The Comparison of Muscle Function Loss of the Respiratory Muscles and Tibialis Anterior

by

Jason S. Chan

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Author’s Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public

Abstract

The respiratory muscles (RM) sustain life by maintaining ventilation but may atrophy faster than other skeletal muscle. Respiratory muscle training (RMT) improves RM strength and attenuates the RM metaboreflex. However, the time course of muscle function loss after detraining is less known. We sought to determine the time course of change in RM strength compared to another skeletal muscle and the respiratory muscle metaboreflex in response to 5 weeks of RMT and 5 weeks of detraining. An experimental group (2F, 6M; 26±4yrs) completed 5 weeks of RMT and tibialis anterior (TA) training (each 5 days/week at 50% of maximal inspiratory pressure (MIP) and 50% maximal isometric force, respectively) followed by 5 weeks of no training (detraining) while a control group (1F, 5M; 24±1yrs) underwent no intervention. Prior to training, post-training, and post-detraining, all participants underwent a resistive breathing task (RBT) to failure (breathing frequency:15 bpm, at 60% MIP, duty cycle 50:50) while heart rate (HR) and mean arterial blood pressure (MAP) were measured. The control group had no change in MIP from pre-training to post training ($p=0.191$) but a small increase from pre-training to post detraining (+11±8%, $p<0.05$) and no changes in TA across pre-training, post training and post detraining. The control group had no changes in HR ($p=0.156$) and MAP ($p=0.758$) response during the RBT on post training and post detraining compared to pre-training. Five weeks of training increased RM (18±8%, $p<0.05$) and TA (+34±15%, $p<0.05$) strength and both remained elevated after 5 weeks of detraining (MIP_{post} vs $MIP_{post\text{-}detraining}$: 154±31vs 153±28 cmH_2O, respectively, $p=0.853$; TA_{post} vs $TA_{post\text{-}detraining}$:86±19 vs 85±16N, respectively, $p=0.982$). The rise in HR was trending but not significantly attenuated after training and detraining (-11±14% and 4±17%, respectively, $p=0.059$). However, the rise in MAP during RBT was attenuated post training (-11±17%, $p<0.01$) and persisted after detraining, during the time matched, final minute of RBT. In conclusion, RM

and TA have similar temporal strength gains and the attenuation of the respiratory muscle metaboreflex remains after 5 weeks of detraining.

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ANOVA	Analysis of variance
F_b	Breathing frequency
COPD	Chronic obstructive pulmonary disease
DBP	Diastolic blood pressure
ECG	Electrocardiogram
HR	Heart rate
IMT	Inspiratory Muscle Training
MAP	Mean arterial blood pressure
MEP	Maximal expiratory pressure
MIP	Maximal inspiratory pressure
P_{mouth}	Mouth pressure
1RM	One repetition maximum
RMT	Respiratory muscle training
SBP	Systolic blood pressure
TA	Tibialis anterior
$T_{exhaustion}$	Time to exhaustion
V_E	Expired ventilation
$\dot{V}O_{2max}$	Maximal oxygen uptake
V_T	Tidal Volume
W_{tot}	Total work

1.0 LITERATURE REVIEW

1.1 Background

Similar to other skeletal muscles, respiratory muscles will hypertrophy and increase in strength in responses to training (7, 34). Respiratory muscle training (RMT) has been adopted by the athletic population with the hope of increasing their athletic abilities (26). For example, 4 weeks of RMT increased cycle endurance time at a moderate intensity by 38% in a trained population (8). This finding is consistent with others who found 6 weeks of RMT resulted in a faster time in a simulated time-trial performance in 16 young healthy males due to the attenuation of the perception of effort during exercise (54). However, the hypothesis that RMT improves athletic performance is equivocal. For example, others demonstrated that 4 weeks of RMT in endurance runners resulted in a 31% increase in maximal inspiratory pressure (MIP), but had no effect on blood lactate or maximal oxygen uptake ($\dot{V}O_{2max}$) during a $\dot{V}O_{2max}$ test (75). Similarly, RMT over 27.5 days in healthy adults increased MIP by 38% but not $\dot{V}O_{2max}$ (22). Likewise, the effects of 4 weeks of RMT on exercise responses in normoxia and hypoxia resulted in a 25% increase in MIP but no change in time to exhaustion (16). In one of the very few placebo controlled studies, 5 weeks of RMT increased MIP (+8%) in the experimental group, with no changes in the control group, yet peak work rate did not differ in either of the two time trial tests between the RMT and control groups (64). As such, RMT consistently results in an increase in respiratory muscle strength (i.e., MIP) but the increase in strength may not necessarily transfer over to overall improved athletic performance.

In addition to athletic populations, RMT is also commonly prescribed to clinical populations such as patients with chronic obstructive pulmonary disease (COPD) (48). Patients with COPD are more inactive compared to a healthy population due to the symptoms of COPD

such as narrowed airways due to inflammation and mucus build up, impaired gas exchange, dyspnea, and peripheral muscle dysfunction (43). When comparing elderly COPD patients to a healthy elderly population there is an increase in time spent in the sitting/lying position (64±15 %/day vs 46±16 %/day, respectively) and a decrease in time spent walking (11±4 %/day vs 6±4 %/day, respectively) (46). Also, as the severity of COPD increased, there was a decrease in both steps taken in a day and physical activity overall during a 960 day period (69% and 61%, respectively) (72). During physical activity, there is an increase in metabolic demand which requires an increased ventilation. Since ventilation is performed by the respiratory muscles, there is an increase in workload for the respiratory muscles during physical activity. The lack of physical activity will reduce the workload on the respiratory muscles, and over a sustained period of time, result in atrophy or disuse. Therefore, commonly associated with COPD patients is a reduction of exercise capacity also due to inspiratory muscle impairment. To counteract the atrophy of the respiratory muscles, RMT is used to target and strengthen these inspiratory muscles to compensate the reduction in exercise training and physical activity (51). RMT has been helpful to COPD patients as it increased their ability to exercise, which is seen as a significant improvement in the distance walked during a 6 minute walk test by +43 m (6). Overall, the benefits of RMT towards the athletic population remains uncertain however RMT is favourable for clinical populations (i.e., COPD patients).

When a training stimulus (i.e., RMT) is removed, the diaphragm like other muscles will undergo atrophy and muscle function loss over time. However, the diaphragm is unique compared to other muscles with its role in ensuring adequate ventilation to sustain life and results in near continuous daily use. Therefore, the diaphragm's responses to training and detraining may potentially be different than other skeletal muscles. Hence, the focus of this thesis will be the

strength response of the respiratory muscles and tibialis anterior, and the response of the respiratory muscle metaboreflex to training and detraining. As such this literature review will focus on the following: 1) characteristics of the diaphragm, 2) characteristics of the tibialis anterior, 3) the respiratory metaboreflex, 4) the effects of respiratory muscle training and 5) the effects of respiratory muscle detraining.

1.2 Characteristics of the Diaphragm

The diaphragm is a skeletal muscle composed of approximately 50% slow twitch (type I) and 50% fast twitch (type II) muscle fibers (37). As the primary muscle for respiration, the combination of slow twitch and fast twitch allows for the diaphragm to manage a wide range of ventilatory demands. The type I and IIa muscle fibers are resistant to fatigue and generate low force, these muscle fibers are beneficial to maintain normal ventilation which requires the diaphragm to be active continuously to maintain appropriate alveolar ventilation (18). The diaphragm also contains type IIx fibers, which are more fatigable but can generate higher forces. The high force generating muscle fibers are mainly used for stabilization during maneuvers generating positive abdomen pressure such as, coughing and sneezing which are important to help clear airways (18).

The diaphragm acts as a primary respiratory muscle pump by generating negative pressure in the thoracic cavity necessary for inspiration (18). During the inspiration portion of the breathing cycle, the diaphragm contracts and moves caudally, which increases the thoracic cavity and generates the negative pressure, resulting in inspiratory airflow. During the expiratory portion of the breathing cycle, the diaphragm relaxes, which decreases the thoracic cavity and generates more positive pressure, resulting in expiratory airflow. During normal resting ventilation, less than 20%

of maximal pressure (19) or force is required, and this relatively low force generation prevents the respiratory muscles from fatiguing.

Due to its continuously active nature, the diaphragm was previously thought to be able to sustain a workload indefinitely and did not develop fatigue. However, it was demonstrated in 3 males that the diaphragm can fatigue when the demand exceeds the supply of energy available during a high workload (>40% max pressure) and prolonged duty cycle (50:50, equal time inspiring and expiring) breathing at rest (55). More importantly, the use of a tension time index to determine if diaphragm fatigue can be elicited at a certain pressure over a certain prolonged duty cycle was introduced. Based on their critical pressure of 40% MIP and a duty cycle of equal inspiring and expiring (i.e., 50:50), they provide a "threshold" tension time index of 0.2. Any tension time index value greater than 0.2 will cause the diaphragm to fatigue and this could be achieved through either increasing the resistive load (%MIP) or the changing the duty cycle (i.e., 75:25, inspiring: expiring vs 50:50). Once the index is greater than 0.2, the high force generated is causing the feed arteries in the diaphragm to compress over a sufficient period of time, resulting in metabolic demand to no longer be met and ultimately, diaphragm fatigue (55). Subsequent to the demonstration that the diaphragm is able to fatigue at rest, it was shown that diaphragm fatigue can develop during strenuous exercise. For example, after the completion of high intensity (85-95% $\dot{V}O_{2max}$) exercise to exhaustion, 12 heathy men at various fitness levels, demonstrated a reduction in transdiaphragmatic pressure (range: -8±3 to -32±5%) via phrenic nerve stimulation (27). Furthermore, the duration of exercise plays a role in the resulting level of diaphragm fatigue. When 8 males performed various constant load time to exhaustion trials at different durations, the result was greater diaphragm fatigue during greater duration of exercise (15.5±5.7%, 23.6±6.4%, and 35.0±12.1% at 50, 75, and 100% of maximal time to exhaustion during the constant load trial

respectively), demonstrating a correlation between the amount of work the diaphragm has done during high intensity exercise and the amount of fatigue the diaphragm experiences (4). Interestingly, diaphragm fatigue during exercise occurs only under specific conditions. Specifically, the diaphragm does not fatigue during a typical $\dot{V}O_{2max}$ test or while mimicking the respiratory work during high intensity exercise in isolation without actually exercising (5). However, diaphragm fatigue can occur in isolation when respiratory work is at levels that well exceed those during exercise (55). Diaphragm fatigue typically only occurs during prolonged high intensity exercises, indicating a need of a high force generated over a longer period of time and likely changes in blood chemistry (i.e. pH, lactic acid, etc.) (14). In 9 males and females comparing diaphragm fatigue at rest with matched frequency and duration of voluntary increases of transdiaphragmatic pressure and during whole body exercise to exhaustion on separate days, there was only significant diaphragm fatigue in the whole-body exercise condition and not in the rest condition (5). The absence of diaphragm fatigue in rest but not during whole body exercise demonstrates that the effects from prolonged high intensity exercise (i.e. metabolites) also plays a role in diaphragmatic fatigue (5). In general, the diaphragm is resistant to fatigue at lower workloads. However, when faced with situations that involve higher workloads, prolonged duty cycles and metabolic consequences (i.e., metabolites, pH changes, etc.), such as during strenuous exercise, diaphragm fatigue can be elicited.

1.3 Characteristics of the Tibialis Anterior

To make appropriate comparisons between the diaphragm and another muscle, the two muscles should have similar fiber types and usages. The relative fiber type composition of the diaphragm is ~50% type 1 and ~50% type II (37). A commonly studied muscle group that is also

a skeletal muscle is the vastus lateralis with a fiber type distribution ~32% type I and ~68% type II (17, 66). However, the quadriceps are muscles commonly used in training programs, which would lead to additional training and/or result in a larger removal of the stimulus during the intervention. In addition, during day-to-day activity, it is unlikely a high fraction of maximal quadriceps strength will be used over long periods of time in individuals with more sedentary lifestyles. In terms of usage, the heart is most comparable to the diaphragm, as they are both used nearly continuously to sustain life. However, the myocardium is composed of cardiac muscle, which is composed of mononucleated branched cells, and contracts involuntarily. Alternatively skeletal muscle is composed of multinucleated non-branched cells, allowing for voluntary contractions. In addition, there are difficulties in objectively assessing the function of the heart due to the involuntary contraction and technical difficulties isolating the force output.

