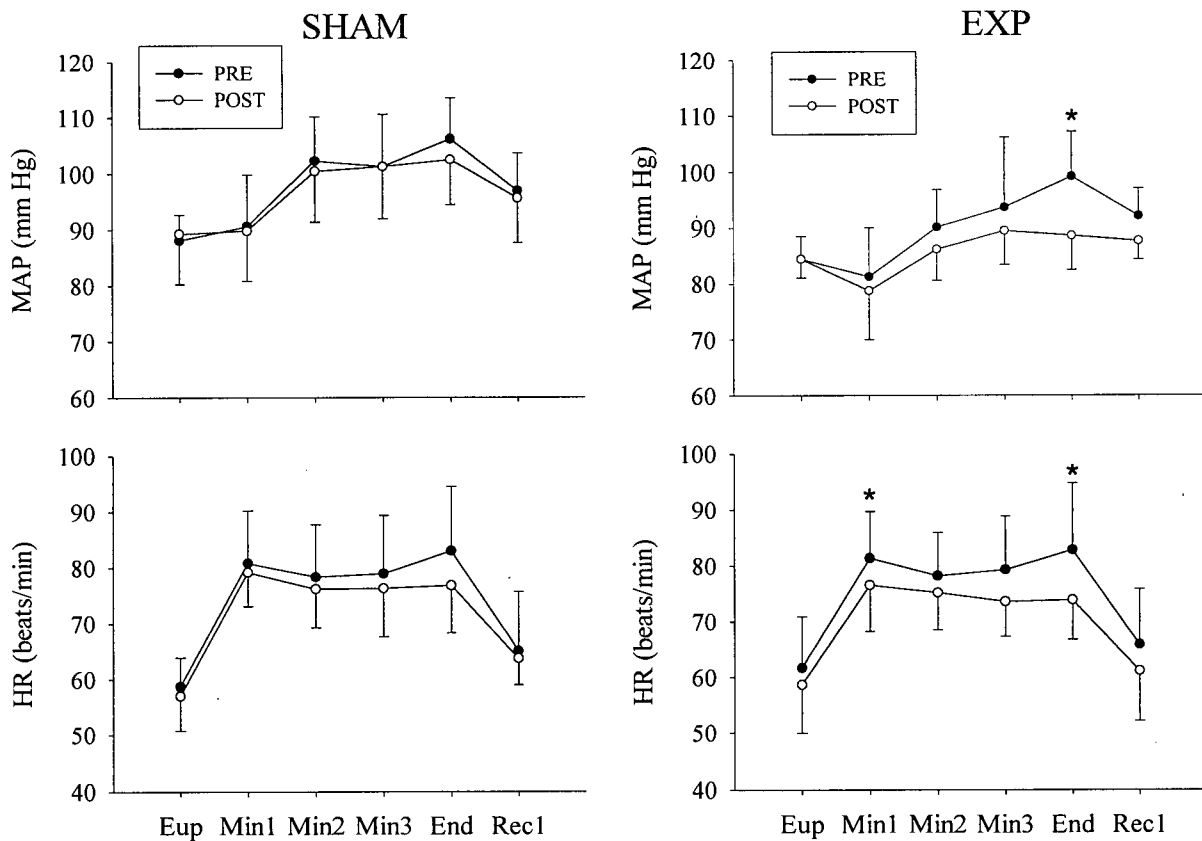
The one-tailed dependent t-tests failed to reveal any significant differences in HR or MAP between the pre and post RBT tests of the SHAM group at any time point.

In contrast to the SHAM group, the EXP group exhibited significantly blunted HR and MAP responses during the RBT$_{POST}$. Over the course of the RBT$_{POST}$, HR rose 27.1%; down 9 beats/min from the RBT$_{PRE}$. In the EXP group, there were significant pre vs. post-

training differences in HR for minute 1 and the final minute of the RBTs. Unlike the MAP responses seen during the pre-training RBTs and the RBT_{POST} of the SHAM group, the RBT_{POST} of the EXP group failed to result in a consistent rise in MAP. From beginning to end, MAP rose only 3.9%; down 11 mmHg from that seen during the RBT_{PRE}. Significant pre vs. post-training differences in MAP for the EXP group were present during only the final minute. Individual data for the RBT_{POST} of the SHAM and EXP groups are displayed in Figures 1B and 2B respectively.

DISCUSSION

The purpose of this study was to investigate the effects of IMT on the cardiovascular responses to resistive work of the inspiratory muscles. To this end, subjects were required to perform resistive inspiratory work before and after a five-week period of inspiratory muscle training. When performing high levels of inspiratory work, subjects in both groups exhibited a time-dependent rise in HR and MAP. Following training in the experimental group, the HR response to the same absolute workload was blunted and the MAP response was nearly abolished. However, in the SHAM group, there were no significant differences in the rise of HR or MAP between baseline and post-training tests. We interpret these findings to indicate a training-induced attenuation of the activity of afferent fibres in response to respiratory muscle resistance work.

Inspiratory Muscle Strength Improvements

In this study we detected an average MIP improvement of approximately 21 cm H_2O in the EXP group. This 17% increase in MIP is within the range of mean training improvements (8-45%) observed by others employing a similar 50% MIP training protocol (32, 44, 45, 70, 88, 89, 106, 123). The non-significant 6% MIP improvement witnessed in

the SHAM group was presumably due to a combination of a MIP learning effect from the weekly MIP assessments, and a placebo effect resulting from our naïve subjects' belief that they were actually engaging in a true IMT protocol. The changes in MIP witnessed in the SHAM group are similar to improvements (-1- 8%) seen by placebo or mock IMT groups in other studies (45, 51, 89, 106, 123). The fact that there were no significant changes in the control strength tests (MEP and HG) in either the EXP or SHAM groups further suggests that the MIP improvements observed in the EXP group were a result of the IMT training.

The MIP test serves as a global measure of inspiratory muscle strength. The MIP improvement observed in the EXP group, therefore, suggests an increase in the global inspiratory muscle strength of this group following five weeks of IMT training. Electrically evoked inspiratory muscle contractile strength was not assessed in this study. As a result, it is not clear what physiological adaptations caused the increase in MIP that we observed in the EXP group. Increased central drive, increased motor unit recruitment, increased inspiratory muscle contractility, and inspiratory muscle hypertrophy are all possible explanations for the MIP increase observed after training.

Inspiratory Muscle Fatigue

In order to elicit diaphragm fatigue, previous researchers (97, 108) have required subjects to attempt to isolate the diaphragm while engaging in breathing protocols performed to task failure and consisting of a prolonged T_I/T_{TOT} of 0.70, a normal f_b of 15 breaths/min and an inspiratory resistance of 60% of MIP. Such a protocol is believed to bring about diaphragm fatigue via drastically increasing the workload of the diaphragm whilst contributing to metabolite accumulation by restricting blood flow to and from this muscle (16, 18).

We attempted to bring about inspiratory muscle fatigue by mimicking the protocol of these previous researchers. Unfortunately, despite encouragement, most subjects failed to achieve the target P_m of 60% of MIP for any appreciable time and instead achieved an average of approximately 50% of MIP throughout the protocol. Given that MIPs require a maximal effort by all inspiratory muscles, and given that during the RBTs the subjects were encouraged to isolate the diaphragm and avoid recruitment of the accessory muscles, this finding is not altogether surprising.

Despite the achieved peak P_m values being slightly lower than the prescribed pressure targets, it is still likely that some degree of diaphragm fatigue was present in our subjects immediately following the RBTs. This presumption is based upon the work of other researchers who have developed an algorithm for determining whether or not a given level of diaphragmatic work performed at a given T_I/T_{TOT} will result in restrictions to diaphragm perfusion and result in diaphragm fatigue. Based on an anaethetized canine model, Bellemare et al. (18) studied the effect of diaphragm contractions on diaphragm blood flow. These researchers determined that blood flow through the diaphragmatic vein was related to the product of T_I/T_{TOT} and the percent of maximal diaphragmatic pressure (P_{di}) being generated; a term they referred to as Ttdi. When Ttdi was above a threshold level of 0.25, diaphragmatic blood flow was found to be restricted and was shown to approach zero flow at a Ttdi of 0.75. Research in humans appears to agree with this observed relationship in canines (16, 17).

In the present study, P_{di} was not measured, nor was diaphragmatic pressure measured during the RBTs. Our best estimate of the diaphragmatic pressures subjects were inspiring at is therefore the %MIP they averaged during the RBTs. Using a %MIP of 50%, and a T_I/T_{TOT} of approximately 0.70, Ttdi is calculated as 0.35; a level believed to be sufficient to

restrict diaphragm perfusion, contribute to metabolite accumulation and result in diaphragm fatigue (16-18).

Additionally, there is reason to suggest that the lack of decline in MIP values following the RBTs does not necessarily indicate that diaphragm fatigue did not occur. MIPs are volitional tests which are purported to be global measurements of respiratory muscle strength and therefore may not be sensitive enough to detect small changes in the diaphragm specific fatigue that we were attempting to elicit (53). Furthermore, the allowable 15% variation in MIP values for a given test may have been too great and introduced a level of variability that precluded any true decline in inspiratory muscle strength from being observed. Lastly, the eight-minute recovery period between the end of the RBT and the measurement of the post-RBT MIPs may have been too long and may have allowed for recovery of the fatigued respiratory muscles. In a recent study by McConnell and Lomax (70), the decline in MIP values observed immediately following a similar diaphragm fatigue protocol was no longer present after five minutes of plantar flexor exercise. Therefore, we believe that although the MIP values did not indicate any appreciable diaphragm fatigue, it is quite possible that diaphragm fatigue did occur but that our measurements techniques were not sensitive enough to detect it and/or we performed the measurements after the muscles had sufficient time to recover.

Cardiovascular Response to Resistive Inspiratory Work

During volitional exercise such as the RBT performed by the subjects in this study, there are both peripheral and central mechanisms governing the rise in HR, blood pressure and sympathetic stimulation that typically occurs (66, 120). Central command arises from the higher brain centres and serves as a top-down controller of cardiovascular activity via recruitment of α-motor neurons (41, 50, 115). Meanwhile, metabolite-sensitive afferent

fibres (chemoreceptors) and mechanically sensitive afferent fibres (mechanoreceptors) arising from the interstitium of the exercising muscle serve as the cardiovascular control mechanisms in the periphery. The thinly-myelinated type III afferents and unmyelinated type IV afferents are believed to respond primarily to mechanical and chemical afferents respectively (61, 72); although both type III and IV afferents may readily respond to either stimuli (5). Type III and IV afferent fibres are known to richly innervate the human inspiratory muscles (34, 52). In this study, it is therefore reasonable to assume that during the RBTs, mechanical deformation of the respiratory muscles, accumulation of muscle metabolites within the inspiratory muscle interstitium, and subject volition each played a role in the observed HR and MAP responses. What is unclear is which of the three pathways were attenuated with training and responsible for the blunted cardiovascular response witnessed in the EXP group post-training.

Researchers investigating the cardiovascular responses to isolated peripheral muscle exercise have developed useful methods of assessing the various contributions of the chemosensitive afferents, the mechanically sensitive afferents, and central activation. In order to determine the role that each of these three cardiovascular effecting pathways has upon exercise, researchers have employed the use of voluntary muscle contractions, electrically evoked muscle contractions, and post-exercise circulatory occlusion (PECO). Voluntary muscle contractions such as those performed in this study are believed to recruit activation of all three pathways of cardiovascular activation, whereas electrically evoked exercise is believed to remove central activation from the equation and involve only the mechanoreceptors and chemoreceptors. During PECO, clearance of muscle metabolites following a bout of exercise is prevented with the supra-systolic inflation of a blood pressure cuff proximal to the site of the previously working muscle. With circulation to the muscle

arrested, the effects of the chemoreceptors can be studied without any interfering influence of either central activation or the mechanically sensitive afferents.

In the study of the intact human inspiratory muscles, use of the above-mentioned designs presents a challenge. For one, unlike the muscles of the periphery, humans are less capable of isolating an individual muscle group during volitional inspiration and therefore the recruitment of diffuse accessory muscles may occur despite subjects' intentions. As a result, electrically evoked contractions of the inspiratory muscles, though performed, do not effectively measure the input of the central activation pathway. Furthermore, PECO is not a feasible option and therefore isolating chemoreceptor input from mechanoreceptor input is considerably more difficult when investigating the inspiratory muscles of the human. Due to these constraints, in order to gain insight into the likelihood that training induced changes to the central and peripheral activation pathways may have occurred in this study one must consult the literature on the training of these responses in peripheral muscles.

Support for a Reduction in Central Activation

Supraspinal activation of the cardiovascular system plays a role in any act of voluntary exercise. As a result of our study paradigm, we cannot rule out the possibility that changes to this top-down processing occurred with training and may have contributed to our observations. Although ratings of perceived exertion were not measured in this study, subjects in the EXP group would be expected to experience a reduced effort requirement during the RBT_{POST} given that these subjects were performing the same absolute level of inspiratory work as during the RBT_{PRE}, but were working at a lower percentage of their MIP due to the training induced inspiratory muscle strength improvements. Due to the placebo effect, the SHAM group may also have experienced a reduction in perceived effort during the RBT_{POST}. Heart rate is believed to be especially susceptible to influence from higher

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brain centres (121) and it is likely that a reduced central activation played a role in the significant reduction in the HR response observed in the EXP group and the trend for a reduction in the HR response of the SHAM group post-training. Furthermore, the brisk HR increase observed in all RBTs is suggestive of a centrally mediated response. Whereas, in the case of MAP, the increase was more gradual and less suggestive of a centrally-mediated response. Central activation has been shown to exhibit less of an influence upon MAP and therefore may not have been the primary mediator of the attenuation observed in this variable (121).

Evidence of changes to central activation with training has been documented in the cardiovascular response to peripheral muscle exercise. Fisher and White (36) were able to demonstrate that after six weeks of unilateral plantar flexor training, diastolic blood pressure was attenuated during electrically evoked contractions of the trained leg whereas systolic blood pressure, diastolic blood pressure, MAP and HR were all attenuated during voluntary exercise of the trained leg. No effects of training were shown during PECO. This study design, therefore, isolated the effects of mechanoreceptor activation, chemoreceptor activation and central activation and suggested that the attenuation of HR and systolic blood pressure that occurred post training were as a result of changes to the central activation pathway. It is possible that, following IMT, a similar adaptation to the central activation pathway may have occurred in the present study.

Support for a Reduction in Mechanoreceptor Afferent Output

Mechanoreceptor activation plays a role in the sympathetic and cardiovascular responses to exercise and it has been proposed that this pathway is also capable of being adapted with training. Following a six-week plantar flexor training period, Fisher and White (36) reported a significant attenuation in the diastolic blood pressure response to electrically

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evoked exercise of the trained leg without a significant attenuation during PECO. These findings suggest that while there was no training effect of the chemoreceptor response, there may have been some desensitization or reduction in responsiveness of the mechanoreceptors. Additionally, in a HG training study, Sinoway et al. (101) required subjects to perform a non-fatiguing voluntary HG task of an intensity insufficient to activate the chemoreflex (14) but of sufficient intensity to bring about small increases in HR, MAP and sympathetic activity via mechanoreceptor activation. After four weeks of HG training there was an attenuation of the MAP, MSNA and norepinephrine spillover responses to the non-fatiguing exercise, demonstrating a training-induced reduction in the outflow of the mechanically sensitive afferents. It has been hypothesized (101) that this effect may be related to the muscle acidosis associated with training, as it has been shown in the anaesthetized feline model that the mechanically sensitive type III afferents have a reduced discharge frequency following repeated lactic acid exposure (102).

In the present study, the inspiratory muscles of both the EXP and SHAM groups observed nearly identical absolute mechanical stimuli during the RBT_{PRE} and RBT_{POST} because peak P_m values were consistent between tests. Therefore, the changes observed in the EXP group were not likely due to changes to the mechanical stimuli during the RBTs. Rather, these findings, like those of Sinoway et al. (101), may be explained by a reduction in activity of the mechanically sensitive afferents to a given amount of mechanical deformation. This may have occurred as a result of repeated exposure to metabolite accumulation or as a result of repeated exposure to the large P_m swings associated with the IMT training.

Support for a Reduction in Chemoreceptor Afferent Output

Although the influence of central activation and the activity of the mechanoreceptors likely contributed to the rise in HR and MAP during the RBTs, there is reason to suggest that metabolite accumulation was the primary driving force behind the substantial increase in HR and MAP in this study. Sheel et al. (97) performed a study which manipulated the P_m values, duty cycles and f_b values subjects were inspiring at during different protocols. During high frequency breathing, and high resistance breathing the mechanoreceptors and central activation pathways were believed to be highly recruited. It was demonstrated by these researchers however, that the increase in MAP and HR in response to such protocols was substantially less than that observed during lower intensity breathing protocols that were associated with metabolite accumulation and respiratory muscle fatigue. The RBTs of this study were based upon the work of these researchers and the time-dependent HR and MAP elevations during the RBTs were similar to those observed during their fatiguing protocols. Therefore, it is believed that metabolite accumulation within the respiratory muscles of our subjects was likely the major contributor to the HR and MAP elevations that we observed.

By association, it is likely that the attenuated HR and MAP response observed in the EXP group was due to a reduction in the activity of chemosensitive afferent fibres. This could occur as a result of reduced metabolite production, increased metabolite clearance and/or a reduced responsiveness of the afferent fibres to the metabolic stimulants. Past studies have shown the inspiratory muscles to be capable of increasing their oxidative capacity. Vrabas et al. (124) performed a ten-week treadmill training study in rats and found greater citrate synthase and superoxide dismutase activities, a greater abundance of type I muscle fibres, and a reduction in type IIb muscle fibres in the diaphragms of those rats exposed to training. Furthermore, Ribera et al. (83) investigated the oxidative capacity of

the inspiratory muscles of patients with COPD; a population expected to exhibit training-like adaptations due to the chronic exposure to high elastic and resistive inspiratory loads. Based on muscle biopsies and oxigraphic chamber analyses, these researchers demonstrated that the patients with COPD had a more highly oxidative muscle fibre profile in the diaphragm, and a greater expression of mitochondrial creatine kinase isoforms in the external intercostal and diaphragm muscles when compared to a group of age-matched controls. Additionally, the maximal oxidative capacity of these muscles was significantly correlated to the patients' pulmonary indexes of obstruction and degree of hyperinflation. Similar aerobic adaptations have been shown to be present in the diaphragms of patients with heart failure (116) who typically experience a chronic hyperventilatory adaptive response to their insufficient cardiac output (69). Furthermore, Spengler et al. (107) illustrated that four weeks of hyperpnoea training in healthy humans results in a significant reduction in blood lactate levels during incremental and steady state cycling to volitional fatigue. Collectively, these results indicate that the five weeks of IMT performed by the EXP group in this study could have potentially resulted in an increased oxidative capacity of the respiratory muscles and could have lessened the production of metabolites at a given workload, thus contributing to the attenuation of the HR and MAP responses during the RBT_{POST}.

A number of training studies investigating attenuation of the HG-induced chemoreflex further support this notion. Mostoufi-Moab et al. (74) found that following four weeks of HG training, the pH and venous lactate responses to rhythmic ischaemic HG exercise were diminished and that this was associated with a reduced MAP response. Similarly, Somers et al. (105) demonstrated that the MSNA response to isometric HG exercise and PECO were both attenuated following six weeks of right arm HG training. Sham training of the left arm failed to result in the same adaptation. Evidence from these

researchers therefore strongly suggests that a reduction in MSNA and MAP responses to exercise may result from a reduction in metabolite accumulation. Although our findings are in agreement with a reduction in metabolite accumulation within the inspiratory muscles, no attempts to measure metabolite levels were made in the present study and therefore no firm conclusions can be drawn in this regard.

Based on a variety of cross-sectional studies, others have suggested that the attenuated chemoreflex response that occurs after training is not solely due to a reduction in metabolite accumulation but that reduced afferent fibre responses to a given level of metabolite accumulation may also be playing a role. In one study (103), the MSNA response to voluntary fatiguing rhythmic ischaemic HG exercise and PECO was assessed in the dominant and non-dominant arms of both body builders and untrained subjects. During the ischaemic exercise, pH changes were similar between body builders and untrained subjects and between dominant and non-dominant arms within subjects. Despite this, the untrained subjects were found to have an MSNA response more than double that of the bodybuilders, and the MSNA response of the dominant arms was markedly lower than that of the non-dominant arms. These results suggest a conditioning induced change in the responsiveness of the type III and IV muscle afferent fibres to the metabolic by-products of exercise. Similarly, Sterns et al. (111) found patients suffering from heart failure to have a normal MSNA response during voluntary isometric handgrip exercise but a reduced MSNA response during the PECO in comparison to a group of age-matched controls. In this study, nuclear magnetic resonance spectroscopy demonstrated that the heart failure subjects and controls experienced similar pH levels during the HG exercise. It was hypothesized that the chronically high muscle acidosis typically experienced by heart failure patients as a result of their poor exercise capacity may have resulted in a blunted chemoreceptor sensitivity in

these subjects. These results suggest that chronic metabolite exposure resulting from localized muscle training may be capable of reducing the activity of the chemosensitive afferents or increasing the threshold of metabolite accumulation required for a response. A similar desensitizing effect of afferent fibres has been shown to occur in the nasal mucosa of humans. In response to a period of systemic capsaicin pre-treatment the usually observed hypertensive response to capsaicin has been shown to be abolished (67). Therefore, in the current study, it is possible that a similar desensitization could have occurred in the chemosenstive afferent fibres of the inspiratory muscles of the EXP group.

A recent study supports the notion that IMT may be able to attenuate the metabolite-mediated cardiovascular response to resistive inspiratory work. McConnell and Lomax (70) studied the effects of the respiratory metaboreflex by using a study design that required inspiratory muscle work to be followed by a plantar flexor exercise task to fatigue. These researchers determined that when inspiratory work similar to that performed in this study, was followed immediately by plantar flexor exercise, time to fatigue of the plantar flexors was reduced. However, when the same absolute inspiratory work was performed after a four-week IMT protocol consisting of 30 breaths, twice daily, at a resistance of 50% of MIP, this finding was no longer present. In a subset of their study population, prior to IMT, they demonstrated that the metaboreflex had been elicited during the prescribed inspiratory muscle work as evidenced by a drop in Q_{leg} and a rise in LVR. They concluded that prior to IMT, the metaboreflex had a prolonged effect which restricted blood flow during the ensuing plantar flexor exercise and contributed to an earlier time to fatigue. Following IMT, although the inspiratory work still contributed to a significant degree of inspiratory muscle fatigue, these researchers suggested that the degree of metabolite accumulation was insufficient to initiate the metaboreflex and, therefore, did not result in a sympathetically

mediated restriction to Q_{leg} during the plantar flexor exercise. The attenuated HR and MAP response observed in the present study is in agreement with the findings of these researchers. We believe that rather than a blunted central activation, the evidence suggests that training induced changes in the cardiovascular response to resistive inspiratory work were acting at the level of the chemo- and mechanoreceptors; either via a reduced metabolite accumulation or a reduced responsiveness to metabolic stimuli.