Conversely, the tibialis anterior (TA) is reasonably similar to the diaphragm and can be used for appropriate comparisons. First, the TA is also a skeletal muscle composed of ~40% type I and 60% type II muscle fibers (77). which is comparable to the diaphragm (37). As such, the TA and the diaphragm should have a similar fatigue resistance and energetics. In addition, similar to the diaphragm, the TA is used daily in most healthy individuals via lifting the foot during locomotion. Specifically, the tibialis anterior is the strongest dorsiflexor in the leg and plays an important role in an individual's gait as it clears the foot from the ground during each step. Thus, in younger healthy individuals who typically walk daily, their TA will be stimulated with a high daily load. Furthermore, during walking a relatively high percent of total TA strength is used. Therefore, even individuals with more sedentary lifestyles are still engaging their TA to a larger degree. The TA is not likely a muscle to be receiving additional training via specific training programs, unlike other skeletal muscles, such as the quadriceps or biceps. The lack of specific

training will minimize the effects of muscle memory, as muscle memory allows for faster gain in muscle mass to a muscle that has been previously trained (20). Finally, the TA is a skeletal muscle and can be voluntarily contracted. Since the TA can voluntarily contract, unlike the heart, force measurements can be objectively measured. Overall, the TA is one of the skeletal muscles used on a daily basis without specific conscious training and similar in terms of composition of muscle fiber types, resulting in a good comparison to the diaphragm.

1.4 Respiratory metaboreflex

During exercise, there is a substantial increase in the metabolic demand of the working muscles. The increased metabolic demands results in greater blood flow to the working muscle (3). This exercise hyperemia is achieved through two mechanisms. The first being increases in cardiac output via greater stroke volume and heart rate. The second mechanism is the increase in sympathetic activity to redirect blood flow to the working muscles. The increase in exercise intensity results in a linear increase of sympathetic outflow (41). As the sympathetic outflow increases, it causes arterioles in inactive tissue beds to vasoconstrict, which reduces blood flow to those inactive tissues. At the same time, in the active musculature local metabolites (i.e. ATP, lactic acid, hydrogen ions, potassium, glucose, prostaglandins, adenosine, etc.) will result in vasodilation in the arterioles resulting in increased blood flow to the active tissue (28, 39). This sympathetically mediated redistribution of blood flow is termed a metaboreflex and is also known to occur with inputs from the respiratory muscles (58). Uniquely though, there are some findings that suggest that the respiratory muscle metaboreflex may be prioritized. The increase of work of breathing has shown to reduce blood flow in other working (locomotor) muscles (15, 23, 58). This competition of blood flow occurs from an increase in sympathetic outflow that will aid in the

redistribution of blood flow back to the respiratory muscles as they begin to fatigue during strenuous exercise (15, 23, 58). There is also evidence that RMT reduced fatigue in other exercising locomotor muscles via improving vascular conductance and blood flow in these muscles (9). Another finding that may suggest that blood flow to the respiratory muscle is prioritized is the potential the difference in sensitivity to vasoconstriction signals in the different vasculature. Specifically, the arterioles in the diaphragm are less sensitive to alpha adrenergic constriction compared to gastrocnemius arterioles, at least in rats (1). The difference in sensitivity results in the arterioles in the diaphragm remaining relatively vasodilated, while the arterioles in the peripheral muscles vasoconstrict. As a result of this difference in sensitivity, blood flow can be preferentially redistributed towards the diaphragm.

The respiratory metaboreflex is dependent on the fatigue from high respiratory muscle work such that occurs during (near) maximal exercise. In 7 male cyclists, an increase in work of breathing (+50%) resulted in an increase in leg vascular resistance (+13%) and a decrease in leg blood flow (-11%) compared to the control during maximal exercise (23). Similarly, in 8 healthy subjects during heavy exercise (90% of maximal work), there is a direct relationship between work of breathing and respiratory muscle blood flow ($r=0.73$), whereas there is an inverse relationship between work of breathing and locomotor muscle blood flow ($r=-0.57$) (15). For example, an increase in work of breathing resulted in an increase in respiratory muscle blood flow and a decrease in locomotor muscle blood flow (15). Together these two studies show vasoconstriction in working limb muscles, resulting in a reduction in blood flow in locomotor muscles due to increasing work of breathing that occurred at (near) maximal exercise. However, during submaximal exercise there are no changes in leg blood flow and leg vascular resistance when the work of breathing was manipulated (74). The absence of changes in blood flow at submaximal

intensities suggests that a higher load is needed to a degree where the increase in cardiac output is insufficient in meeting metabolic needs for the respiratory metaboreflex to occur. Ultimately, this occurrence during strenuous exercise will reduce the working locomotor muscle's ability to meet metabolic demands, which increases locomotor fatigue and decreases performance (58). Furthermore, this finding is supported by a study, where they investigated the effects of inspiratory muscle fatigue on resting limb vasculature in 6 healthy humans at rest. A tension to time index of 0.24 and 0.42 (both at workload of 60% MIP, duty cycle of 0.4 and 0.7, respectively) were used during the trial of respiratory muscle loading fatigue compared to their control trials with a tension to time index of 0.008 and 0.014 respectively (both at workload of 2% MIP, duty cycle of 0.4 and 0.7, respectively). The tension time index for the respiratory muscle loading trials were above the 0.2 threshold while the control trails were not, which resulted in only the respiratory muscle loading trials to develop diaphragm fatigue. There was an increase in sympathetic outflow, seen in the increase in mean arterial blood pressure (MAP) (+4-13 mmHg) and heartrate (+16-20 beats/mins) in the diaphragm fatigue trials and no change in the control trials (59). Once the diaphragm began to fatigue, there was a reduction in resting limb blood flow (-30%) and an increase in limb vascular resistance (+50-60%) compared to the control and this occurred after 2 minutes of respiratory loading. Since the changes only occurred after 2 minutes of respiratory loading, it indicates that there is a time dependency on the increase in sympathetic outflow in the working limb muscle while performing high resistance prolonged duty cycle breathing, which results in diaphragm fatigue(59). This time dependency with the changes in limb vascular resistance, suggest it was not likely due to the effect of central command. Additionally, with the absence of inspiratory muscle fatigue, there was no change in resting limb blood flow and limb vascular resistance, seen in control trials (59). Similarly, it was found in 4 women and 3 men during

respiratory loading and measuring limb muscle nerve sympathetic activity at rest, that the increase in sympathetic outflow in working limb muscle did not occur until the diaphragm began to fatigue (65). This redistribution of blood flow results in a competition of metabolic needs, which can exacerbate the fatigue in the working limb muscles (52, 59). It was found in 8 male cyclists that a higher force output of inspiratory muscles (inspiratory loading), exacerbated muscle fatigue in the working limbs during exercise (52). Whereas, a lower force output (inspiratory unloading), attenuated muscle fatigue in the working limbs during exercise (52).

1.5 Respiratory muscle training

Any kind of training should follow the key training principles of: overload, reversibility, progression, individualization, periodization, and specificity, including the respiratory muscles (30). Overload involves applying a workload (i.e., pace, resistance, repetition) higher than baseline to stimulate adaptations. Reversibility is when the stimulus (i.e., workload) is lowered or removed, it will result in the loss of the adaptations. Progression involves the increase of stimulus to continuously stress the muscle to provoke further adaptation. Individualization is the concept that training should be changed based on the individual. Periodization is having a planned training program to provide structure (i.e., when to train, for how long, when to rest, etc.) to the training being done. Lastly, specificity is the concept that training should be done for a specific task/goal to allow for the most direct transfer of the adaptations (i.e., movement patterns, skills) to the actual task. The same principles apply to the respiratory muscles.

With RMT, the respiratory muscle will hypertrophy and become stronger. The increase in strength allows the generation of a greater negative pressure during inspiration but is dependent on intensity and frequency of the training. For example, after 5 weeks of RMT training at 50%

MIP and 60 repetitions a day, 25 young healthy males demonstrated an ~16% increase in MIP (Table 1) (50). A longer training period appears to result in larger increases in MIP, where 9 weeks of RMT also at 50% MIP resulted in an increase of 64 ± 3% in 6 healthy individuals (Table 1) (53). Additionally, at similar duration and load, 5 weeks of RMT at 50% MIP but only ~40 repetitions a day in 9 cyclists resulted only a 8% increase in MIP (Table 1) (64). RMT has also been shown to have an effect on the cardiovascular response during rest and exercise. In 25 healthy young males and females 6 weeks of inspiratory muscle training (IMT) increased MIP by 34% and reduced systolic (SBP) and diastolic blood pressure (DBP) by 4.3 and 3.9 mmHg, (SBP_{pre}: 112.5±10.4 mmHg vs SBP_{post}:108.3±12.9 mmHg; DBP_{pre}:67.7±8.5 vs DBP_{post}:63.8±9.8 mmHg) respectively at rest (13).

Five weeks of RMT attenuates the respiratory metaboreflex during a fatiguing protocol via resistive respiratory work in 16 humans. The training group had an increase of 17% in MIP (pre: −125 ± 10 vs post: −146 ± 12 cmH_2O) and an attenuation of the respiratory metaboreflex (HR_{Pre}: 62±3 to 83±4beats/min vs HR_{Post}: 59±3 to 74±2 beats/min; MAP_{pre}: 84±1 to 99±3mmHg vs MAP_{post}: 84±1 to 89±2 mmHg) (76). This attenuation of the respiratory metaboreflex, is thought to aid in exercise performance as it will reduce the competition for blood flow between working locomotor muscles and respiratory muscles. However as detailed above an increase in strength (MIP) may not lead to an increase in exercise performance. For example, 5 weeks of RMT at 50% MIP resulted in the training group and the control group both improving in terms of $\dot{V}O_{2max}$ (26% and 16%, respectively) and time trial over time, but the changes were not different between groups. Therefore, they concluded no significant difference between the training group and the control group in terms of performance during exercise after the 5 weeks of RMT. Additionally, there was

no significant difference over the 5 weeks based on HR, ventilation, blood lactate, and workrate during the incremental test (64).

Overall, RMT has been shown to be effective in increasing strength and reducing the magnitude of the respiratory metaboreflex over 4-9 weeks of training in various studies and these benefits could attenuate the competition of blood flow between respiratory muscle and other working limb muscles during strenuous exercise (76). However, the effect of RMT on exercise performance is still controversial. One concern seen in various studies mentioned above is the adaptations resulting from RMT has a large variation between individuals. At similar time frame of training (5 weeks), there is a large range in MIP adaptations. The average increase of MIP between these studies can range from 8% to 59% (13, 50, 53, 64, 76).

Table 1. Summary of various RMT studies.