Respiratory Muscle Metaboreflex and Exercise

Many studies have investigated the possibility of improved whole-body exercise endurance performance following a period of IMT. This body of literature has produced conflicting findings, the strengths and weaknesses of which are beyond the scope of this thesis [interested readers are referred to Sheel, (2002) (96)]. In defense of the hypothesis that IMT is capable of significantly improving exercise performance, many have suggested a possible role for increased respiratory muscle fatigue resistance and attenuated metaboreflex responses with IMT. In light of the effects that high levels of inspiratory muscle work performed during whole body exercise have upon restricting Q_{leg} (47), limiting whole-body exercise performance (49), and contributing to peripheral muscle fatigue (87), such a hypothesis seems plausible. However, direct research investigating this potential mechanism has been rather absent from the literature. Recently, using cervical and thoracic magnetic stimulation techniques, Verges et al. (118) showed that the degree of diaphragm and abdominal muscle fatigue in response to endurance cycling is capable of being significantly reduced with hyperpnoea training. Furthermore, as elaborated on earlier in this thesis, McConnell and Lomax (70) have shown that IMT can abolish the effects that prior inspiratory muscle work can have on reducing the time-to-fatigue during plantar flexor exercise. As a whole, these findings and those of our own study, suggest that there is good

reason to believe that IMT is capable of increasing the fatigue resistance of the inspiratory muscles and attenuating the metaboreflex response during whole-body endurance exercise.

A reduction in sympathetic influences arising from the respiratory muscles during intense whole body exercise may contribute to improved endurance performance following IMT. Although no measurements of the state of the sympathetic nervous system were directly made in this study, the evidence from the HR and MAP responses suggest that, following IMT, there was an attenuation of the sympathetic response to high levels of inspiratory muscle work. If during sustained and intense whole-body exercise ($> 80\%$ VO_2max) this attenuated sympathetic response also held true, this would prevent the increase in vascular resistance and reduction in locomotor muscle blood flow that is believed to typically occur. An increase in the delivery of arterial blood to the exercising muscles in the face of the high respiratory muscle demands associated with intense endurance exercise would theoretically increase exercise capacity. Our findings, therefore, may help to explain those of previous researchers who have documented improved whole-body endurance exercise performance following IMT. However, the suggestion that our findings apply to the scenario of intense whole-body exercise is purely speculative and deserves further attention in future studies.

Limitations

There are a number of limitations to this study that are worthy of mention. As noted previously, inspiratory muscle fatigue was not directly assessed in this study. The use of the MIP test as a measure of inspiratory muscle fatigue is a rather insensitive test and does not reveal anything about the specific type of fatigue present (low-frequency, high-frequency, or central fatigue) and does not provide specific information as to which inspiratory muscles are fatigued. Therefore, in this study we cannot be confident as to whether inspiratory

muscle fatigue did or did not occur, and can certainly not make any claims about the type of fatigue that may or may not have been present. However, as we elaborated on previously in this document, we would speculate that based on the peak P_m values, T_I/T_{TOT} values and duration of the RBTs, subjects in this study likely did encounter some degree of diaphragm fatigue and the MIP test either failed to detect it or was executed after the muscles had recovered.

Due to the nature of studying the respiratory muscle systems of intact humans, we were unable to determine what cardiovascular effecting pathway was contributing to the rise in HR and MAP observed in this study. Furthermore, we can only hypothesize as to the cause for the attenuation in the HR and MAP response in the EXP group post-training. Although we postulate that the altered HR and MAP responses were primarily mediated by a reduced metabolite accumulation or a reduced afferent fibre responsiveness to metabolites, we cannot rule out the possibility that changes in central activation or central processing of afferent input may have significantly contributed to our observations.

Subjects in this study were not truly randomized into EXP and SHAM groups. Due to logistical concerns, the first eight subjects were assigned to the EXP group and the remainder were assigned to the SHAM group. We do not believe that this resulted in different subject populations within each group as the spirometric and physical characteristics between groups were not different. However, as the EXP subjects were all tested prior to the SHAM subjects we cannot deny that there may have been some learning effect on behalf of the experimenters. Furthermore, although subjects in this study were blinded to their treatment group, the experimenters in this study were not blind to the condition that the subjects had been assigned. Although these issues had the potential to influence the outcomes of this study, the primary variables (HR and MAP) measured in this

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study were not believed to be vulnerable to experimenter influence. Also, subjects in this study served as their own controls and therefore any effect of learning on behalf of the experimenters would likely have been apparent in the results of both study groups.

A final limitation of this investigation is that it is limited in scope to the study of healthy, college-aged men. As such, the findings from this research may not be directly applicable to women, older or younger aged men, or clinical populations. Future study of these specific populations is warranted.

Conclusion

In summary, our findings show that five weeks of resistive IMT is capable of increasing inspiratory muscle strength and attenuating the time-dependent rise in HR and MAP that occurs with resistive respiratory exercise in healthy males. Five weeks of sham training failed to result in the same HR and MAP attenuation and failed to result in a significant improvement in inspiratory muscle strength. We speculate that the attenuated cardiovascular response following training may have been due to a reduced activity of the type III and IV afferent fibres innervating the inspiratory muscles. This may have been caused by an increased oxidative capacity of the inspiratory muscles, resulting in a reduced metabolite accumulation with resistive work. Alternatively, this may be due to a desensitization or decline in responsiveness of the type III and IV afferents to chemical stimulants, resulting from a conditioned response to repeated exposure to the accumulated metabolites associated with the IMT. Our findings of reduced cardiovascular responsiveness to inspiratory work suggest that IMT may have the potential to improve whole-body exercise performance.

REFERENCES

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

2. NHLBI Workshop summary. Respiratory muscle fatigue. Report of the Respiratory Muscle Fatigue Workshop Group. *Am Rev Respir Dis* 142: 474-480, 1990.

3. Standardization of Spirometry, 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med* 152: 1107-1136, 1995.

4. **Aaron EA, Seow KC, Johnson BD, and Dempsey JA**. Oxygen cost of exercise hyperpnea: implications for performance. *J Appl Physiol* 72: 1818-1825, 1992.

5. **Adreani CM and Kaufman MP**. Effect of arterial occlusion on responses of group III and IV afferents to dynamic exercise. *J Appl Physiol* 84: 1827-1833, 1998.

6. **Allen GM, Hickie I, Gandevia SC, and McKenzie DK**. Impaired voluntary drive to breathe: a possible link between depression and unexplained ventilatory failure in asthmatic patients. *Thorax* 49: 881-884, 1994.

7. **Aubier M, Farkas G, De Troyer A, Mozes R, and Roussos C**. Detection of diaphragmatic fatigue in man by phrenic stimulation. *J Appl Physiol* 50: 538-544, 1981.

8. **Babcock MA, Johnson BD, Pegelow DF, Suman OE, Griffin D, and Dempsey JA**. Hypoxic effects on exercise-induced diaphragmatic fatigue in normal healthy humans. *J Appl Physiol* 78: 82-92, 1995.

9. **Babcock MA, Pegelow DF, Harms CA, and Dempsey JA**. Effects of respiratory muscle unloading on exercise-induced diaphragm fatigue. *J Appl Physiol* 93: 201-206, 2002.

10. **Babcock MA, Pegelow DF, Johnson BD, and Dempsey JA**. Aerobic fitness effects on exercise-induced low-frequency diaphragm fatigue. *J Appl Physiol* 81: 2156-2164, 1996.

11. **Babcock MA, Pegelow DF, McClaran SR, Suman OE, and Dempsey JA**. Contribution of diaphragmatic power output to exercise-induced diaphragm fatigue. *J Appl Physiol* 78: 1710-1719, 1995.

12. **Babcock MA, Pegelow DF, Taha BH, and Dempsey JA**. High frequency diaphragmatic fatigue detected with paired stimuli in humans. *Med Sci Sports Exerc* 30: 506-511, 1998.

13. **Badier M, Guillot C, Lagier-Tessonnier F, and Jammes Y**. EMG changes in respiratory and skeletal muscles during isometric contraction under normoxic, hypoxemic, or ischemic conditions. *Muscle Nerve* 17: 500-508, 1994.

14. **Batman BA, Hardy JC, Leuenberger UA, Smith MB, Yang QX, and Sinoway LI**. Sympathetic nerve activity during prolonged rhythmic forearm exercise. *J Appl Physiol* 76: 1077-1081, 1994.

15. **Bellemare F and Bigland-Ritchie B**. Central components of diaphragmatic fatigue assessed by phrenic nerve stimulation. *J Appl Physiol* 62: 1307-1316, 1987.

16. **Bellemare F and Grassino A**. Effect of pressure and timing of contraction on human diaphragm fatigue. *J Appl Physiol* 53: 1190-1195, 1982.

17. **Bellemare F and Grassino A**. Evaluation of human diaphragm fatigue. *J Appl Physiol* 53: 1196-1206, 1982.

18. **Bellemare F, Wight D, Lavigne CM, and Grassino A**. Effect of tension and timing of contraction on the blood flow of the diaphragm. *J Appl Physiol* 54: 1597-1606, 1983.

19. **Belman MJ and Mittman C**. Ventilatory muscle training improves exercise capacity in chronic obstructive pulmonary disease patients. *Am Rev Respir Dis* 121: 273-280, 1980.

20.**Bigland-Ritchie B**. EMG and fatigue of human voluntary and stimulated contractions. *Ciba Found Symp* 82: 130-156, 1981.

21.**Bigland-Ritchie B, Johansson R, Lippold OC, Smith S, and Woods JJ**. Changes in motoneurone firing rates during sustained maximal voluntary contractions. *J Physiol* 340: 335-346, 1983.

22.**Bigland-Ritchie B, Johansson R, Lippold OC, and Woods JJ**. Contractile speed and EMG changes during fatigue of sustained maximal voluntary contractions. *J Neurophysiol* 50: 313-324, 1983.

23.**Bigland-Ritchie B, Jones DA, Hosking GP, and Edwards RH**. Central and peripheral fatigue in sustained maximum voluntary contractions of human quadriceps muscle. *Clin Sci Mol Med* 54: 609-614, 1978.

24.**Bigland-Ritchie B, Jones DA, and Woods JJ**. Excitation frequency and muscle fatigue: electrical responses during human voluntary and stimulated contractions. *Exp Neurol* 64: 414-427, 1979.

25.**Bigland-Ritchie BR, Dawson NJ, Johansson RS, and Lippold OC**. Reflex origin for the slowing of motoneurone firing rates in fatigue of human voluntary contractions. *J Physiol* 379: 451-459, 1986.

26.**Bilodeau M, Henderson TK, Nolta BE, Pursley PJ, and Sandfort GL**. Effect of aging on fatigue characteristics of elbow flexor muscles during sustained submaximal contraction. *J Appl Physiol* 91: 2654-2664, 2001.

27.**Boutellier U, Buchel R, Kundert A, and Spengler C**. The respiratory system as an exercise limiting factor in normal trained subjects. *Eur J Appl Physiol Occup Physiol* 65: 347-353, 1992.

28.**Boutellier U and Piwko P**. The respiratory system as an exercise limiting factor in normal sedentary subjects. *Eur J Appl Physiol Occup Physiol* 64: 145-152, 1992.

29.**Brown GL and Burns BD**. Fatigue and neuromuscular block in mammalian muscles. *Proc R Soc Lond [Biol]* 136: 182-195, 1949.

30. **Chen R, Collins SJ, Remtulla H, Parkes A, and Bolton CF**. Needle EMG of the human diaphragm: power spectral analysis in normal subjects. *Muscle Nerve* 19: 324-330, 1996.

31.**Dempsey JA, Sheel AW, St Croix CM, and Morgan BJ**. Respiratory influences on sympathetic vasomotor outflow in humans. *Respir Physiol Neurobiol* 130: 3-20, 2002.

32.**Downey AE, Chenoweth LM, Townsend DK, Ranum JD, Ferguson CS, and Harms CA**. Effects of inspiratory muscle training on exercise responses in normoxia and hypoxia. *Respir Physiol Neurobiol*, 2006.

33. **Dunn FT**. The blockage of motor impulses in an asynchronized volley at the neuromuscular junction. *J Cell Comp Physiol* 46: 348-250, 1955.

34.**Duron B**. Intercostal and diaphragmatic muscle afferents. In: *Regulation of Breathing, Part I*, edited by Hornbein TF. New York: Marcel Decker, 1981, p. 473-540.

35.**Edwards RH, Hill DK, and Jones DA**. Effect of fatigue on the time course of relaxation from isometric contractions of skeletal muscle in man. *J Physiol* 227: 26P-27P, 1972.

36.**Fisher WJ and White MJ**. Training-induced adaptations in the central command and peripheral reflex components of the pressor response to isometric exercise of the human triceps surae. *J Physiol* 520 Pt 2: 621-628, 1999.

37.**Fuller D, Sullivan J, and Fregosi RF**. Expiratory muscle endurance performance after exhaustive submaximal exercise. *J Appl Physiol* 80: 1495-1502, 1996.

38. **Gandevia SC**. Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev* 81: 1725-1789, 2001.

39. **Gandevia SC, Allen GM, Butler JE, and Taylor JL**. Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. *J Physiol* 490 (Pt 2): 529-536, 1996.

40. **Gandevia SC, Macefield G, Burke D, and McKenzie DK**. Voluntary activation of human motor axons in the absence of muscle afferent feedback. The control of the deafferented hand. *Brain* 113 (Pt 5): 1563-1581, 1990.

41. **Goodwin GM, McCloskey DI, and Mitchell JH**. Cardiovascular and respiratory responses to changes in central command during isometric exercise at constant muscle tension. *J Physiol* 226: 173-190, 1972.

42. **Grimby L and Hannerz J**. Firing rate and recruitment order of toe extensor motor units in different modes of voluntary conraction. *J Physiol* 264: 865-879, 1977.

43. **Grimby L, Hannerz J, Borg J, and Hedman B**. Firing properties of single human motor units on maintained maximal voluntary effort. *Ciba Found Symp* 82: 157-177, 1981.

44. **Guenette JA, Martens AM, Lee AL, Tyler GD, Richards JC, Foster GE, Warburton DER, and Sheel AW**. Variable effects of respiratory muscle training on cycle exercise performance in men and women. *Canadian Journal of Applied Physiology* In press, 2005.

45. **Hanel B and Secher NH**. Maximal oxygen uptake and work capacity after inspiratory muscle training: a controlled study. *J Sports Sci* 9: 43-52, 1991.

46. **Haouzi P, Hill JM, Lewis BK, and Kaufman MP**. Responses of group III and IV muscle afferents to distension of the peripheral vascular bed. *J Appl Physiol* 87: 545-553, 1999.

47. **Harms CA, Babcock MA, McClaran SR, Pegelow DF, Nickele GA, Nelson WB, and Dempsey JA**. Respiratory muscle work compromises leg blood flow during maximal exercise. *J Appl Physiol* 82: 1573-1583, 1997.

48. **Harms CA, Wetter TJ, McClaran SR, Pegelow DF, Nickele GA, Nelson WB, Hanson P, and Dempsey JA**. Effects of respiratory muscle work on cardiac output and its distribution during maximal exercise. *J Appl Physiol* 85: 609-618, 1998.

49. **Harms CA, Wetter TJ, St Croix CM, Pegelow DF, and Dempsey JA**. Effects of respiratory muscle work on exercise performance. *J Appl Physiol* 89: 131-138, 2000.

50. **Hobbs S**. Central command during exercise: Parallel activation of the cardiovascular and motor systems by descending command signals. In: *Circulation, Neurobiology and Behavior*, edited by Smith OA GR, Weiss SM. New York: Elsevier Science Publishing Co, Inc, 1982, p. 213-256.

51. **Inbar O, Weiner P, Azgad Y, Rotstein A, and Weinstein Y**. Specific inspiratory muscle training in well-trained endurance athletes. *Med Sci Sports Exerc* 32: 1233-1237, 2000.

52. **Jammes Y and Speck DF**. Respiratory control by respiratory and diaphragmatic muscle afferents. In: *Regulation of Breathing*, edited by Dempsey JA and Pack AI. New York: Marcel Dekker, 1995, p. 543-582.

53. **Johnson BD, Babcock MA, Suman OE, and Dempsey JA**. Exercise-induced diaphragmatic fatigue in healthy humans. *J Physiol* 460: 385-405, 1993.

54. **Johnson BD, Saupe KW, and Dempsey JA**. Mechanical constraints on exercise hyperpnea in endurance athletes. *J Appl Physiol* 73: 874-886, 1992.

55.**Jones D**. Muscle fatigue due to changes beyond the neuromuscular junction. In: *Human Muscle Fatigue: Physiological Mechanisms*, edited by Porter RaWJ. London: Pitman Medical, 1981, p. 178-190.

56.**Jones DA**. High-and low-frequency fatigue revisited. *Acta Physiol Scand* 156: 265-270, 1996.

57.**Jones DA and Bigland-Ritchie B**. Electrical and contractile changes in muscle fatigue. In: *Biochemistry of Exercise: INternational series on Sport Sciences*, edited by Saltin B. Champaign, IL: Human Kinetics Publishers, 1986, p. 377-392.

58.**Jones DA, Bigland-Ritchie B, and Edwards RH**. Excitation frequency and muscle fatigue: mechanical responses during voluntary and stimulated contractions. *Exp Neurol* 64: 401-413, 1979.

59.**Jones DA, Newham DJ, and Torgan C**. Mechanical influences on long-lasting human muscle fatigue and delayed-onset pain. *J Physiol* 412: 415-427, 1989.

60.**Jonville S, Jutand L, Similowski T, Denjean A, and Delpech N**. Putative protective effect of inspiratory threshold loading against exercise-induced supraspinal diaphragm fatigue. *J Appl Physiol* 98: 991-998, 2005.

61.**Kaufman M and Forster H**. Reflexes controlling circulatory, ventilatory and airway responses to exercise. In: *Handbook of Physiology, Section 12, Exercise Regulation and integration of multiple systems*, edited by Rowell LSJ. Bethesda, MD, USA: American Physiological Society, 1996, p. 381-447.

62.**Kaufman M, Forster, HV**. *Reflexes controlling circulatory, ventilatory and airway responses to exercise*. New York: Oxford University Press for the American Physiological Society, 1996.

63. **Kawakami Y, Amemiya K, Kanehisa H, Ikegawa S, and Fukunaga T**. Fatigue responses of human triceps surae muscles during repetitive maximal isometric contractions. *J Appl Physiol* 88: 1969-1975, 2000.

64.**Krnjevic K and Miledi R**. Presynaptic failure of neuromuscular propagation in rats. *J Physiol* 149: 1-22, 1959.

65.**Laghi F, D'Alfonso N, and Tobin MJ**. Pattern of recovery from diaphragmatic fatigue over 24 hours. *J Appl Physiol* 79: 539-546, 1995.

66. **Lind AR, Taylor SH, Humphreys PW, Kennelly BM, and Donald KW**. The Circulatiory Effects Of Sustained Voluntary Muscle Contraction. *Clin Sci* 27: 229-244, 1964.

67.**Lundblad L, Hua XY, and Lundberg JM**. Mechanisms for reflexive hypertension induced by local application of capsaicin and nicotine to the nasal mucosa. *Acta Physiol Scand* 121: 277-282, 1984.

68. **Mador MJ, Magalang UJ, Rodis A, and Kufel TJ**. Diaphragmatic fatigue after exercise in healthy human subjects. *Am Rev Respir Dis* 148: 1571-1575, 1993.

69.**Mancini DM**. Pulmonary factors limiting exercise capacity in patients with heart failure. *Prog Cardiovasc Dis* 37: 347-370, 1995.

70.**McConnell AK and Lomax M**. The influence of inspiratory muscle work history and specific inspiratory muscle training upon human limb muscle fatigue. *J Physiol* 577: 445-457, 2006.

71. **McKenzie DK, Bigland-Ritchie B, Gorman RB, and Gandevia SC**. Central and peripheral fatigue of human diaphragm and limb muscles assessed by twitch interpolation. *J Physiol* 454: 643-656, 1992.

72. **Mense S and Stahnke M**. Responses in muscle afferent fibres of slow conduction velocity to contractions and ischaemia in the cat. *J Physiol* 342: 383-397, 1983.

73. **Morgan DW, Kohrt WM, Bates BJ, and Skinner JS**. Effects of respiratory muscle endurance training on ventilatory and endurance performance of moderately trained cyclists. *Int J Sports Med* 8: 88-93, 1987.

74. **Mostoufi-Moab S, Widmaier EJ, Cornett JA, Gray K, and Sinoway LI**. Forearm training reduces the exercise pressor reflex during ischemic rhythmic handgrip. *J Appl Physiol* 84: 277-283, 1998.

75. **Moxham J, Morris AJ, Spiro SG, Edwards RH, and Green M**. Contractile properties and fatigue of the diaphragm in man. *Thorax* 36: 164-168, 1981.

76. **Moxham J, Wiles CM, Newham D, and Edwards RH**. Sternomastoid muscle function and fatigue in man. *Clin Sci (Lond)* 59: 463-468, 1980.

77. **O'Donnell DE, D'Arsigny C, Raj S, Abdollah H, and Webb KA**. Ventilatory assistance improves exercise endurance in stable congestive heart failure. *Am J Respir Crit Care Med* 160: 1804-1811, 1999.

78. **O'Leary DS**. Point: the muscle metaboreflex does restore blood flow to contracting muscles. *J Appl Physiol* 100: 357-358; discussion 360-351, 2006.

79. **Ozkaplan A, Rhodes EC, Sheel AW, and Taunton JE**. A comparison of inspiratory muscle fatigue following maximal exercise in moderately trained males and females. *Eur J Appl Physiol* 95: 52-56, 2005.

80. **Ozkaplan A, Rhodes, EC**. Exercise induced respiratory muscle fatigue- A review of methodology and recent findings. *Biology of Sport* 21: 207-230, 2004.

81. **Radegran G and Saltin B**. Muscle blood flow at onset of dynamic exercise in humans. *Am J Physiol* 274: H314-322, 1998.

82. **Ray CA and Gracey KH**. Augmentation of exercise-induced muscle sympathetic nerve activity during muscle heating. *J Appl Physiol* 82: 1719-1725, 1997.

83. **Ribera F, N'Guessan B, Zoll J, Fortin D, Serrurier B, Mettauer B, Bigard X, Ventura-Clapier R, and Lampert E**. Mitochondrial electron transport chain function is enhanced in inspiratory muscles of patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 167: 873-879, 2003.

84. **Richardson RS, Sheldon J, Poole DC, Hopkins SR, Ries AL, and Wagner PD**. Evidence of skeletal muscle metabolic reserve during whole body exercise in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 159: 881-885, 1999.

85. **Ries AL and Moser KM**. Comparison of isocapnic hyperventilation and walking exercise training at home in pulmonary rehabilitation. *Chest* 90: 285-289, 1986.

86. **Rodman JR, Henderson KS, Smith CA, and Dempsey JA**. Cardiovascular effects of the respiratory muscle metaboreflexes in dogs: rest and exercise. *J Appl Physiol* 95: 1159-1169, 2003.

87. **Romer LM, Lovering AT, Haverkamp HC, Pegelow DF, and Dempsey JA**. Effect of inspiratory muscle work on peripheral fatigue of locomotor muscles in healthy humans. *J Physiol* 571: 425-439, 2006.

88. **Romer LM and McConnell AK**. Specificity and reversibility of inspiratory muscle training. *Med Sci Sports Exerc* 35: 237-244, 2003.

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

90. **Rosenbluth A**. The electrical excitability of mammalian striated muscles. *Am J Physiol* 129: 22-38, 1940.

91.**Roussos C, and Moxham, J.** Respiratory muscle fatigue. Chapter 27. In: *The Thorax*, edited by Roussos C; Macklem P. New York: Marcel Dekker, 1985, p. 829-870.

92.**Saxton JM and Donnelly AE.** Characteristics of impaired voluntary torque generating capacity after maximum voluntary eccentric muscular work in man. *Journal of Physiology* 477: 57-58p, 1994.

93.**Scherer TA, Spengler CM, Owassapian D, Imhof E, and Boutellier U.** Respiratory muscle endurance training in chronic obstructive pulmonary disease: impact on exercise capacity, dyspnea, and quality of life. *Am J Respir Crit Care Med* 162: 1709-1714, 2000.

94.**Seals DR.** Robin Hood for the lungs? A respiratory metaboreflex that "steals" blood flow from locomotor muscles. *J Physiol* 537: 2, 2001.