Author	Sample Size	Intensity	Frequency	Duration	Outcome
Witt et al. 2007	16 (8 control, 8 exp) Healthy, young	Control = 10% MIP, EXP = 50% MIP	3 sets of 75 reps, 6 days/week	5 weeks	**MIP:** +17% -Increase in HR and MAP were attenuated **HR:** Pre IMT = +33% Post IMT= +25% **MAP:** Pre IMT = +17% Post IMT = +6%
Ramsook et al 2017	25 (12 exp, 13 control) Healthy, young males	EXP = 50% MIP	2 sets of 30 a day (one in the morning and one in evening), 5 times/week	5 weeks	- **MIP** = +16% - **IMT** had no effect on ventilatory responses or neuromechanical coupling of the respiratory system during incremental cycle exercise.
Gee et al. 2019	6 athletes (5M, 1F) with cervical spinal cord injury	30 breaths (<6/10 on Modified Borg Dyspnea Scale)	2 sets of 30 repetition, 5 days/week	6 weeks	**-MIP =** +39% **-MEP =** +24%

Mills et al 2014	34 older adults 65-75 yrs old	50% MIP	2 sets of 30 reps	8 weeks	-**MIP** = +34%
DeLucia et al 2018	Healthy 12 IMT (6M, 6F), 13 control	75% MIP	5 sets of 60 breaths/day, 5 days/week,	6 weeks	-**Post IMT**: Decrease in resting SBP and DBP -**MIP** = +34%
Sonetti et al. 2001	9 male cyclists 8 controls	~50% until failure, ~40 reps or 3-5 mins	5 days a week,	5 weeks	- **MIP** = +8% - No difference in control group (placebo) - Endurance test time = +26% - peak workrate (until $\dot{V}O_{2max}$) = +9%
Ferreira et al. 2011	13 hypertensive patients	30% MIP	(15-20 breaths/min)30 mins, 7 days/week	8 weeks	-**MIP =** +47% -**SBP Pre:**133.2±9.9 **Post:** 125.2±13.0 mmHg -**DBP Pre:**80.7±12.3 **Post:** 75.2±1.0 mmHg -**Daytime SBP Pre:** 136.8±12.2 **Post:** 127.6±14.2 mm Hg, -**Daytime DBP Pre:** 83.3±13.1 **Post:** 77.2±12.2 mmHg
Bailey et al. 2010	16 recreational active individuals (age 22+/-4)	EXP – 50% MIP, Control – 15% MIP	EXP – 2 sets of 30 breaths, 7 days/week Control – 1 set of 60 breaths	4 weeks	-**MIP** = +17%
Edward et al. 2013	36 healthy male (age 24 ± 4)	EXP – 55%MIP CON – 10%	1 set of 30 reps, 7 days/week	4 weeks	- Increase in time to volitional exhaustion (lower RPE) -**MIP** = +15% -no change in $\dot{V}O_{2max}$/spirometry
Romer et al. 2003	24 healthy individuals (13M, 11F)	A – 10 sets, 3 rep B – 2 sets 30 reps, no resistance C – 50%, 30 reps	2 sets a day, 6 days/week MR – 2 days/week	9 weeks training, 9 weeks of detraining	**-MIP:** groups A, B, C by 48 ± 3%, 25 ± 3%, 64 ± 3%, respectively. - **Detraining =** -7% MIP from peak levels

Abbreviations: MIP, maximal inspiratory pressure; HR, heart rate; MAP, mean arterial blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; MEP, maximal expiratory pressure; IMT, inspiratory muscle training.

1.6 Respiratory muscle detraining

Similar to any response to training, once the stimulus (i.e., training) is removed, the adaptations will dissipate over time (40). This is termed as detraining. During detraining the previously trained muscle will undergo atrophy and muscle function loss. Although the impact of detraining on other skeletal muscles is relatively well known, there are few studies specific to the respiratory muscles due to the invasiveness of the methods used to reduce the workload for respiratory muscles. Specifically, to completely eliminate diaphragmatic work would require the use of mechanical ventilators. Whereas the methods for other skeletal muscle such as a biceps are non-invasive (i.e., putting a cast on a limb). Currently, only one study has assessed the effect of detraining on respiratory muscle function and found that 9 weeks of detraining (post 9 weeks of training), decreased MIP by 7% from peak levels however, there were no further significant decreases over another 9 weeks of detraining (18 weeks total) (53). In addition, a reduction of the training frequency by 66% was sufficient to sustain adequate stimulus to maintain the increased inspiratory muscle function up to 18 weeks (53). However, the response of the metaboreflex to detraining remains unknown. Since the inspiratory muscle strength can be retained via lower stimulus and/or will be retained to a lower degree even without maintaining any stimulus, the cardiovascular adaptations (i.e., attenuation of the respiratory metaboreflex) should also be retained to a similar degree (greater than baseline).

1.7 Application of the findings

Determining this time course of function loss (cardiovascular and strength) would help in deciding if individuals such as athletes and/or COPD patients who completed RMT need to sustain a certain degree of the training to maintain their adaptations. One of the adaptations of RMT was

an increase in strength (Table 1) and the gain in strength is lost over time once the stimulus (RMT) is removed. However, maintaining two thirds of their training load will help retain their inspiratory muscle strength gain from the training (53). Another adaptation of RMT is the attenuation of the respiratory metaboreflex, which is achieved after 5 week of training (76). However, it is currently unknown if the cardiovascular response (i.e., attenuation of the metaboreflex) will persist while the strength response (i.e., increase in MIP) remains elevated to some degree after detraining.

The implications of respiratory muscle detraining extend beyond athletics or those in pulmonary rehabilitation. Individuals who undergo mechanical ventilation have a rapid atrophy in their diaphragm. The inactivity of the diaphragm from 18-69 hours of mechanical ventilations results in 57% and 53% reduction in slow twitch and fast twitch muscle fibers, respectively, compared to those who were only limited to 2-3 hours of mechanical ventilation (31). In addition, the largest decrease in diaphragm thickness occurred within the first 72 hours of mechanical ventilation (57). Furthermore, the atrophy of the diaphragm is more rapid, than the peripheral muscle suggesting the response to detraining may be different. For example, with ICU admission, up to 30% of muscle mass is lost within 10 days and the largest reduction is in the rectus femoris (45). The atrophy of the diaphragm is more rapid than other skeletal muscles when looking at the removal of the stimulus, which may suggest that the diaphragm is unique to other skeletal muscles.

2.0 STUDY RATIONALE

Due to the unique nature of the diaphragm and its role to be constantly active to sustain life, suggests that the diaphragm is unique compared to other skeletal muscles. One gap in the literature is research investigating effect of detraining on respiratory muscles, specifically on the diaphragm due to the invasiveness of the interventions used to decrease the workload of the diaphragm over a period of time, which is achieved through mechanical ventilation. In addition, the response of the respiratory metaboreflex has been established with relationship to RMT however, the relationship between the respiratory metaboreflex and detraining remains unknown. Therefore, the purpose of this thesis is to investigate the time course of muscle function loss due to detraining of the diaphragm and if that time course is different to a comparable skeletal muscle (matched with muscle fiber type and usage), the tibialis anterior, and whether the attenuation of the respiratory metaboreflex from RMT is sustained after detraining.

3.0 RESEARCH QUESTIONS AND HYPOTHESIS

3.1 Research Questions

1. Will the diaphragm lose strength during detraining at a similar rate as the tibialis anterior?
2. Will the attenuation of the respiratory muscle metaboreflex persist after 5 weeks of detraining?

3.2 Hypothesis

1. The time course of muscle function loss of the diaphragm following detraining will be similar to the tibialis anterior due to similar daily activity and muscle fiber type.
2. The attenuation of the respiratory muscle metaboreflex will persist after 5 weeks of detraining but at a lesser magnitude than peak training levels due to the elevated muscle strength compared to baseline.

4.0 METHODS

4.1 Ethics

The experimental procedures for this study were approved by the Office of Research Ethics at the University of Waterloo (ORE #41928). The research methods and protocols adhered to the recommendations outlined by the Declaration of *Helsinki* concerned with the use of human participants, except for registration in a database.

4.2 Subjects

A sample size calculation for a one tailed paired t-test was done using G*Power. Based on Witt et al.'s finding on the changes of MIP (+17% of MIP) and attenuation of metaboreflex (Pre vs post RMT, +18% vs +6% MAP) with RMT, a sample of N=6 per group is sufficient power to investigate the effects of RMT on MIP and the metaboreflex over 5 weeks (76). A total of 14 young, healthy participants were recruited in this study, with 6 participants in the control group and 8 in the experimental group. This study included males and females under the age of 40 years old, as ageing can influence the pulmonary function at the beginning of the 5^{th} decade. Females were included in this study only if they are taking a form of monophasic contraceptives to minimize the known effects of the menstrual cycle on the metaboreflex (44). Inclusion and exclusion criteria are found in Table 2 below.

Table 2. Participant inclusion and exclusion criteria.

Inclusion Criteria	Exclusion Criteria
• Age <40 years old • Participates in physical activity 2+ days a week	• Individuals with cardiovascular, metabolic, gastrointestinal or respiratory conditions • Participants that are taking prescription or non-prescription medication that could interfere with the cardiorespiratory response on a daily basis (excluding monophasic birth control). • Females on any other types of birth control, including non-hormonal, progesterone only, or triphasic tablets, or if the participant is not normally menstruating • Individuals with arthritis • Smokers • Female participants, if they are pregnant or nursing.

4.3 Experimental overview

Participants were randomly assigned into either the control or experimental group. The participants in the experimental groups completed a 10-week protocol, which included 5 weeks training (week 1-5) followed by 5 weeks detraining (week 6-10) and 3 separate metaboreflex testing days (pre-training, post-training, and post-detraining) (Fig.1). Whereas the participants in the control group only participated in the 3 testing days over the 10 weeks (Fig 2). Both groups were asked to maintain physical activity levels throughout the protocol.

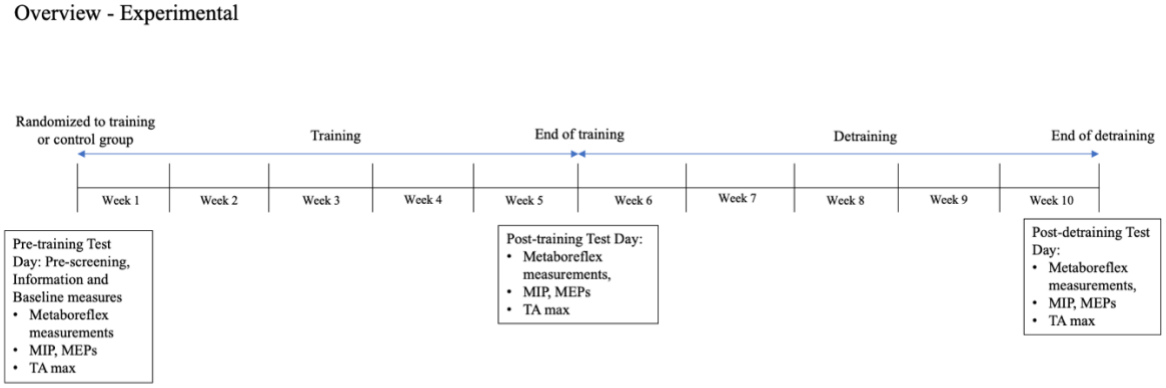

Figure 1. Experimental group protocol schematic.

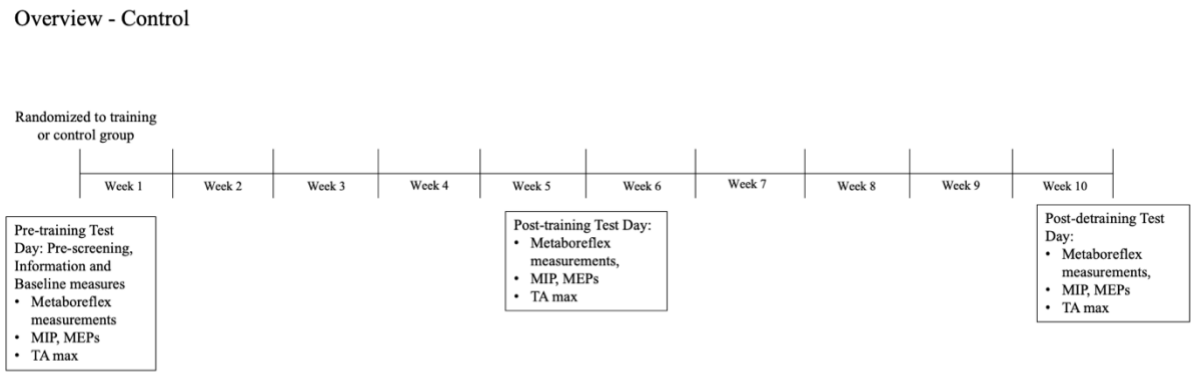

Figure 2. Control group protocol schematic.

4.3.1 Testing Day protocol

On each testing days participants were asked to avoid caffeine and large meals prior to arriving to the testing day. On the first testing day (pre-training), baseline measurements were taken, which included height, weight, maximal inspiratory pressure (MIP), maximal expiratory pressure (MEP), maximal isometric strength of dorsi flexor, and a respiratory metaboreflex test (Fig. 1). On each of the subsequent 2 testing days (post-training and post-detraining), identical measurements were taken.