95. **Seals DR.** Sympathetic neural discharge and vascular resistance during exercise in humans. *J Appl Physiol* 66: 2472-2478, 1989.

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

97.**Sheel AW, Derchak PA, Morgan BJ, Pegelow DF, Jacques AJ, and Dempsey JA.** Fatiguing inspiratory muscle work causes reflex reduction in resting leg blood flow in humans. *J Physiol* 537: 277-289, 2001.

98. **Sheel AW, Derchak PA, Pegelow DF, and Dempsey JA.** Threshold effects of respiratory muscle work on limb vascular resistance. *Am J Physiol Heart Circ Physiol* 282: H1732-1738, 2002.

99.**Shoemaker JK, Hodge L, and Hughson RL.** Cardiorespiratory kinetics and femoral artery blood velocity during dynamic knee extension exercise. *J Appl Physiol* 77: 2625-2632, 1994.

100.**Simon M, LeBlanc P, Jobin J, Desmeules M, Sullivan MJ, and Maltais F.** Limitation of lower limb VO(2) during cycling exercise in COPD patients. *J Appl Physiol* 90: 1013-1019, 2001.

101.**Sinoway L, Shenberger J, Leaman G, Zelis R, Gray K, Baily R, and Leuenberger U.** Forearm training attenuates sympathetic responses to prolonged rhythmic forearm exercise. *J Appl Physiol* 81: 1778-1784, 1996.

102.**Sinoway LI, Hill JM, Pickar JG, and Kaufman MP.** Effects of contraction and lactic acid on the discharge of group III muscle afferents in cats. *J Neurophysiol* 69: 1053-1059, 1993.

103. **Sinoway LI, Rea RF, Mosher TJ, Smith MB, and Mark AL.** Hydrogen ion concentration is not the sole determinant of muscle metaboreceptor responses in humans. *J Clin Invest* 89: 1875-1884, 1992.

104.**Sinoway LI, Smith MB, Enders B, Leuenberger U, Dzwonczyk T, Gray K, Whisler S, and Moore RL.** Role of diprotonated phosphate in evoking muscle reflex responses in cats and humans. *Am J Physiol* 267: H770-778, 1994.

105.**Somers VK, Leo KC, Shields R, Clary M, and Mark AL.** Forearm endurance training attenuates sympathetic nerve response to isometric handgrip in normal humans. *J Appl Physiol* 72: 1039-1043, 1992.

106.**Sonetti DA, Wetter TJ, Pegelow DF, and Dempsey JA.** Effects of respiratory muscle training versus placebo on endurance exercise performance. *Respir Physiol* 127: 185-199, 2001.

107.**Spengler CM, Roos M, Laube SM, and Boutellier U.** Decreased exercise blood lactate concentrations after respiratory endurance training in humans. *Eur J Appl Physiol Occup Physiol* 79: 299-305, 1999.

108.St Croix CM, Morgan BJ, Wetter TJ, and Dempsey JA. Fatiguing inspiratory muscle work causes reflex sympathetic activation in humans. *J Physiol* 529 Pt 2: 493-504, 2000.

109. St Croix CM, Satoh M, Morgan BJ, Skatrud JB, and Dempsey JA. Role of respiratory motor output in within-breath modulation of muscle sympathetic nerve activity in humans. *Circ Res* 85: 457-469, 1999.

110.Stephens JA and Taylor A. Fatigue of maintained voluntary muscle contraction in man. *J Physiol* 220: 1-18, 1972.

111.Sterns DA, Ettinger SM, Gray KS, Whisler SK, Mosher TJ, Smith MB, and Sinoway LI. Skeletal muscle metaboreceptor exercise responses are attenuated in heart failure. *Circulation* 84: 2034-2039, 1991.

112. Stuessi C, Spengler CM, Knopfli-Lenzin C, Markov G, and Boutellier U. Respiratory muscle endurance training in humans increases cycling endurance without affecting blood gas concentrations. *Eur J Appl Physiol* 84: 582-586, 2001.

113.Supinski GS, DiMarco AF, and Altose MD. Effect of diaphragmatic contraction on intramuscular pressure and vascular impedance. *J Appl Physiol* 68: 1486-1493, 1990.

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

115.Thornton JM, Aziz T, Schlugman D, and Paterson DJ. Electrical stimulation of the midbrain increases heart rate and arterial blood pressure in awake humans. *J Physiol* 539: 615-621, 2002.

116. Tikunov B, Levine S, and Mancini D. Chronic congestive heart failure elicits adaptations of endurance exercise in diaphragmatic muscle. *Circulation* 95: 910-916, 1997.

117.Travaline JM, Sudarshan S, and Criner GJ. Recovery of PdiTwitch following the induction of diaphragm fatigue in normal subjects. *Am J Respir Crit Care Med* 156: 1562-1566, 1997.

118.Verges S, Lenherr O, Haner AC, Schulz C, and 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.

119.Verin E, Ross E, Demoule A, Hopkinson N, Nickol A, Fauroux B, Moxham J, Similowski T, and Polkey MI. Effects of exhaustive incremental treadmill exercise on diaphragm and quadriceps motor potentials evoked by transcranial magnetic stimulation. *J Appl Physiol* 96: 253-259, 2004.

120.Victor RG, Bertocci LA, Pryor SL, and Nunnally RL. Sympathetic nerve discharge is coupled to muscle cell pH during exercise in humans. *J Clin Invest* 82: 1301-1305, 1988.

121.Victor RG, Pryor SL, Secher NH, and Mitchell JH. Effects of partial neuromuscular blockade on sympathetic nerve responses to static exercise in humans. *Circ Res* 65: 468-476, 1989.

122.Victor RG and Seals DR. Reflex stimulation of sympathetic outflow during rhythmic exercise in humans. *Am J Physiol* 257: H2017-2024, 1989.

123.Volianitis S, McConnell AK, Koutedakis Y, McNaughton L, Backx K, and Jones DA. Inspiratory muscle training improves rowing performance. *Med Sci Sports Exerc* 33: 803-809, 2001.

124.Vrabas IS, Dodd SL, Powers SK, Hughes M, Coombes J, Fletcher L, Demirel H, and Reid MB. Endurance training reduces the rate of diaphragm fatigue in vitro. *Med Sci Sports Exerc* 31: 1605-1612, 1999.

125. **West J**. *Respiratory Physiology- The Essentials*. Baltimore, MD: Williams & Wilkins, 1995.

126. **Westerblad H and Allen DG**. Recent advances in the understanding of skeletal muscle fatigue. *Curr Opin Rheumatol* 14: 648-652, 2002.

127. **Westerblad H, Duty S, and Allen DG**. Intracellular calcium concentration during low-frequency fatigue in isolated single fibers of mouse skeletal muscle. *J Appl Physiol* 75: 382-388, 1993.

128. **Westerblad H, Lee JA, Lamb AG, Bolsover SR, and Allen DG**. Spatial gradients of intracellular calcium in skeletal muscle during fatigue. *Pflugers Arch* 415: 734-740, 1990.

129. **Wetter TJ, Harms CA, Nelson WB, Pegelow DF, and Dempsey JA**. Influence of respiratory muscle work on VO(2) and leg blood flow during submaximal exercise. *J Appl Physiol* 87: 643-651, 1999.

130. **Wood SA, Morgan DL, and Proske U**. Effects of repeated eccentric contractions on structure and mechanical properties of toad sartorius muscle. *Am J Physiol* 265: C792-800, 1993.

APPENDIX A – REVIEW OF LITERATURE

Maintaining homeostasis of the blood oxygen and carbon dioxide partial pressures within the human body requires adequate ventilation. This responsibility falls largely upon the muscles of inspiration. At a state of rest, this is not a difficult task in healthy humans. However, during maximal exercise there can be as much as a five-fold increase in tidal volume (V_t), and a three fold increase in breathing frequency (f_b), requiring the respiratory muscles to contract both stronger and faster in order to maintain blood oxygen and carbon dioxide levels within the normal range.

The dramatic increase in the demands placed upon the respiratory muscles with exercise is reflected in a large increase in the oxygen requirements of these muscles. During rest, the oxygen cost of breathing is believed to be less than 5% of the body's total oxygen consumption (VO_2) (125). However, during maximal exercise this proportion jumps to as high as 15% of the maximal VO_2 (VO_{2max}) (4) and up to 14-16% of the total cardiac output (48). Therefore during exercise, the oxygen demands of the respiratory muscles increase disproportionately to that of the rest of the body.

The diaphragm is the primary muscle of inspiration. Fortunately, it is well suited to the demands placed upon it during exercise. Histological analysis has shown that type I slow-oxidative muscle fibres comprise approximately 50% of the diaphragm, with the other 50% consisting of an equal proportion of fast twitch type IIa and type IIb fibres (2). Although this data is limited to the characteristics of the diaphragm, the other most relevant muscles of inspiration, the external intercostals, generally coincide with what is observed in the diaphragm (2). The high proportion of type I muscle fibres and dense capillarization of the inspiratory muscles contribute to its highly oxidative capacity and general resistance to fatigue under normal circumstances. Further contributing to the fatigue resistant nature of

the diaphragm, is the tendency of this muscle to operate at a mechanically advantageous length-tension relationship and at a mechanically efficient portion of the pressure-volume curve during periods of high ventilatory demand (53, 54). Despite these safeguards, it is widely accepted that during the high levels of inspiratory work associated with prolonged intense exercise or inspiratory resistance protocols, fatigue of the inspiratory muscles can occur.

Given the life-sustaining role served by the inspiratory muscles, it is important to understand under what circumstances inspiratory muscle fatigue (IMF) occurs, how it is best detected, and how the human body responds to it. As the muscles of inspiration are themselves skeletal muscles it is important to first address the large body of literature on skeletal muscle fatigue (SMF).

Skeletal Muscle Fatigue

In general, the occurrence of SMF is dependent upon the balance between energy demands (strength, efficiency, and frequency of contractions) and energy availability (oxygen and substrate usage and delivery), although numerous other factors, including those that mediate the level of electrical activation from the central nervous system and/or effect the excitation-contraction coupling process of the muscle also play a major role (91). SMF can be classified into three categories: central fatigue, low-frequency peripheral fatigue, and high-frequency peripheral fatigue. Each of the three forms of SMF is associated with different physiological responses.

Central fatigue often occurs in response to sustained maximal contractions. In response to such contractions there is a reduction in motor unit firing rate (22, 40, 43) that can exceed the reduction in contraction times and increase in relaxation rates (21, 22, 35) normally observed. When a mismatching between motor unit firing rate and contractile

speed occurs, force production is reduced and central fatigue will occur (38). Both spinal and supraspinal factors play a role in this inability of the motorneurons to maintain their initially high firing rates. Spinal factors include the intrinsic behaviour of the motorneuron, recurrent inhibition, reflex input reaching α- and γ-motoneurons and their presynaptic modulation, as well as other neuromodulatory influences acting on motorneurons and spinal circuitry (38). At the supraspinal level, descending pathway outputs to motorneurons and the regulators that influence such outputs are the factors that may affect motorneuron firing rates during central fatigue (38). Whatever the root cause, the asynchrony between the motorneuron firing rate and contractile speed results in the reduced contractile strength observed during central fatigue.

Central fatigue plays an especially important role in IMF, with approximately 50% of the force reduction observed in some diaphragm fatiguing protocols believed to be attributable to reductions in central motor drive (2). The occurrence of central fatigue is best assessed with a twitch-interpolation technique involving the detection of the increase in muscle force production that occurs when electrical impulses stimulate a muscle during maximal voluntary contractions. Although even in the rested subject differences in voluntary and electrically stimulated force production values are likely to occur, the observed difference is exaggerated in the presence of central fatigue making this technique a valuable assessment tool (38). In addition to the changes observed with the twitch interpolation technique, other hallmarks of central fatigue are an increase in the fluctuations of force outputs during sustained maximal voluntary contractions (38), and the increase in force output observed when subjects are encouraged to perform "super" efforts (20, 24).