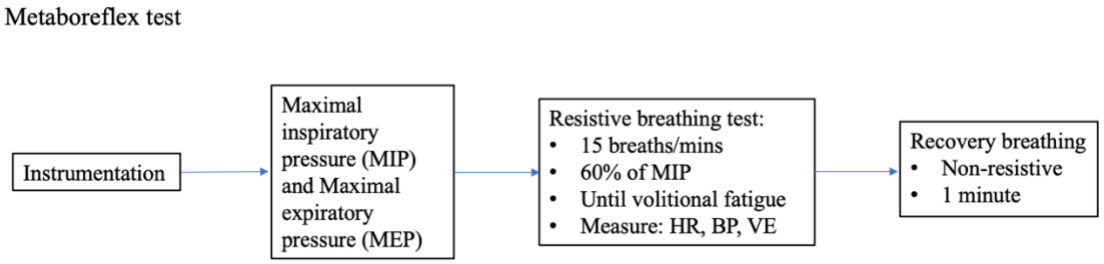

Figure 3. Metaboreflex Test Schematic.

4.3.2 Maximal inspiratory and expiratory pressure

Mouth pressure was measured via a calibrated differential pressure transducer (DP15-32; Validyne Engineering, Northridge, CA) connected to a port in the mouthpiece during each of the 3 test day visits (pre-training, post-training, post detraining). To perform the MIP and MEP maneuver, participants were in an upright seated position with nose clips worn. At the end of normal expiration (at functional residual capacity), the participant immediately put on a mouthpiece and was told to inhale as hard as possible with verbal encouragement. In between each MIP, participants were given 30 seconds to one minute of rest. The average of three consistent (<5% difference) MIPs were taken and used as the MIP value. The MEP maneuver was performed by participants at total lung capacity, and they immediately put on a mouthpiece and were told to exhale as hard as possible with verbal encouragement. Participants then had 30 seconds to one minute of rest in between each MEP maneuver and the average of three consistent (<5% difference) MEPs were taken as the MEP value. The above procedures are consistent with standards set by the *American Thoracic Society* (19).

4.3.3 Maximal isometric strength of the dorsi flexors

Maximal isometric strength of dorsi flexors were measured with a shin isolator (Sky King, Oseola, IN) attached to a load cell (interface MFG, USA) anchored to a metal platform. The participant was in the seated position and a Velcro strap was used to keep the participant's leg on the chair to minimize the recruitment of other muscles. The knee and ankle were kept at a 90^{o} and 340^{o} (20^{o} down) angle, respectively, while performing the dorsi flexion. The participant was reminded to not move the leg and only hinge at the ankle joint. The average of three consistent (<5% difference) forces generated with the shin isolator were used as the maximal isometric strength value. The predetermined weight to force relationship is obtained via the force generated through incremental increases of weights. These predetermined values of force that correspond to a certain weight was strongly correlated (Appendix 1, $R^2 = 0.9994$).

4.3.4 Respiratory metaboreflex testing exercise

Participants were in an upright seated position during the metaboreflex testing. First, a minimum of 10 minutes of eupnea was collected to ensure steady baseline cardiorespiratory measurements on the threshold loader (fig. 4). MIPs were then be measured as described above. The predetermined weight to pressure relationship was obtained via lifting incremental increases of weights with a standard three-liter syringe, which establishes the inspiratory pressure needed to lift the weight to allow for airflow. These predetermined values of weights that correspond to a certain MIP value were strongly correlated (Appendix 2, $R^2 = 0.9932$). The weight used for the respiratory loading test was equal to 60% of this corresponding weight.

For the respiratory loading test, participants inspired against a resistive load (weights) equal to 60% of MIP, based on the predetermined values, at a breathing frequency of 15 breaths per

minute (duty cycle of 50:50) (76). During the metaboreflex testing exercise, a small CO_2 line was connected to the threshold loader (Fig 4) to titrate CO_2 to ensure participants are isocapnic throughout the exercise. A guideline is provided on a computer monitor at a precalculated mouth pressure to guide the necessary force needed to be generated by the subject. An audio cue was used to signal the subject to inspire or expire to ensure a consistent breathing frequency. Participants continued this breathing exercise until voluntary exhaustion.

For the whole duration of the breathing exercise, cardiorespiratory parameters were measured. A calibrated pneumotachometer (model 3813; Hans Rudolph) was used to collect expired flow and was used to calculate breathing frequency, expired volume, and minute ventilation. The pneumotachometer was placed distal to the one-way valve to ensure it was not exposed to the substantial negative pressure (Fig 4). A calibrated gas analyzer (CD-3Am; Applied Electrochemistry, Bastrop, TX) was used to measure expired carbon dioxide and estimate end-tidal CO_2 concentrations. An electrocardiogram (ECG) in the three-lead configuration, the right lead (RA) was placed just below the right clavicle, the left lead (LA) was placed right below the left clavicle and the other left lead (LL) was placed on the left lower side of the rib cage (ISO-4 Isolation Preamplifier, CWE inc) was used determine heart rate. Photoelectric plethysmography (Human NIBP Nano Interface, ADInstruments) was used to non-invasively measure blood pressure via a cuff placed on their middle finger on their hand to collect beat-by-beat blood pressure through the entire duration of the testing days. Before collecting any data, the correct cuff size was determined via the finger cuff guide provided, the height correction unit was used to account for any height changes of the finger and calibrated according to the instructional guide. Throughout the duration of the data collection, subjects were reminded to keep their hand as still and relaxed as possible.

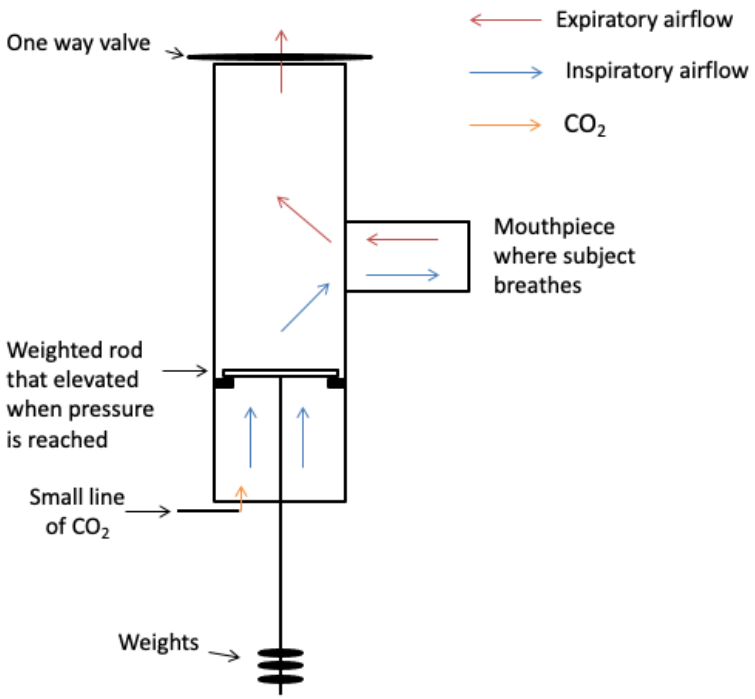

Figure 4. Schematic of Threshold loader

4.3.5 Training Day protocol

The training protocol occurred during the first 5 weeks of the 10 week protocol (Fig. 5) for the training group. Participants completed their training program 5 days a week at home through 2 exercises. The first exercise included 2 sets of 30 repetitions at 50% of their MIP using a commercially available respiratory muscle trainer (ModeL PBK3; PowerBreathe). The other exercise included 2 sets of 30 repetitions of dorsiflexion at 50% of maximal isometric strength of the dorsi flexor to train the TA of the dominant leg. To maintain training progression, weekly baseline measurement for MIP and 1RM were taken in order to adjust the training for the week. For example, as the subjects showed progression through increases in their MIP, their 50% load increased as it will be equal to 50% of this weekly measurement of MIP. Similarly, to MIPs, the weekly progression of dorsiflexion was 50% of their weekly maximal isometric strength

measurement. The respiratory training was tracked and logged onto the respiratory trainers directly. Daily emails were sent out as reminders to ensure participants completed their training program. The detraining protocol was the last 5 weeks of the 10-week protocol (Fig. 1). This involved the participants ending the inspiratory muscle and tibialis anterior training and were asked to keep other physical activity constant. During both the training and detraining, those in the control group did not perform any respiratory or tibialis anterior training.

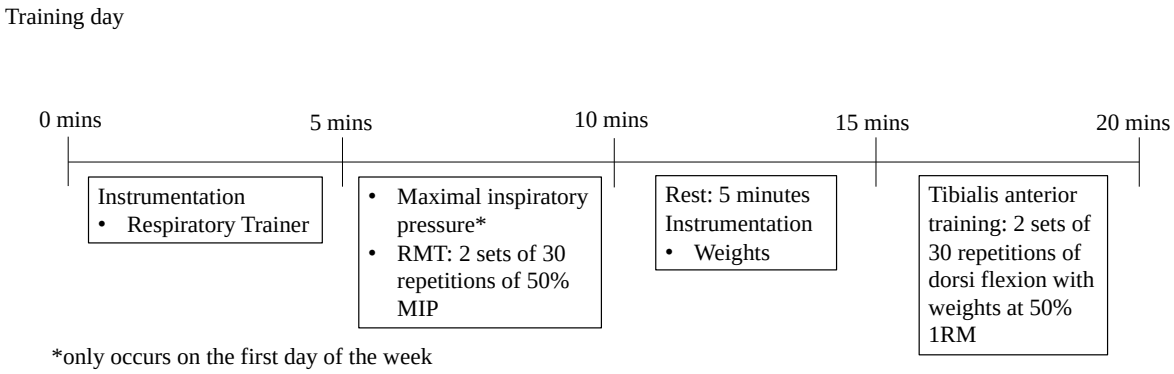

Figure 5. Training day protocol schematic.

4.4 Data analysis

The mean value of MIPs and MEPs were calculated from the average of a one second nadir and peak pressures of each MIP & MEP taken from three consistent maneuvers (<5% difference). Maximal isometric strength of the TA was taken from the highest force generated over one second. A mean was then calculated from three consistent trials (<5% difference).

The following variables, HR, MAP, and V_E were taken from the average of one-minute bins of the last minute of baseline and then one-minute bins of minute 1-3 and the last minute of the exercise. The one minute bins provided the participant's baseline and then minute 1, 2, 3 provided progression of the response to the breathing exercise and finally the final minute provided the final

response. Since the duration of the breathing exercise during the metaboreflex trial varied between individuals as they went to voluntary exhaustion, the last minute of the test used was the time reached in the pre-training trial. This matched the times of the exercise between the pre-training, post-training and post-detraining trial (Fig. 6). For example, if the last minute was taken from minute 10 of the metaboreflex test on the pre-training day, then minute 10 was when the variables were taken for the post training and post-detraining days regardless how long the participant's time to exhaustion was. In addition to matching the time the amount of respiratory work done was matched (<10% difference). The respiratory work was calculated via the integral of the mouth pressure generated for each breath and then used in the following equation to determine the overall amount of work done.

$$\text{Eq 1. } W_{tot} = (\int P_{mouth})(F_b)(T_{exhaustion}).$$

Heart rate was calculated from the R-R interval measured from the electrocardiogram. Minute ventilation was calculated from multiplying the breath frequency with the tidal volume. SBP and DBP were derived from the pulse pressure measured beat by beat with the photoelectric plethysmography. Mean arterial blood pressure (MAP) is calculated via measurements collected by photoelectric plethysmography and LabChart.

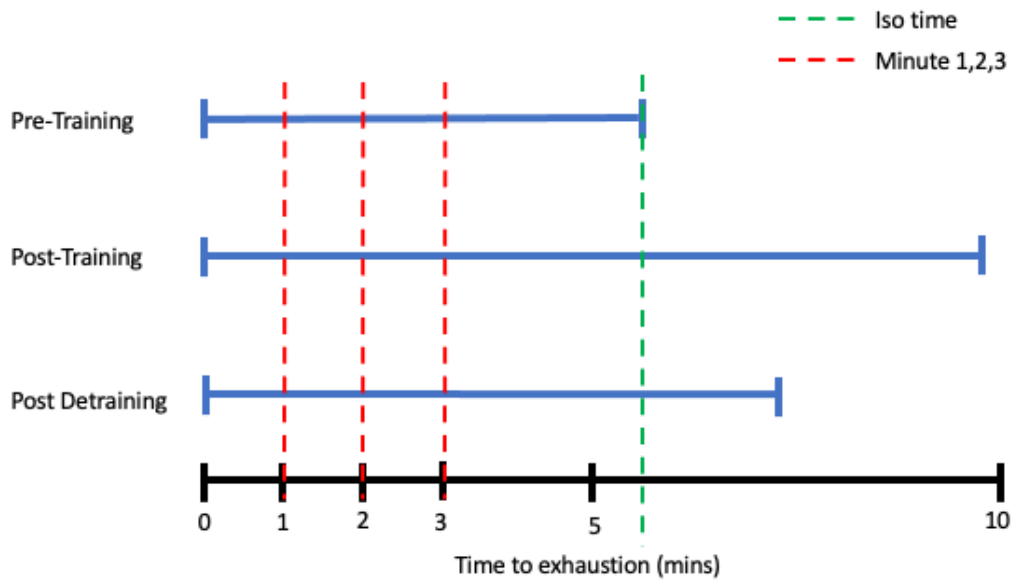

Figure 6. Example of time points compared between trials

4.5 Statistical analysis

The peak MAP and HR at the end of metaboreflex, MIP, MEP, and maximal isometric strength of the TA were compared with a 1-way repeated measure ANOVA. When significant F-ratios are detected a Tukey post-hoc test was used to determine where the difference lay. An independent T-test was used to compare the participant demographics between groups. Correlations were done to determine the relationship between inspiratory muscle strength (MIP) and the respiratory metaboreflex (HR and MAP), the change in MAP and change in time to exhaustion, the starting strength (TA and MIP) and the change in strength (TA and MIP). Significance was set at $P<0.05$.

5.0 RESULTS

5.1 Baseline Data

Eight participants (6M,2F) completed the experimental group protocol, and six participants (5M,1F) completed the control group protocol. Demographic and baseline measurements can be seen in Table 3. All demographic and baselines measurements were not different between groups ($p>0.05$). There was no difference in HR at rest (10 minuutes of seated, eupnea) on pre-training, post training and post detraining in the experimental (76±7 vs 79±10 vs 82±10 bpm respectively, $p=0.314$) and control group (80±12 vs 77±7 vs 78±13 bpm, $p=0.16$). Mean arterial blood pressure was also not different across pre-training, post training and post detraining in the experimental (92±7 vs 95±11 vs 89±7 mmHg, $p=0.08$) and control group (89±10 vs 87±7 vs 87±9 mmHg, $p=0.978$).

Table 3. Demographic and baseline values of the control and experimental group

	Control	Experimental
Participants	5M,1F	6M, 2F
Age (years)	24±1	26±4
Height (cm)	173±8	176±6
Weight (kg)	75±16	76±12
BMI (kg/m^2)	25±6	25±3
MIP (cmH_2O)	111±32	132±27
TA strength (N)	82±18	65±14
HR (bpm)	79±11	76±7
MAP (mmHg)	92±11	94±9

Abbreviations: BMI, body mass index; MIP, maximal inspiratory pressure; TA, tibialis anterior strength; HR, heart rate; MAP, mean arterial blood pressure. Values are reported as mean ± *SD*.
*Represents significant differences ($p<0.05$) from control group.

5.2 Tibialis Anterior Strength measurements

The experiment group had a significant increase of 21±13N (+34±15%) in TA strength (Fig. 7, $p<0.001$) from pre training to post training. Their TA strength remained elevated and not significantly different after 5 weeks of detraining (-1±9N between the post training and post detraining; Fig. 7, $p=0.982$). The post detraining TA strength remained elevated compared to the pre training levels (Fig. 7, $p<0.001$). There was no correlation between the initial TA strength and the strength gain from TA training (Appendix 3, $R^2=0.003394$, $p=0.891$) in the experimental subjects. The control group had no changes in TA strength over the period of training and detraining (Fig. 7, $p=0.125$).

5.3 Respiratory Muscle Strength measurements (MIPs and MEPs)

The experimental group had a significant increase of 23±11cmH_2O (+18±8%) in MIP (Fig. 7, $p<0.001$) from pre training to post training. The MIP was not significantly different from post training to post detraining (-2±9 cmH_2O, -1±7%, $p=0.853$). The post detraining levels remained elevated compared to the pre training (Fig. 7, p<001). The control had no changes from pre training to post training (Fig. 7, $p=0.191$). However, their post detraining MIP was elevated (+11±8%) from pre training levels (Fig. 7, $p=0.006$). There was no correlation between the starting MIP and the strength gain from RMT (Appendix. 4, $R^2=0.04878$, $p=0.5992$). The MEP was not different at pre training, post training or post detraining for the experimental and the control group (Fig. 9, $p=0.603$, $p=0.571$, for the experimental and control groups respectively).

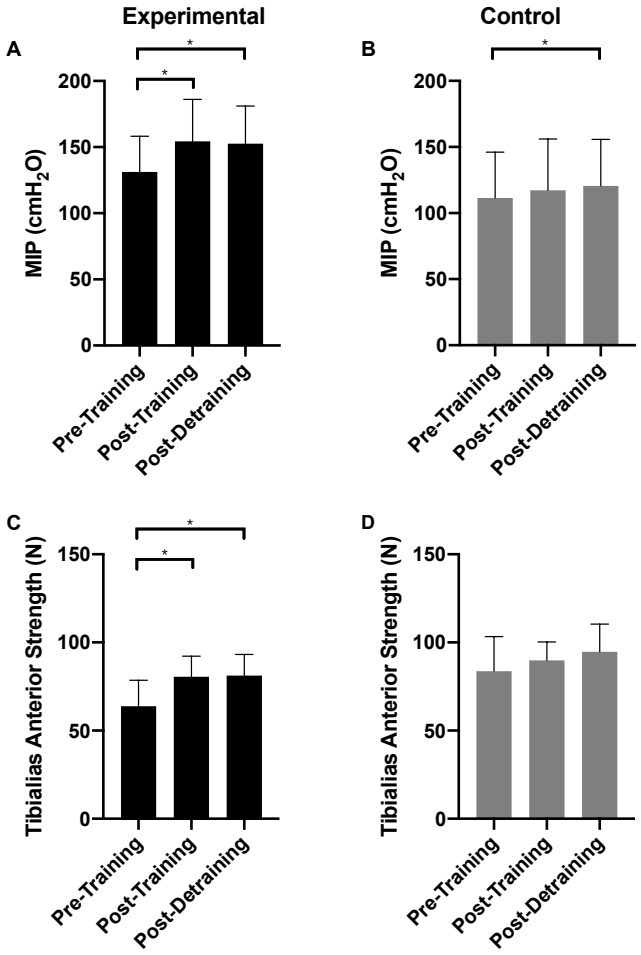

Figure 7. Mean maximal inspiratory pressure (MIP) and tibialis anterior strength over various days for both groups. Panel A and B show the mean MIP at pre-training, post training and post detraining for the experimental (black, Panel A) group and control (grey, Panel B) group. Panel C and D show the mean TA strength at pre-training, post training and post detraining in the experimental (black, Panel C) group and control (grey, Panel D). *Represents significant differences ($p<0.05$) from pre-training values.

5.4 Respiratory Muscle Metaboreflex measurements

Figure 8 is a representative image of an experimental participant during the resistive breathing task on post detraining day that demonstrates the attenuation of the respiratory metaboreflex. The dotted and solid lines show the HR and MAP response on the pretraining and post training day, respectively. There was no difference between the respiratory muscle work completed during the resistive breathing task on either day for the experimental or control group

when comparing at iso time (Fig. 9, p=0.520, p=0.422, for the experimental and control respectively).

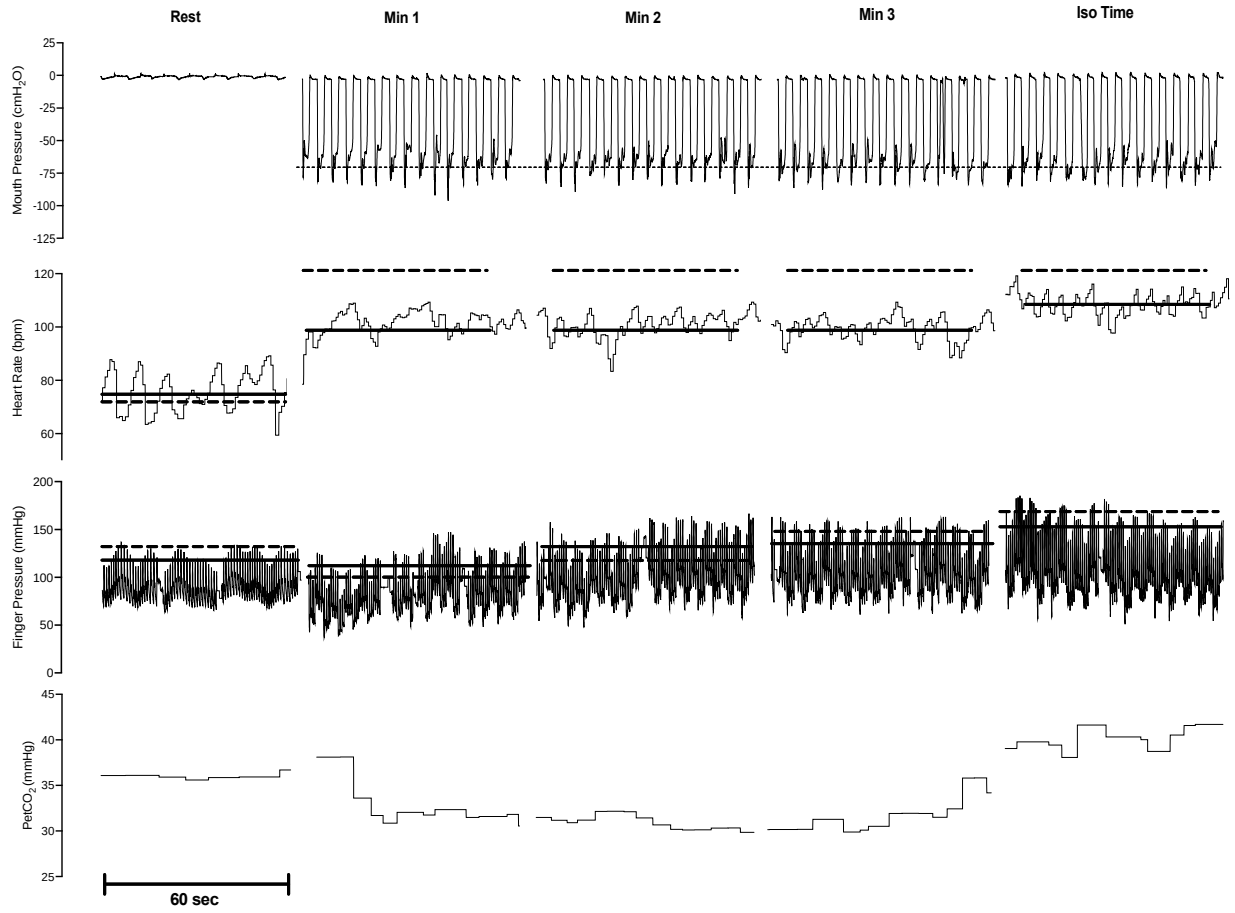

Figure 8. Raw data of a male participant from the experimental group at various time points during a resistive breathing task on the post detraining day. Resistive breathing was performed at 60% maximal inspiratory pressure with a 0.5 duty cycle at 15 breaths per minutes. Dotted line indicates mean value of the 1 minute bin from pre-training and solid line indicates mean values from the post training.