In contrast to central fatigue, peripheral muscle fatigue is identified by reductions in the force produced during electrical stimulation of the muscle at a given frequency or set of frequencies (91). That is, the frequency-force curve is found to be depressed. With the occurrence of peripheral muscle fatigue, often times the frequency-force curve is not uniformly depressed in comparison to control values. Usually the depressions occur in specific stimulation frequency ranges that allow for the differentiation between the two subtypes of fatigue; low-frequency (1-20 Hz) fatigue and high-frequency fatigue (50-100 Hz) (80).

Low-frequency fatigue typically involves an impaired excitation-contraction coupling mechanism induced by a series of forceful yet submaximal contractions (55). The two most likely mechanisms responsible for low-frequency fatigue are reduced Ca^{2+} levels within the fatigued muscle fibres, and damage to sarcomeres in the middle of the muscle fibre (56).

Given that the binding of Ca^{2+} to troponin represents an essential step in the excitation contraction coupling process, it had long been recognized that reductions in Ca^{2+} levels or in the affinity of Ca^{2+} for troponin could result in a reduction in muscle contraction strength (55). Evidence of Ca^{2+} dependent mechanism of low-frequency fatigue has been eluded to in a study by Westerblad et al (127) in which intracellular Ca^{2+} levels were directly sampled in fatigued and non-fatigued single mammalian muscle fibres. At a given stimulation frequency the Ca^{2+} levels in the non-fatigued muscles were significantly higher than those in the fatigued muscles. Furthermore, the absence of any changes in intracellular Ca^{2+} buffering, and the maintenance of the relationship between Ca^{2+} and muscle tension in the fatigued fibres indicated that the fatigue was related to a reduced Ca^{2+} release from the sarcoplasmic reticulum rather than a change in the characteristics of the Ca^{2+}-troponin

interaction. Thus it appears that reduced Ca^{2+} release may be at least one of the mechanisms explaining the reduction in contractile strength observed during low-frequency fatigue.

Sarcomere damage may also play a role in low-frequency fatigue. It has been noted that low-frequency fatigue often occurs in response to muscles fatigued by stretching or by isometric contractions performed at long lengths (56). The sarcomere damage hypothesis suggests that when elongated muscle fibres contract there is a stretching of the series elements in the mid-sections of the muscle fibre by the stronger sarcomeres located at the ends of the fibre (59). The overstretching of the middle sarcomeres is believed to result in the end sarcomeres having to compensate for this lack of contractile function by adopting a shorter length than is found in their non-fatigued state. At a shorter length however, the sarcomeres are not contracting optimally and exhibit a shift in the force frequency curve. Such a shift is a defining characteristic of low-frequency fatigue. In further support of this hypothesis, studies have shown that the greatest loss in force production resulting from low-frequency fatigue occurs with the muscle tested at short lengths (130). Evidence supporting a role for sarcomere damage in low-frequency fatigue also comes from the time course of the recovery, which in some cases may last for over 24 hours (55, 65, 92, 130). Such a long recovery time would be required to repair the structural damage that is proposed to occur to the sarcomeres. It is likely that both sarcomere damage and inhibited Ca^{2+} release from the sarcoplasmic reticulum have a mechanistic relationship to the occurrence of low-frequency peripheral muscle fatigue.

High-frequency peripheral muscle fatigue occurs in response to high-frequency muscle stimulation and is characterized by a slowing of the waveform and loss of amplitude of the muscle action potential (24, 58) as well as the presence of a rapid recovery (24, 58). The proposed mechanism behind high-frequency fatigue is a failure of transmission across

the neuromuscular junction (29, 33, 64, 90, 110). To support this contention, Westerblad et al. (128) found the existence of Ca^{2+} gradients in the cells of muscles fatigued with high-frequency tetanus; whereas a much more homogenous Ca^{2+} distribution was present in rested muscles and muscles fatigued through intermittent titanic stimuli (i.e. those exhibiting low frequency fatigue). The Ca^{2+} distribution in the high-frequency fatigued muscles was such that there was greater Ca^{2+} in the periphery of the muscle cell and diminished Ca^{2+} in the core, suggesting problems with impaired conduction along the muscle cell t-tubules. This finding, and the behavioural characteristics of high-frequency muscle fatigue are in accordance with abnormal interfiber cation concentrations (increased K^+ and reduced Na^+ concentration) interfering with conduction along the surface membrane and t-tubules of the muscle (24, 56, 57). It is doubtful whether high frequency fatigue is a common mechanism of skeletal fatigue, as the stimulation frequencies (approximately 50 Hz or greater) (25) often required for this form of fatigue to be observed are not within the normal physiological range of motor unit firing rates (approximately 5-30 Hz) (42). Consequently, high-frequency muscle fatigue has not received the same research attention that low-frequency muscle fatigue has garnered and may not be as relevant to the study of IMF.

The pathways involved in SMF are complex and remain incompletely understood. Therefore, the mechanisms responsible for IMF are also unclear. It is likely however, that the IMF observed in response to high intensity exercise or resistance breathing protocols is likely to involve a combination of the above-proposed mechanisms. In order to better understand IMF it is worthwhile to discuss the various methods in which IMF may be assessed.

Methods of Assessing Inspiratory Muscle Fatigue

There are three commonly used methods of assessing IMF: volitional tests of inspiratory pressure, electromyography (EMG) recordings, and supramaximal bilateral phrenic nerve stimulation (BPNS). These methods are often combined or modified in order to gain a more complete assessment of IMF to suit the demands of a specific study.

Voluntary maximal inspiratory pressures (MIPs) are the most convenient method of inspiratory muscle strength testing. For these tests, subjects are instructed to expire to their residual volume and then perform a maximal inspiratory effort through a mouthpiece with only a small pinhole-sized inlet. The MIP sustained for a 1-second period is recorded (1). To assess fatigue, MIP values following a fatiguing protocol can be compared with those obtained during a baseline rested state. The use of MIP values to assess fatigue is problematic because the volitional nature of the test creates significant variation in values that is related to subject motivation and the degree of central rather than peripheral fatigue. Furthermore, the non-specific causes of the observed reductions in MIP values cannot be easily attributed to any one form of skeletal muscle fatigue. The benefit of the MIP test is that it represents a global measure of IMF, rather than representing the response of a single inspiratory muscle, and it is convenient and easy to use allowing for measurements of fatigue to be made quickly, prior to any immediate recovery.

With fatigue of skeletal muscles there is a corresponding shift in the EMG power spectrum from higher to lower frequencies (13) and as a result, surface EMG recordings can be used to identify fatigue. This technique has the benefit of being able to identify changes in the contractile properties of the muscles although contamination of the signal due to extraneous noise and cross-talk from nearby contracting muscles often represents a significant challenge. With recent use of needle EMG to pinpoint and actually penetrate the

inspiratory muscles of interest there is evidence indicating that these issues may be partially resolved (30).

The most accurate and objective method of assessing IMF is to assess transdiaphragmatic pressure (P_{di}) during bilateral phrenic nerve stimulation (BPNS). In this technique, two balloon tipped catheters are connected to pressure transducers and passed intranasally; one is positioned in the oesophagus while the other is positioned in the stomach. The difference between the gastric and oesophageal pressures is considered to be an accurate measure of P_{di}. Based on the changes in P_{di} values in response to electrical or magnetic bilateral supramaximal stimulation of the phrenic nerve, diaphragm fatigue can be diagnosed. The ability to assess the twitch response, as well as the response to paired stimuli makes this an ideal technique for the study of high-frequency and low-frequency muscle fatigue as well as central fatigue (9). Limitations to its use in assessing IMF are that it is an invasive and rather complicated procedure and that it only provides insight into diaphragm fatigue, disregarding the state of the other inspiratory muscles.

Inspiratory Muscle Fatigue: Causes and Consequences

Through the use of the various methods of assessing IMF very informative data has been obtained about the factors that contribute to IMF and ways in which the human body responds to such fatigue. One study by Johnson and co-workers (53) employed the BPNS technique to assess the presence of low-frequency diaphragm fatigue by measuring the P_{di} of individuals of varying fitness levels in response to 1 Hz, 10 Hz and 20 Hz stimuli, both before and after exercise of varying intensities. The results from this study indicated that sustained, near maximal exercise (>85% VO_{2max}) is capable of inducing diaphragm fatigue, with a greater incidence and greater degree of fatigue occurring at increasing exercise intensities. Exercise below the 85% VO_{2max} threshold, despite the onset of whole body

fatigue, was not found to induce low-frequency diaphragm fatigue. A similar study involved subjects exercising at 95% VO_{2max} and tested the P_{di} response at 10, 20, 50, 70, and 100 Hz and showed evidence for the occurrence of high-frequency diaphragm fatigue following near maximal intensity exercise(12).

Interestingly, the act of generating such high levels of ventilation as that seen during high intensity exercise is, by itself, not sufficient to induce IMF. Although it has been demonstrated that increased inspiratory resistance or high ventilation during exercise can both induce low-frequency IMF, when a non-exercising subject hyperventilates to a degree that matches that found during maximal exercise there is little evidence to indicate any degree of low-frequency fatigue occurring (11). In a non-exercising state, evidence suggests that diaphragmatic work must actually double that observed during maximal exercise in order for diaphragm fatigue to arise. This suggests that the metabolic milieu and/or blood flow competition and/or limits to oxygen availability that coincide with exercise play a crucial role in the induction of low-frequency IMF.

In order to investigate this phenomenon further, it has been shown that by altering the duty cycle (time spent on inspiration divided by the time spent on both inspiration and expiration, T_I/T_{TOT}) at which subjects respire at it is possible to restrict blood flow to the diaphragm. A study performed in anaesthetized canines showed that despite diaphragm intramuscular pressure swings being significantly lower than that seen in peripheral skeletal muscles, diaphragm contraction was sufficient to mechanically hinder phrenic artery blood flow (113). Additionally, it has been found that the time to diaphragm fatigue (T_{lim}) during resistive breathing protocols is inversely related to the product of T_I/T_{TOT} and the mean P_{di} during each inspiration (16, 17). This product has been termed the tension time of the diaphragm (TT_{di}). The relationship between T_{lim} and the inverse of the TT_{di} has been shown

to be best described by a quadratic hyperbolic function (16). Therefore, both the strength of the diaphragm contraction, as well as the timing of its contractions contribute to the occurrence of fatigue. A critical TT_{di} of 0.15 has also been identified in which TT_{di} values below this are associated with T_{lim} approaching infinity, and TT_{di} values greater than 0.15 being associated with a T_{lim} of less than 45 minutes (16). As a result of this research into the effect that altering T_I/T_{TOT} has on the occurrence of IMF there is strong evidence to indicate that blood flow restriction is associated with an earlier diaphragm fatigue.

To specifically study the role that oxygen restriction plays in this relationship, studies have been done assessing the effect of hypoxic conditions on IMF. Babcock et al. required subjects to perform exercise tests at 85% VO_{2max} under two different conditions; a normoxic condition and a hypoxic condition consisting of a 15% fraction of inspired oxygen (8). Diaphragm fatigue was assessed by detecting reductions in P_{di} in response to BPNS at three different stimulation frequencies: 1 Hz, 10 Hz and 20 Hz. Under hypoxic conditions subjects were found to have an earlier occurrence of diaphragm fatigue at 1 Hz and 10 Hz stimulation frequencies, and a more prolonged P_{di} recovery period than that found with normoxia. This work highlights the role that limitations in oxygen availability have in the occurrence of low-frequency IMF and its recovery. Given that the earlier occurrence of diaphragm fatigue was also associated with a shorter time to volitional whole body fatigue on the exercise cycling test, this study also shed some light on the notion that the occurrence of IMF may in some instances limit exercise performance.