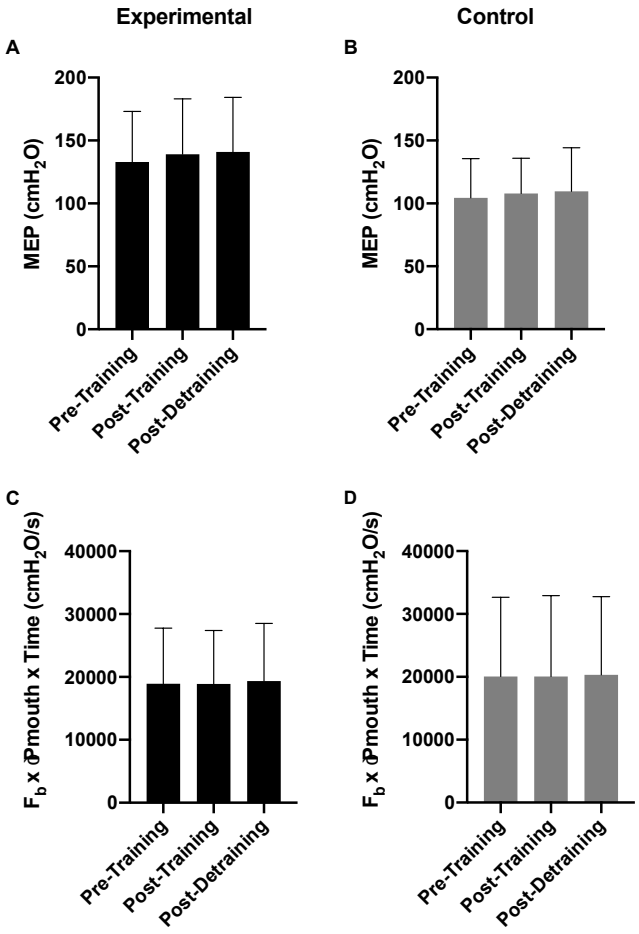

Figure 9. Mean maximal expiratory pressure (MEP) and respiratory work (Fb x ∫Pmouth x Time; cmH2O/s) completed during the resistive breathing task various days for both groups. Panels A & B show the mean MEP for the experimental group at pre-training, post training and post detraining in the experimental (black, Panel A) group and the control (grey, Panel B) group. Panels C and D show the mean respiratory work completed at iso time at pre-training, post training and post detraining in the experimental (black, Panel C) group and the control (grey, Panel D) group. *Represents significant differences ($p<0.05$) from pre-training values.

5.4.1 Heart Rate

The percent change in HR response of the experimental group over pretraining, post training and post detraining from rest to iso time can be seen in figure 10. The experimental group had a nonsignificant attenuation of HR of -11±14% from pre training to post training levels ($p=0.059$) at iso-time during the metaboreflex testing. There was a lesser and still nonsignificant decrease of 4±17% from post training to post detraining levels and it was still not different from

pre training level (p=0.059). The control had a nonsignificant attenuation of HR (-5±7%) from pre training to post training levels (Fig.10, p=0.156). There was a lesser nonsignificant decrease of 1±9% from post training to post detraining levels.

The absolute change in HR response of the experimental group over pretraining, post training and post detraining from rest to iso time can be seen in figure 10. The rise in HR was significantly less (8±9 bmp) in the experimental group from pre training to post training (p=0.036). There was a nonsignificant decrease in the rise of 3±10 bpm from post training to post detraining (p=0.147). There was no relationship between the changes in MIP and changes in HR (Fig.12, R^2=0.3237, p=0.14). There were no significant differences in the absolute change in HR for the control group across pre training, post training and post detraining levels (Fig.10, p>0.05).

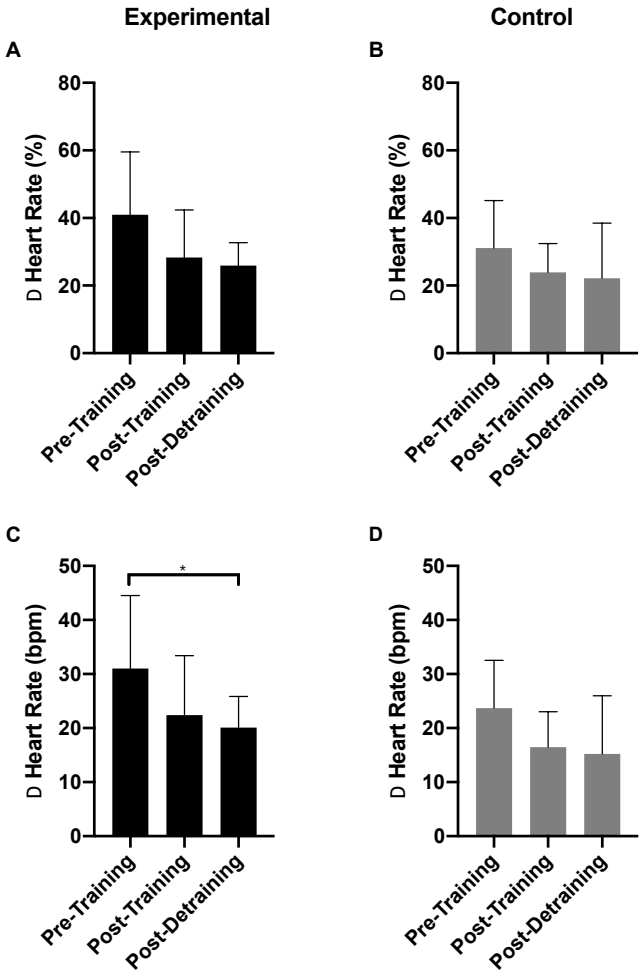

Figure 10. Mean percent change of heart rate (HR) and absolute change of heart rate from rest during the resistive breathing task over various days for both groups. Panel A and B represent the mean percent change of HR from rest to iso time of the experimental (black, Panel A) group and control (grey, Panel B) at pre-training, post training and post detraining. Panel C and D represent the mean change of absolute HR from rest to iso time of the experimental (black) group and control (grey, Panel D) at pre-training, post training and post detraining. *Represents significant differences ($p<0.05$) from pre-training values.

5.4.2 Mean Arterial Pressure

Absolute MAP values are shown in Table 4 and 5 for control and experimental groups, respectively, during each of the resistive breathing tasks. There was a general trend of MAP dropping in the first minute or two and then climbing back up and eventually reaching a rise in MAP at iso time and the final minute. The SBP and DBP increased over the resistive task. There was a trend for a lesser increase in the post training and post detraining compared to pre-training

in SBP (10±14, 14±10 vs 22±12) and DBP (6±9, 9±5 vs 15±9) at iso time in the experimental group. This trend was not seen in the SBP (17±27, 10±9 vs 16±26 for post training, post detraining and pre-training, respectively) and DBP (9±15, 8±7 vs 12±1 for post training, post detraining and pre-training, respectively) of the control group at iso time.

Table 4. Mean cardiovascular measurements of the control group at pre-training (Pre), post training (Post) and post detraining (De) during the resistive breathing task

	Day	HR (bpm)	MAP (mmHg)	SBP (mmHg)	DBP (mmHg)	ΔSBP (mmHg)	ΔDBP (mmHg)
Rest	Pre	80±12	89±10	130±16	73±10	-	-
	Post	77±7	87±7	129±12	72±7	-	-
	De	78±13	87±9	125±15	71±8	-	-
Min 1	Pre	94±17	90±19	131±29	74±17	0.1±26	1±14
	Post	90±6	87±17	127±30	71±13	-2±26	-1±14
	De	92±12	85±17	117±25‡	70±14	-9±15	-2±8
Min 2	Pre	95±16	94±22	137±37	76±18	7±34	3±16
	Post	93±9	91±20	130±36	74±16	0.3±30	2±16
	De	93±9	90±15	122±18	74±14	-3±7	2±7
Min 3	Pre	97±15	97±20	142±32	79±16	11±30	6±14
	Post	94±8	95±16	137±29	78±12	8±25[a]	6±14[a]
	De	93±12	93±14	126±20†	76±12	1±8	4±5
Iso Time	Pre	103±15*	103±18[a]	146±27	85±15[a]	16±26[a]	12±11[a,b]
	Post	95±7*	100±17[a]	146±31[a,b]	82±14[a]	17±27[a,b]	9±15[a,b]
	De	95±11*	99±17	136±23*[a]	80±14	10±9[a,b]	8±7[a]
Final Minute	Pre	103±15*	103±18[a]	146±27	85±15[a]	16±26[a]	12±11[a,b]
	Post	95±10	101±19[a,b]	145±34[a,b]	82±14[a]	15±29[a,b,c]	9±14[a,b]
	De	95±13*	99±18*[a]	141±27*[,a,b,c,]	81±14*[a]	16±13[a,b]	10±8[a]

Abbreviations: HR, heart rate; MAP, mean arterial blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; Values are reported as mean ± *SD*.*Represents significant differences from rest ($p<0.05$), [a,b,c] Represents significant differences from minutes 1, minute 2, and minute 3, respectively ($p<0.05$). †Represents significant difference from pre-training ($p<0.05$).

Table 5. Mean cardiovascular measurements of the experimental group at pre-training (Pre), post training (Post) and post detraining (De) during the resistive breathing task

	Day	HR (bpm)	MAP (mmHg)	SBP (mmHg)	DBP (mmHg)	ΔSBP (mmHg)	ΔDBP (mmHg)
Rest	Pre	76±7	94±8	136±9	80±6	-	-
	Post	80±9	101±9	142±15	80±8	-	-
	De	83±11	92±10†	129±13†	76±9	-	-
Min 1	Pre	98±14	91±13	127±19	77±11	-9±18	-4±10
	Post	97±12	95±10	134±17	79±8	-8±16	-1±6
	De	103±17	84±10	120±16‡	72±11	-10±13	-4±9
Min 2	Pre	100±14	101±9	137±10	83±8	2±13	3±9
	Post	98±13	102±5	141±13	85±4	-1±15	5±6
	De	103±16	93±9†,‡	126±9‡	77±8	-4±13	1±8
Min 3	Pre	102±12*	105±8	144±11^{a}	88±9^{a}	8±11	8±8
	Post	99±13	106±5	146±14	89±4	4±19	7±6
	De	104±13	99±10	136±11	82±9	6±11	6±7
Iso Time	Pre	107±15*	115±11*^{a,b}	157±11*^{a,b}	95±12*^{a,b}	22±12	15±9
	Post	101±13*	111±7*^{a}	152±14^{a}	93±6*^{a,b}	10±14	6±9
	De	104±13*	103±12*^{a,b}	143±14^{a,b}	85±10*^{a,b}	14±10	9±5
Final Minute	Pre	107±15*	115±11*^{a,b}	157±11*^{a,b}	95±12*^{a,b}	22±12	15±9
	Post	109±22*	117±15*^{a,b}	161±18^{a,b}	98±14*^{a,b,c}	19±17	18±10
	De	105±15*	105±13‡*	139±24$^{\dagger,\ddagger a}$	85±12*^{a,b}	9±20	9±7

Abbreviations: HR, heart rate; MAP, mean arterial blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; Values are reported as mean ± *SD*.*Represents significant differences from rest ($p<0.05$), ^{a,b,c} Represents significant differences from minutes 1, minute 2, and minute 3, respectively ($p<0.05$). †,‡ Represents significant difference from pre-training and post training, respectively ($p<0.05$).

The percent change in MAP response over pre training, post training and post detraining for the experimental group from rest to iso time can be seen in figure 11. The experimental group had a significant decrease in the rise of MAP by 11±7% from pre training to post training ($p=0.003$). There was a nonsignificant decrease of 1±6% from post training to post detraining ($p=0.836$). The post detraining levels were still lower than the pre training levels ($p=0.007$). The

control group experienced no changes in MAP response at pre training, post training and post detraining levels (Fig.11 p=0.758).

The absolute change in MAP response over pre training, post training and post detraining for the experimental group from rest to iso time can be seen in figure 11. The experimental group had a decrease in the rise of MAP by 8±7 mmHg from pre training to post training (p=0.01). From post training to post detraining there was a not significant decrease of 1±4mmHg (p=0.868). There is a strong inverse relationship between the changes in MAP and the changes in MIP from pre training to post training in the experimental group (Fig.12, R^2=0.7473, p<0.01). There were no differences in the absolute changes in MAP between pre training, post training and post detraining (Fig.11, p=0.829) in the control group.