In support of the idea that increased work of the inspiratory muscles may result in exercise limitations, it has been shown that in some patients suffering from severe chronic obstructive pulmonary disease (COPD) a plateau in leg oxygen uptake (VO_{2legs}) and leg blood flow (Q_{leg}) occurs during cycling exercise despite continual increases in workload

(100). This suggests that these subjects are likely experiencing limitations in Q_{leg} due to the increased metabolic demands associated with their high work of breathing. Furthermore, it appears that the limitation of Q_{leg} is related to exercise failure as those COPD patients that did not exhibit a plateau response were found to have a greater exercise capacity.

The belief that the work of breathing is in some way related to exercise performance has been more closely investigated in a series of studies by Harms and colleagues (47, 48). With the use of proportional assist ventilators that assist with inspiration in proportion to the inspiratory airflow and volume generated by subjects, these researchers were able to reduce the work of breathing during exercise. Adding to their experimental design, mesh screens were used in order to increase the work of breathing. Using these techniques, the work of breathing was able to be manipulated and consequent changes in Q_{leg}, LVR, VO_{2legs}, norepinephrine spillover and VO_{2max} were capable of being observed. It was shown that with respiratory muscle unloading at an exercise intensity corresponding to VO_{2max}, there was a 14-16% reduction in cardiac output and a 7% reduction in VO_{2max}; differentials that represent the approximate the demand that the respiratory muscles normally place upon the cardiovascular and respiratory systems of the body during maximal exercise. In addition, by performing a thermodilution technique and blood draws from the femoral and brachial arteries to assess Q_{leg} and VO_{2legs}, it was determined that these two variables had strong and significant inverse correlations to the work of breathing. A direct correlation was also found between the work of breathing and LVR, and between the degree of norepinephrine spillover and LVR. With less oxygen available to, and taken up by, the locomotor muscles during conditions of increased inspiratory work it was hypothesized that performance would likely suffer. Such a hindrance of performance is believed to occur in patients with COPD (84) and chronic heart failure (77) with evidence indicating that unloading of the inspiratory

muscles can result in dramatic improvements in endurance exercise performance in these populations. Thus, it appears that high levels of inspiratory muscle work are related to sympathetic and peripheral vascular changes that may limit exercise performance and contribute to locomotor muscle fatigue.

Despite the strength of these studies there is a limitation to the amount of insight they provide into the condition of inspiratory muscle fatigue. Firstly, the technique of norepinephrine spillover provides a general index of the sympathetic state of the muscle but does not provide specific information about the source of norepinephrine or the firing frequency or amplitude of the sympathetic nerve action potentials. Furthermore, although these studies were capable of carefully manipulating the work of breathing they did not take into account the effects that increased respiratory motor output or IMF may have had on the observed reductions in Q_{leg} and VO_{2legs}.

To more clearly address the sympathetic changes occurring during inspiration, St. Croix et al. performed studies using a delicate microneurography technique that employs the use of intraneural needle electrodes inserted in the peroneal nerve of the leg in order to take direct recordings of peripheral muscle sympathetic nerve activity (MSNA). During the MSNA recordings the V_t, f_b and end-tidal CO_2 levels were controlled while respiratory muscle output was manipulated with procedures consisting of either mechanical ventilation, voluntary hyperventilation or inspiratory resistance (109). These researchers discovered respiratory periodicities in MSNA that were unaffected by intrathoracic pressures or respiratory motor output. Instead, variation in MSNA was associated with lung volumes and therefore attributed to changes in feedback from baroreceptors and pulmonary stretch receptors.

Having shown that respiratory muscle output was not by itself a determinant of MSNA, these researchers next set out to elucidate the effects of diaphragm fatigue on MSNA. Using the microneurography technique once again, St. Croix and colleagues required subjects to perform various breathing protocols that manipulated breathing resistance and the occurrence of diaphragm fatigue, in addition to manipulations of respiratory motor output (108). Although increases in HR were found to occur during all trials of increased inspiratory resistance, increases in blood pressure and MSNA were only found to coincide with diaphragm fatigue; supporting the contention that the occurrence of diaphragm fatigue is linked to changes in sympathetic outflow and vascular resistance. Unfortunately, volitional measures of IMF were used in this study and as a result, it is impossible to tell what form of fatigue was taking place.

In an attempt to more clearly assess the changes in Q_{leg} that may occur with low-frequency IMF, Sheel et al. devised a study consisting of several different inspiratory protocols and the BPNS method of assessing IMF (97). One protocol involved subjects breathing at 60% of MIP with a T_I/T_{TOT} of 0.70, while another protocol required subjects to breathe at the same MIP with a T_I/T_{TOT} of 0.40. Both of these protocols were found to induce a 25-40% reduction in diaphragm force output as assessed by 1 Hz stimuli. In addition, these researchers required subjects to perform other non-fatiguing tasks that manipulated T_I/T_{TOT} or central inspiratory motor output through increases in inspiratory flow rate or force output. Large reductions in Q_{leg} (approximately 30%) and large increases in LVR, and MAP were only observed during the two fatiguing protocols. In a follow up study, these researchers showed the effects of diaphragm fatigue on LVR and Q_{leg} to dissipate within 30 seconds of recovery (98). Thus, low frequency IMF appears to have an acute, yet meaningful effect on both MSNA and Q_{leg}.

Collectively, the above findings support the existence of a sympathetically mediated inspiratory muscle metaboreflex. The inspiratory muscle metaboreflex hypothesis holds that the accumulation of metabolic by-products that contribute to IMF also stimulate the activity of the chemosensitive afferent type III or IV fibres innervating the diaphragm. Group III and IV afferents serve as sensory fibres that also exist in other skeletal muscles throughout the body. They are believed to fire in response to stimuli such as metabolic by-products (104), mechanical deformation (122), temperature (82), and vascular distension during muscular contraction (46). It is suspected that the activation of these afferent fibres occurs under conditions when blood supply to the inspiratory muscles is insufficient and metabolite accumulation occurs, resulting in sympathetically mediated excitation to most systemic vascular beds. Presumably, the end result is a vasoconstriction in the splanchnic, renal, and mesenteric vasculatures as well as the vascular beds of inactive skeletal muscles. This response possibly serves as an adaptive response (31, 62) to allow blood to be diverted towards the fatigued inspiratory muscles at a cost to the other muscles and organs of the body.

There is a sufficient amount of evidence to indicate that this metaboreflex response likely does occur. A similar metaboreflex is known to occur in response to fatiguing work performed by other skeletal muscles such as the formearm muscles involved in handgrip exercise (74). Recently, the inspiratory muscle metaboreflex hypothesis has also been upheld in work by Rodman et al. in which lactic acid was infused into the phrenic artery supplying the diaphragm of dogs both at rest and during exercise (86). In response to the transient lactic acid infusions, the dogs exhibited an increase in sympathetic activity and a reduction in hind limb blood flow that was abolished with the administration of sympathetic

blockade. Such findings are in accordance with the existence of an inspiratory muscle metaboreflex.

Based on the mechanism of the inspiratory muscle metaboreflex it seems intuitive that any changes to the diaphragm, whether at the cellular level or tissue level, that could increase its capacity for fatigue resistance would capable of attenuating this response. There is evidence from literature in rats indicating that tissue and cellular changes to the diaphragm are possible with endurance training. A study by Vrabas et al. (124) found that 10 weeks of treadmill endurance training led to increases in citrate synthase and superoxide dismutase enzyme activities of the rat diaphragm. In addition, decreases in type IIb and increases in type I myosin heavy chains of the rat diaphragm were also found to occur. These changes would be expected to increase the oxidative capacity of the diaphragm and the ability of the diaphragm to prevent the accumulation of the metabolic bi-products associated with the respiratory muscle metaboreflex.

In humans, some studies have found specific inspiratory muscle training (IMT) to be capable of improving the functional characteristics of the respiratory muscles, such as increasing the maximum voluntary ventilation values (107), MIP values (44) and the T_{lim} during resistance breathing protocols (27, 28). The findings from research into possible whole body exercise performance benefits from IMT have been more equivocal [see (Sheel, 2002) for review, (96)]. Evidence to indicate that IMT may be capable of altering the tissue of the diaphragm is available from the muscle biopsies of patients who have died of heart failure. These individuals who suffer from a poor ventricular function exhibit a compensatory hyperventilation in response to their inadequate cardiac output (69). As a result they are exposed to chronic "hyperventilation training". In these individuals, muscle diaphragm biopsies have revealed an increased mitochondrial content and increased aerobic

capacity of the diaphragm (116). Despite the evidence that IMT may elicit physiological changes, there has been little research into whether or not IMT may alter the IMF metaboreflex response.

Training of the inspiratory muscles could increase their capacity for oxidative metabolism and thus reduce the oxygen and blood flow requirements during intense work. As a result, the metaboreflex response to IMF may occur at a higher workload or may occur on a smaller magnitude. Thus, IMT could allow blood flow to remain high in other skeletal muscles, possibly increasing the capacity for whole-body work to be performed.

Evidence in support of the possibility that IMT could attenuate the metaboreflex comes from the literature on patients with COPD and literature on the forearm metaboreflex. As mentioned previously, the evidence indicates that patients with COPD encounter greater blood flow competition by their inspiratory muscles and, as a result, a greater metaboreflex response during exercise that serves to limit their exercise performance (100). Therefore, one would expect that IMT in these patients would result in greater physiological gains than that seen in the healthy population. As expected, a study by Scherer et al. found that IMT in COPD patients lead to significant exercise performance improvements and an average VO_{2peak} increase of 19% (93).

With regards to the handgrip literature, Moustoufi-Moab and co-workers found that after a period of four weeks of forearm training, five days a week, at 30-35% of maximum voluntary contraction, subjects not only exhibited a greater time to fatigue during a fatiguing protocol but they also exhibited a reduction in lactate accumulation and a smaller increase in MAP in response to exercise under a state of impeded forearm blood flow (74). The authors suggested that in response to the training regimen, there was a reduced metaboreceptor activity present during exercise under conditions of mismatched muscle metabolism and

flow. Therefore, it is possible that training of the inspiratory muscles may be capable of eliciting similar adaptations.

Indeed, recent research suggests that IMT may have an effect on the respiratory muscle metaboreflex in healthy humans. McConnell and Lomax (70) have demonstrated that the time to fatigue during plantar flexion exercise to task failure is reduced by prior fatiguing inspiratory work. These researchers studied this effect in subjects before and after a four-week period of IMT and found that the same absolute amount of inspiratory work failed to result in an earlier time to plantar flexor fatigue following IMT. However, in this study hallmarks of the metaboreflex were only measured pre-training and as a result it is unclear if, or how, IMT affected the metaboreflex response to the inspiratory muscle work. As such, it remains unclear in what way the metaboreflex response may be altered following a period of IMT.

APPENDIX B –RAW DATA

Table 8. Individual anthropometric and descriptive characteristics. Definition of abbreviation: BMI = body mass index.

Subject	Group	Age (yrs)	Height (cm)	Mass (kg)	BMI (kg/m^2)
1	EXP	23	170.0	63.4	21.93771626
2	EXP	28	183.9	80.2	23.71433277
3	EXP	24	173.5	68.4	22.72255396
4	EXP	25	199.0	80.2	20.25201384
5	EXP	24	172.0	75.6	25.5543537
6	EXP	21	174.5	65.4	21.47765618
7	EXP	29	175.8	75.2	24.33212838
8	EXP	26	180.0	79.4	24.50617284
9	SHAM	24	190.2	90.2	24.9336301
10	SHAM	27	165.5	66.4	24.24220297
11	SHAM	29	187.0	105.8	30.25536904
12	SHAM	23	178.5	76.6	24.04098894
13	SHAM	27	176.6	78.6	25.20235632
14	SHAM	25	173.2	75.8	25.26814906
15	SHAM	24	178.4	71.2	22.37125219
16	SHAM	34	186.5	77.2	22.19522889

Table 9. Individual pulmonary function data. Definitions of abbreviations: FVC = forced vital capacity; $FEV_{1.0}$ = forced expiratory volume in 1 second.

Subject	Group	FVC (L)	FEV1.0 (L)	FEV1.0/FVC (%)	FEV1.0/FVC (% Predicted)
1	EXP	4.05	3.31	81.7	94
2	EXP	5.39	4.42	82.0	100
3	EXP	4.30	3.74	87.0	101
4	EXP	7.68	6.34	82.6	102
5	EXP	6.04	4.60	76.2	88
6	EXP	4.13	3.65	88.4	103
7	EXP	4.97	3.94	79.3	95
8	EXP	7.19	5.59	77.7	94
9	SHAM	5.41	4.45	82.3	97
10	SHAM	4.27	3.75	87.8	104
11	SHAM	5.29	4.69	88.7	108
12	SHAM	5.50	4.71	85.6	100
13	SHAM	5.31	4.85	91.3	109
14	SHAM	5.42	4.27	78.8	94
15	SHAM	5.65	4.80	85.0	99
16	SHAM	5.76	4.68	81.3	100

Table 10. Individual weekly average maximal inspiratory pressure values. Values are presented in units of cm of H_2O. Definitions of abbreviations: Fam = familiarization; Wk = weeks of training.

Subject	Group	Fam	Baseline	1 Wk	2 Wk	3 Wk	4 Wk	5 Wk
1	EXP	-114.9	-145.8	-144.2	-148.4	-156.2	-153.7	-147.4
2	EXP	-176.5	-183.1	-207.8	-201.0	-218.9	-215.6	-214.5
3	EXP	-109.9	-114.1	N/A	-118.5	-130.0	-131.0	-141.5
4	EXP	-106.3	-112.5	-106.7	-111.0	-109.0	-132.6	-149.1
5	EXP	-106.1	-120.9	-120.5	-117.6	-112.4	-129.5	-147.1
6	EXP	-101.2	-109.3	-108.6	-109.9	-111.3	-111.0	-115.3
7	EXP	-98.9	-88.8	-103.1	-95.2	-85.1	-102.6	-100.2
8	EXP	-111.5	-125.7	-136.2	-143.6	-144.5	-152.7	-149.1
9	SHAM	-149.4	-182.6	-178.2	-180.1	-185.5	-184.2	-156.4
10	SHAM	-83.6	-92.8	-83.0	-81.4	-88.8	-89.0	-92.4
11	SHAM	-166.1	-178.5	-191.5	-185.5	-181.5	-183.4	-190.8
12	SHAM	-136.2	-128.7	-152.1	-136.9	-134.0	-135.5	-142.8
13	SHAM	-160.4	-141.0	-137.3	-134.6	-142.9	-140.3	-140.5
14	SHAM	-132.5	-132.9	-122.8	-118.0	-133.1	-109.1	-116.5
15	SHAM	-105.4	-110.5	-133.5	-121.3	-138.6	-162.6	-163.0
16	SHAM	-178.7	-162.5	-181.3	-169.2	-165.9	-183.0	-177.7

Table 11. Individual heart rate values from the EXP group pre and post training. Values are in beats/min. Definitions of abbreviations: Min = minutes of RBT; Rec1 = first minute of recovery.

	Subject 1		Subject 2		Subject 3		Subject 4		Subject 5		Subject 6		Subject 7		Subject 8	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Eupnea	63.5	57.8	45.6	49.1	65.1	57.8	53.0	44.7	72.9	68.8	60.7	61.1	59.9	59.7	72.7	70.2
Min 1	86.8	84.6	71.0	70.8	81.7	73.1	71.3	62.9	91.4	86.2	79.2	73.6	76.6	75.5	92.5	85.1
Min 2	78.9	75.6	68.6	70.7	78.0	75.3	68.1	63.2	90.0	82.2	76.4	73.9	77.1	75.3	87.9	84.5
Min 3	78.4	72.9	66.7	68.9	79.5	76.6	71.1	62.3	92.0	79.1	72.1	72.1	80.3	73.6	93.7	82.9
Min 4	74.4	75.5	64.8	69.7	82.6	70.7	68.8	60.8	91.6	77.6	72.0	73.6	78.3	74.8	93.5	83.7
Min 5	76.9	74.4	63.3	68.9	81.7	74.6	69.0	60.9	90.6	79.9	73.7	73.7	78.1	74.3	93.8	82.6
Min 6	82.7	76.0	64.9	70.7	81.6	74.7	68.0	62.9	91.6	84.0	72.2	75.8	78.2	74.6	94.7	82.8
Min 7	81.6	73.1	64.2	73.1	80.0	74.6	71.8	62.8	93.1	80.9	72.4	79.6	78.4	74.4	99.1	85.0
Min 8	82.0	71.9	63.9	69.2	83.7	77.2	68.8	61.7	95.3	85.1	73.6	69.1	75.4	73.5	100.6	84.2
Min 9	84.5	72.8	61.0	68.2	86.6	75.1	69.9	61.5	97.1	85.0	73.3	70.2				
Min 10	84.1	74.9	68.4	69.3			70.1	63.2	97.8	81.0	73.0	70.5				
Min 11	83.0	74.1	65.9	71.9			69.5	64.0	93.9	81.5	72.7	71.7				
Min 12	84.2	71.7	69.3	65.2					92.8	83.9	73.3	70.6				
Min 13	84.8	68.8	72.6	72.3					97.3	83.0						
Min 14	82.3	69.2	72.5	72.9					99.9	82.4						
Min 15	81.0	71.6							98.9	83.2						
Min 16	84.1	67.0														
Min 17	85.1	67.1														
Rec 1	67.9	58.1	46.9	53.2	68.0	63.6	58.5	46.0	76.8	67.3	64.0	62.7	66.0	61.7	78.3	75.9

Table 12. Individual heart rate values from the SHAM group pre and post training. Values are in beats/min. Definitions of abbreviations: Min = minutes of RBT; Rec1 = first minute of recovery.

	Subject 9		Subject 10		Subject 11		Subject 12		Subject 13		Subject 14		Subject 15		Subject 16	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Eupnea	52.2	61.2	57.1	57.7	57.9	55.5	58.5	61.3	53.0	46.4	68.6	65.4	60.7	58.1	61.4	50.0
Min 1	71.4	74.7	73.9	74.1	82.2	79.1	79.5	79.1	73.5	72.1	78.0	78.0	100.2	89.0	87.6	86.7
Min 2	68.0	72.9	71.5	72.6	83.4	75.8	77.4	80.9	67.6	65.9	76.4	73.3	93.3	89.1	88.5	78.6
Min 3	68.1	74.3	72.1	71.3	81.2	75.8	83.9	83.1	66.6	59.4	74.1	77.6	96.2	88.4	88.9	80.1
Min 4	69.0	73.8	74.2	66.8	83.0	75.4	90.4	81.6	64.1	60.5	77.0	76.4	94.3	88.6	92.7	79.3
Min 5	68.8	74.2	74.7	65.7	84.2	77.9	89.2	80.9	66.1	67.2	74.7	76.1	95.3	86.4	93.1	78.8
Min 6	68.0	74.4	74.5	68.9	85.0	81.2			67.1	61.1	77.4	75.2			97.0	85.0
Min 7	71.8	75.7	74.6	68.8	84.9	77.7			67.2	60.7	81.2	78.3			98.0	82.9
Min 8	71.7	75.5									77.5	78.3			100.1	84.6
Min 9											80.5	80.3				
Min 10											80.3	79.4				
Rec 1	56.8	61.8	66.2	68.5	61.6	61.6	55.8	65.3	56.4	54.2	65.8	64.1	69.6	64.2	88.2	69.0

Table 13. Individual MAP values from the EXP group pre and post training. Values are in mmHg. Definitions of abbreviations: Min = minutes of RBT; Rec1 = first minute of recovery.

	Subject 1		Subject 2		Subject 3		Subject 4		Subject 5		Subject 6		Subject 7		Subject 8	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Eupnea	87.4	86.4	81.2	83.9	88.0	83.2	81.1	80.2	84.0	86.7	80.5	80.2	91.6	89.9	82.1	85.0
Min 1	78.3	78.8	77.5	77.1	85.3	84.4	88.2	81.6	81.8	80.6	67.5	59.3	96.3	79.0	75.3	89.2
Min 2	88.9	90.7	84.8	86.7	94.1	82.8	99.0	86.2	85.7	88.0	80.7	74.8	98.9	87.0	89.0	93.7
Min 3	89.7	94.9	82.1	88.8	96.1	87.7	100.3	89.9	84.0	89.0	86.0	76.4	121.0	95.5	90.0	94.1
Min 4	92.9	94.2	85.9	85.2	98.5	85.9	99.0	86.0	83.5	85.7	90.1	76.8	124.7	95.5	92.2	94.9
Min 5	97.4	96.6	85.8	88.2	95.6	88.0	97.8	85.4	85.7	86.5	87.4	74.8	119.5	94.1	95.8	94.3
Min 6	93.2	94.6	85.0	84.6	96.1	83.5	98.4	87.6	86.7	89.4	91.7	76.5	123.7	93.4	95.2	94.6
Min 7	94.4	93.5	89.9	90.8	97.8	85.8	100.4	89.5	87.2	87.3	88.2	76.8	120.6	92.0	97.5	94.0
Min 8	96.5	90.8	90.7	90.1	100.6	87.0	96.6	86.9	87.8	89.5	88.3	72.3	112.4	89.1	100.4	93.7
Min 9	104.3	92.4	85.5	86.0	104.9	91.1	101.1	93.6	90.5	88.0	85.8	73.6				
Min 10	107.0	94.2	90.0	85.2			100.8	92.2	90.1	84.1	87.0	74.5				
Min 11	109.0	93.5	92.5	85.3			98.6	92.6	90.9	85.6	89.5	73.8				
Min 12	109.5	92.3	93.5	84.8					89.1	86.3	87.3	74.8				
Min 13	107.9	92.5	95.8	86.7					90.2	85.6						
Min 14	105.3	92.3	96.1	87.5					88.7	86.7						
Min 15	110.3	95.1							90.5	87.4						
Min 16	105.5	89.7														
Min 17	103.4	93.3														
Rec 1	93.5	90.1	91.7	85.5	97.9	87.9	88.6	88.5	86.8	88.4	91.4	80.6	100.4	90.8	86.9	90.1

Table 14. Individual MAP values from the SHAM group pre and post training. Values are in mmHg. Definitions of abbreviations: Min = minutes of RBT; Rec1 = first minute of recovery.

	Subject 9		Subject 10		Subject 11		Subject 12		Subject 13		Subject 14		Subject 15		Subject 16	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Eupnea	96.0	104.1	93.3	80.6	85.9	90.5	89.4	92.9	83.5	85.2	86.4	77.8	83.0	84.4	87.1	98.5
Min 1	92.8	96.0	93.4	80.0	89.2	92.3	106.9	104.6	74.0	79.9	84.3	81.4	92.7	88.4	91.2	95.8
Min 2	105.8	100.2	102.3	90.0	111.6	105.7	111.8	114.6	94.4	97.5	88.9	86.8	103.6	103.0	99.8	105.7
Min 3	104.4	101.9	102.2	93.5	101.4	109.7	120.8	116.7	93.8	97.3	89.0	86.7	100.8	101.0	97.8	103.2
Min 4	103.5	102.6	107.2	91.0	105.8	113.3	121.1	114.1	95.3	98.6	91.4	92.0	105.0	103.3	102.0	98.7
Min 5	105.3	99.5	108.6	89.7	109.1	112.2	121.3	112.2	95.5	96.9	94.3	93.6	105.4	102.3	101.4	100.9
Min 6	102.0	102.7	109.1	89.1	109.6	115.2			95.7	102.0	94.3	92.1			104.1	98.5
Min 7	103.1	104.7	109.1	89.1	108.2	113.0			96.0	102.1	96.9	98.1			104.6	104.0
Min 8	103.3	105.1									99.4	98.8			101.9	101.1
Min 9											104.3	96.1				
Min 10											104.3	95.2				
Rec 1	100.5	110.0	101.3	90.1	88.0	99.8	100.2	97.9	92.8	90.3	106.7	87.7	87.7	88.2	98.2	100.8

APPENDIX C –

UBC RESEARCH ETHICS BOARD'S CERTIFICATE OF APPROVAL