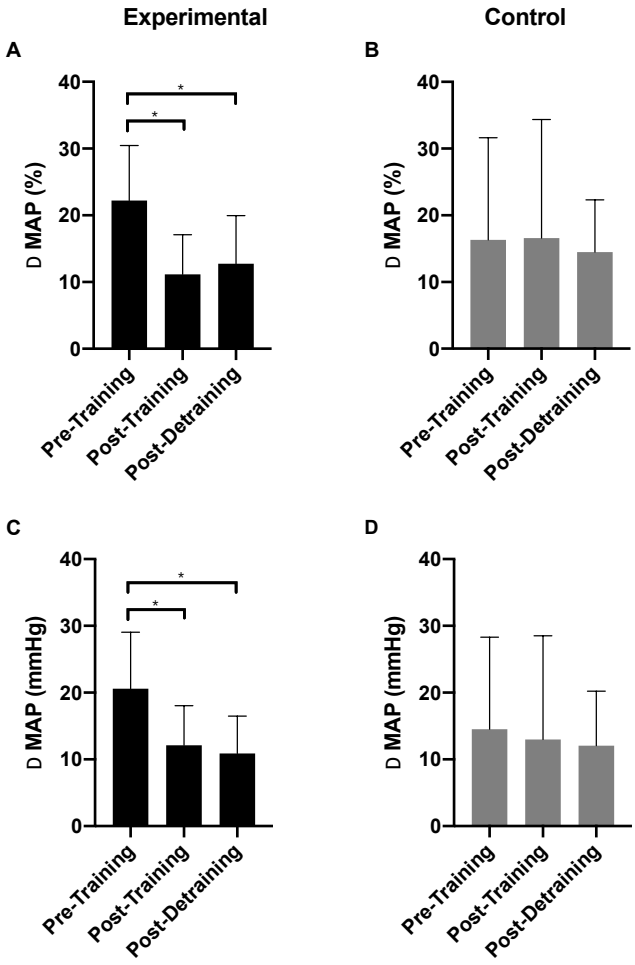

Figure 11. Mean percent change of mean arterial blood pressure (MAP) and absolute change of MAP from rest during the resistive breathing task over various days both groups. Panel A and B represent the mean percent change of MAP from rest to iso time of the experimental (black) group and control (grey, Panel B) at pre-training, post training and post detraining. Panel C and D represent the mean change of absolute MAP from rest to iso time of the experimental (black) group and control (grey, Panel D) at pre-training, post training and post detraining. *Represents significant differences ($p<0.05$) from pre-training.

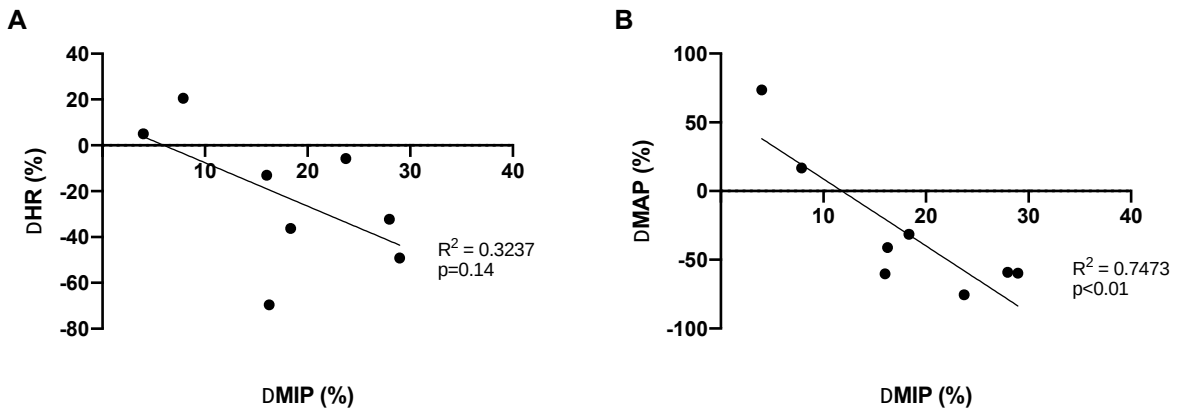

Figure 12. Relationship between the change in cardiovascular variables during the resistive breathing task from pre-training to post training. (A) No relationship between the percent change in heart rate change in maximal inspiratory pressure of the experimental group from pre-training to post training (p=0.14). (B) Strong relationship between the percent change of mean arterial blood pressure and change in maximal inspiratory pressure of the experimental group (p<0.01).

5.5 Time to exhaustion

The time to exhaustion of the experimental group across pre training, post training and post detraining can be seen in figure 13. The experimental group had a significant increase in time to exhaustion of 8.1±5.1 minutes from pre training to post training (p<0.001). There was a nonsignificant decrease of 4.1±5.9 minutes from post training to post detraining (p=0.077). Also, the time to exhaustion at post detraining was not different from pre training levels however, still elevated by 4.05 minutes (p=0.077). There was no relationship between the change in MAP and the time to exhaustion (Appendix 5, R^2=0.0003, p=0.9668). There was no difference in the time to exhaustion in the control group across pre training, post training and post detraining (Fig.13, p=0.10).

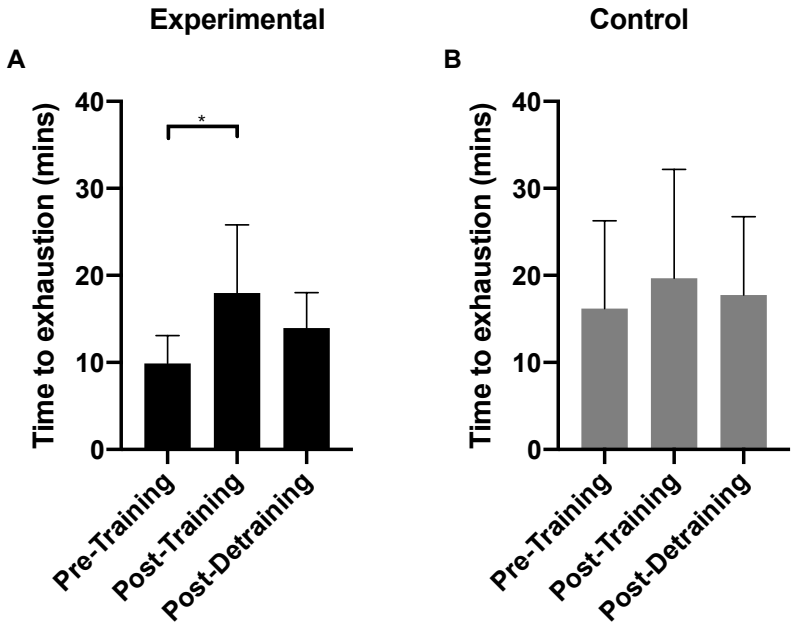

Figure 13. Mean time to exhaustion of the resistive breathing task over various days for both groups. (A) Mean time to exhaustion at pre-training, post training and post detraining for the experimental (black) group. (B) Mean time to exhaustion at pre-training, post training and post detraining for the control (grey) group. *Represents significant differences ($p<0.05$) from pre-training.

5.6 Ventilation during resistive breathing task (metaboreflex)

Ventilation data measured for rest, minute 1,2,3, iso time and final minute are shown in Table 6 and 7 for the experimental and control group, respectively. No differences were found in end-tidal CO_2 at all time points in the experimental group during the pretraining day ($p=0.104$). During post training, there was a small decrease in end-tidal CO_2 from rest to min 1,2, and 3 (5±5 mmHg, 5±4 mmHg, 4±3 mmHg, respectively, Table 7, $p<0.05$). And a small increase from min 1, 2, 3, to iso time and final minute (iso: 5±3 mmHg, 5±3 mmHg, 4±2 mmHg; final: 6±5mmHg, 6±5mmHg, 5±4mmHg, respectively, Table 7, $p<0.05$). During post detraining, there was a small significant decrease in end tidal CO_2 from rest to minute 1 and then no difference comparing iso time and final minute to rest (38.2 mmHg vs 33.9 mmHg, $p<0.05$ and 38.8, 39.8 mmHg vs 38.2

mmHg, $p>0.05$, respectively). End-tidal CO_2 was not different at rest to iso time and final minute in both post training and post detraining measurements ($p>0.05$). There was no difference in end-tidal CO_2 at all time points in the control group during the pre and post training day (Table 6, $p>0.05$). There was a small significant increase in end-tidal CO_2 on the post detraining day at the iso time and final minute compared to minute 1 (40.8 vs 36.4, $p<0.05$). Breathing frequency was not different when comparing minute 1,2,3, iso time and final minute in both the control and experimental group at pre-training, post training and post detraining (Table 7, $p>0.05$). No differences were found in tidal volume comparing minute 1,2,3, iso time and final minute in the experimental group at pre-training, post training and post detraining ($p>0.05$). There was a small significant difference between minute 2 and rest in the pre training, post training and post detraining (0.811 vs 0.592, $p=0.02$ and 1.16 vs 0.577, $p=0.047$, 0.981 vs 0.565, $p<0.05$, respectively). No differences were found in the tidal volume comparing rest, minute 1,2,3, iso time and final minute in the control group at pre-training, post training and post detraining (Table 6, $p>0.05$). Minute ventilation was not different when comparing minute 1,2,3, iso time and final minute in the control and experimental group at pre-training, post training and post detraining (Table 6 and 5, $p>0.05$).

Table 6. Mean ventilation data of the control group at pre-training (Pre), post training (Post) and post detraining (De) during the resistive breathing task

	Day	F_b (bpm)	V_T (L)	$\dot{V}_e$ (L/min)	$PetCO_2$ (mmHg)	Work (cmH_2O/s)	Total Time (mins)
Rest	Pre	9±4	0.54±0.26	4.3±1.3	37±6	-16±8	-
	Post	7±2	0.68±0.37	4.5±2.2	39±5	-15±6	-
	De	7±3	0.66±0.35	4.2±1.4	38±4	-15±13	-
Minute 1	Pre	14±1.2*	0.60±0.28	8.6±4.3*	39±1	-96±33*	-
	Post	14±0.5*	0.86±0.41	12.6±6.5*	37±2	-108±28*	-
	De	14±0.5*	0.79±0.27	11.5±3.8*	36±4	-118±31*†	-
Minute 2	Pre	15±0.5*	0.71±0.28	10.5±4.1*	39±3	-101±30*	-
	Post	15±0.1*	0.83±0.33	12.3±4.9*	36±3	-110±30*	-
	De	15±0.4*	0.76±0.27	11.1±4.0*	38±4	-111±28*	-
Minute 3	Pre	15±0.6*	0.74±0.22	10.9±3.6*	39±3	-104±33*	-
	Post	15±0.7*	0.77±0.29	11.5±4.6*	39±2	-104±29*	-
	De	15±0.5*	0.73±0.22	10.7±3.5*	39±3	-115±27*	-
Iso Time	Pre	15±0.4*	0.82±0.12	12.1±2.0*	39±4	-103±26*	-
	Post	15±0.5*	0.89±0.24	13.1±3.8*	39±2	-108±26*	-
	De	15±1.4*	0.66±0.31	9.84±5.1*	41±3	-109±30*	-
Final Minute	Pre	15±0.4*	0.82±0.12	12.1±2*	39±4	-103±26*	16.2±10
	Post	15±0.5*	0.78±0.25	11.9±3.8*	39±3	-109±27*	19.7±13
	De	15±0.8*	0.63±0.34	9.15±4.9*	41±3[a]	-109±28*	17.8±9

Abbreviations: F_b, breathing frequency; V_T, tidal volume; $\dot{V}_e$, minute ventilation; $PetCO_2$, end-tidal carbon dioxide; Work, respiratory work completed at iso time during the resistive breathing task (F_b x ∫Pmouth x Time). Values are reported as mean ± *SD*. *Represents significant differences from rest ($p<0.05$), [a]Represents significant differences from minutes 1 ($p<0.05$). †Represents significant difference from pre-training ($p<0.05$).

Table 7. Mean ventilation data of the experimental group at pre-training (Pre), post training (Post) and post detraining (De) during the resistive breathing task.

	Day	F_b (bpm)	V_T (L)	$\dot{V}_e$ (L/min)	$PetCO_2$ (mmHg)	Work (cmH_2O/s)	Total Time (mins)
Rest	Pre	8±2	0.57±0.21	4.4±1.95	36±4	-10±5	-
	Post	8±4	0.58±0.45	4.0±8.4	39±3	-10±2	-
	De	7±3	0.60±0.27	4.1±4.31	38±3	-10±4	-
Minute 1	Pre	15±0.6*	0.84±0.56	12.2±8.0*	38±4	-123±37*	-
	Post	15±0.8*	1.1±0.34	16.3±4.9*	34±4*†	-110±23*	-
	De	15±0.7*	1.1±0.26	17.2±3.8*	34±4*†	-115±35*	-
Minute 2	Pre	15±0.2*	0.92±0.34	13.5±4.6*	38±4	-124±41*	-
	Post	15±0.4*	1.1±0.30	16.4±4.5*	34±4*†	-109±24*	-
	De	16±1.5*	1.1±0.25	17.0±3.3*†	35±7	-117±36*	-
Minute 3	Pre	15±0.7*	0.92±0.40	13.1±4.7*	39±5	-128±39*	-
	Post	15±0.5*	1.1±0.39	16.5±5.6*	35±4*	-109±22*	-
	De	16±1.8*	1.0±0.22	16.3±3.0*	36±4	-121±38*	-
Iso Time	Pre	15±0.5*	0.90±0.47	14.5±6.5*	40±6	-131±43*	-
	Post	15±0.8*	1.1±0.33	16.0±5.5*	39±3[a,b,c]	-114±26*	-
	De	15±0.9*	0.92±0.14	13.8±2.2*	39±4	-123±36*	-
Final Minute	Pre	15±0.5*	0.90±0.47	14.5±6.5*	40±6	-131±43*	9.9±3.2
	Post	16±2*	1.4±0.68	21±9.6*	39±5[a,b,c]	-113±32*	18.0±7.8†
	De	15±2*	0.96±0.49	14±7.0*	40±4	-125±41*	13.9±4.1

Abbreviations: F_b, breathing frequency; V_T, tidal volume; $\dot{V}_e$, minute ventilation; $PetCO_2$, end-tidal carbon dioxide; Work, respiratory work completed at iso time during the resistive breathing task (F_b x ∫Pmouth x Time). Values are reported as mean ± *SD*. *Represents significant differences from rest ($p<0.05$), [a,b,c] Represents significant differences from minutes 1, minutes 2, minutes 3 respectively ($p<0.05$). †Represents significant difference from pre-training ($p<0.05$).

6.0 DISCUSSION

6.1 Main finding

The purposes of this thesis were 1) to determine whether the strength response to training and detraining of respiratory muscles were similar to an akin locomotor muscle (TA) and 2) whether the attenuation of the respiratory muscle metaboreflex persisted after 5 weeks of detraining. The main findings in this thesis are twofold. First, that both RMT and TA training resulted in a significant increase in the strength after 5 weeks of training (Fig. 7) and the strength in both muscles remained elevated after 5 weeks of detraining. The lack of differences in the temporal response of strength between the respiratory muscle and TA supports that respiratory muscles have a similar response to training as other similar skeletal muscles (Fig. 7). Secondly, 5 weeks of training resulted in an attenuation of the respiratory muscle metaboreflex (Fig. 11) that persisted after detraining. After 5 weeks of detraining, the attenuation of the respiratory muscle metaboreflex may aid in reducing the competition for blood with locomotor muscles during strenuous exercise 5 weeks (without any training) after 5 weeks of RMT (Fig. 11). Overall, the respiratory muscle strength response to training and detraining is similar to other skeletal muscles (TA) and the potential benefits of the attenuation of the respiratory metaboreflex remains after 5 weeks of detraining.

6.2 Respiratory muscle and TA strength response

The strength response indicates that respiratory muscles and TA have a similar temporal strength response to training and detraining. This was expected due to their comparable traits such as muscle fiber types (~50 type I and ~50% type II vs ~60 type I vs ~40 type II, for the diaphragm and TA, respectively), activity (continuously vs daily for the diaphragm and TA, respectively),

and both skeletal muscles. The increase of MIP with RMT is similar with Ramsook, 2017 (+16%) and Witt, 2007 (+17%) studies with similar training protocol (5 weeks, 2 sets of 30 repetitions at 50% MIP) (50, 76). This strength gain doesn't seem to be near the plateau point of strength gains as a longer training will result in higher strength gains with 9 weeks of training, at 50% at 30 repetitions a day resulted in a 64±3% increase in MIP and suggest that the strength gains will plateau at 6 weeks of training (54). There was no difference in the MEPs in both groups (Fig, 9), indicating that our RMT protocol, which targeted the inspiratory muscles, was isolated to the inspiratory muscles, and did not increase function of the expiratory muscles. More importantly, the increase in MIP without changes in MEP confirms the increase of MIP is due to the training protocol and not due to other factors (i.e., familiarization, other training).

The percent change (gain) in MIP did not have a relationship with the starting MIP (Appendix 4). The gain of MIP varied from individuals and one possibility for this difference is that the starting MIPs are absolute values and not relative values. Therefore, even though one participant may have a higher MIP value, it does not necessarily indicate that they are more trained as it does not take into consideration of other factors (i.e., height, size). The difference of the respiratory muscle being "trained" is likely the reason for the differences in MIP gain.

Both the strength of the respiratory muscle and TA remained elevated after the 5 weeks of detraining. There was no difference in MIPs from post training to post detraining, indicating the respiratory muscle strength remained elevated after 5 weeks of detraining. This finding is supported with the finding of another study, where they found MIPs remained elevated after 9 weeks of detraining (54).

Five weeks of training resulted in an increase of 34±15% vs 18±8% for TA and respiratory muscle, respectively. Even though the TA and respiratory muscles have comparable traits they are

still different. They are both active daily, however, there was greater gain of strength in the TA compared to respiratory muscles likely because the TA is not continuously active (i.e., when sitting, sleeping, etc.) whereas the respiratory muscles are continuously active. The difference in activity results in respiratory muscles to be relatively more trained than the TA, which leads to the more trained muscle (respiratory muscle) to have a lesser muscle strength gain than the less trained muscle (TA) (2). In other skeletal muscles, it is shown in an untrained population that 8 weeks of training of low load, high repetition (30% of 1RM, 12 sets of 8 repetitions, 3 times a week) resulted in an increase in 40.9% of 1RM in quadriceps (knee extensors) (25). Since the quadriceps are a large muscle group and usually experience a small workload throughout the day (i.e., standing up), they are likely less trained in the untrained population compared to respiratory muscle and the TA, which is likely why they have the greater strength gains.

The increase in strength seen in both respiratory muscles and TA is not likely due to hypertrophy as minimal hypertrophy occurs until 6-7 weeks of training (35). Since the training protocol in this study was only 5 weeks of training the increase in strength is expected to be as a result from neurological adaptations, as these are dominant in the early stages of training (38). Some of the neurological adaptation would include improved motor unit firing synchronization, faster firing frequency, and decrease agonist-antagonist coactivation (21, 29, 36). Any one of these neurological adaptations will improve muscle strength. However, it is likely a combination of all these adaptations. These neural adaptations can vary between different groups of muscles that are used to different degrees daily. A muscle that is used more often on a regular basis would likely be more neurologically adapted therefore, when introducing a training stimulus, it is likely to have an overall smaller neurological adaptation.

6.3 Resting HR and MAP

Five weeks of RMT did not change resting HR and MAP on either day in both groups ($p>0.05$). Six weeks of IMT found no difference in HR which is in line with the finding of this thesis (13). However, the same study showed that 6 weeks of IMT resulted in reductions of resting blood pressure (SBP, -4.3mmHg; DBP, -3.9mmHg) (13). This difference could be due the differences in training protocol. The current training protocol was 5 weeks (5 days/week, 2 sets of 30 repetitions at 50% MIP) whereas, the other study's training protocol was 6 weeks (5 days/week, 30 repetition at 75% MIP). The higher intensity of the training could be the reasoning for the different finding between the studies. Their protocol resulted in an increase of 34% MIP and this difference in increase in MIP (17% vs 34%) indicates that a greater stimulus (training intensity) resulted in a greater response (MIP), this may suggest that the training intervention in the current study was insufficient to lower resting SBP/DBP. Moderate-intensity dynamic resistance training and low-intensity isometric resistance training both have been shown to be able to decrease resting SBP and/or DBP (11). More importantly, RMT has been shown to improve resting SBP and DBP in an older population with higher blood pressure or obstructive sleep apnea and the improvement of resting blood pressure is due to the improvements of vascular endothelial functions post RMT (12, 49). Another possibility for the difference in findings could be the sample size. In DeLucia 2017's study they have a sample size almost twice as large (12 experimental, 13 control) compared to this thesis (8 experimental, 6 control) (13). In this study, the resting MAP somewhat trended towards being significant but was not different between pre-training and post training (92 ± 7 vs 89 ± 7, respectively, $p=0.08$), which suggests that the effect of RMT on resting MAP is likely small and this thesis is likely underpowered to show this effect as it was not the aim of the study.

6.4 Respiratory muscle metaboreflex.

There was no significant difference in change in HR (percent and absolute, Fig.10) in both experimental and control group. However, there is a trend towards being significantly different as seen in of an attenuation of -11±14% from pre training to post training levels in the experimental group ($p=0.059$) and only an attenuation of -5±12% in the control group ($p=0.156$). No difference but trending was also seen in the absolute change of HR (-8±9 bpm, $p=0.147$) in experimental group from pre-training to post training. However, it was significantly lower in the post-detraining compared to pre-training (-11±13 bpm, $p<0.05$). There was no difference in the change in HR in the attenuation of 3±10 bpm ($p=0.09$) in the control group. One possible reasoning for this trend, despite not being significantly different, is the control of HR is heavily influenced by central command.

One study that was used to demonstrate the influence of central command on HR was through hand grip exercise (71). During static hand gripping exercise, with a neuromuscular blockade, demonstrated no force was generated even at maximal effort contractions (71). Since no force was generated during this maximal effort contraction, no work was being done by the participant. However, it still resulted in an increase in HR, similar to the increase resulting from 30% of maximal voluntary contraction, indicating the rise in HR is due to central command (71). On the other hand, MAP had minimal increase during the contraction with neuromuscular blockade and was significantly lower compared to the 30% maximal voluntary contraction. These findings indicate that HR is more affected by the central command compared to MAP (71). When performing the same task, the effects of central command should be a similar magnitude each time. Since HR values in this thesis were compared at iso time where the work completed were the same for pre-training and post training; the contribution of central command to the response of HR

between the pre-training and post training trials should be similar. The small differences in the attenuation of HR response are likely due the neural adaptations (i.e., improved recruitment patterns) and contributed to minimal differences to the HR (-11±14%). Furthermore, the effects of central command on MAP would be minimal.

There was an attenuation of MAP from pre-training to post training by 11±7% ($p=0.003$), which is similar to Witt's finding of an attenuation of 11% in MAP after 5 weeks of RMT (76). This is expected as identical training protocols were used. There was no difference in the attenuation from post training to post detraining ($p=0.836$) and the post detraining levels were still lower than the pre training levels ($p=0.007$). The attenuation of MAP is also seen in the change of absolute values. The experimental had an attenuation of 8±7 mmHg from pre training to post training ($p=0.01$). From post training to post detraining there was a not significant decrease of 1±4mmHg ($p=0.868$). The increase of MAP is contributed from increases in SBP and DBP in both groups (Table 4 and 5), which indicates an increase in sympathetic response.

One explanation for the attenuation in MAP is after training the MIP has increased, therefore the same resistance during the resistive breathing task is now at a lower relative intensity. There is a strong inverse relationship between the changes in MAP and the changes in MIP from pre training to post training (Fig.13, $R^2=0.7473$, $p<0.01$). This inverse relationship between the change in MAP and the change in MIP indicates that the greater increase in MIPs results in a greater attenuation of MAP during the resistive breathing task. This inverse relationship between the change in MAP and change in MIP supports that the lower relative intensity likely plays in a role in the attenuation of the respiratory muscle metaboreflex, which is seen in this current longitudinal study. In agreement, this is also seen in a cross-sectional study, that a lower relative intensity will result in a lower MAP response seen in dynamic contractions in humans (67).

Another explanation for the attenuation in MAP is the training resulted in the improvement in the recruitments and changes in composition. With neurological adaptations such as, improved motor unit firing synchronization, faster firing frequency, and decrease agonist-antagonist coactivation, respiratory muscles would have improved its ability to manage the same stress, therefore resulting in lower metabolite productions at the same amount of work/stressor (21, 29, 36). It is shown in clinical populations, where they are chronically experiencing higher workloads (i.e., in chronic heart failure/COPD patients), they experience changes in muscle fiber types from type II to type I, which will increase their oxidative capacity (32, 69). The reduction in metabolites due the increase in oxidative capacity would explain the attenuation of MAP seen in the experimental group. However, these changes are seen in patient with these condition over long periods of time (months to years). It is unlikely seen in 5 weeks as over 6 weeks of strength training only resulted in a shift to type IIa from type IIx and no change in type I fibers (47).

The diaphragm is rich in group III and group IV afferent nerve fibers. During diaphragm fatigue, there is increase in the type IV (metabolic) afferent activity while there will be no change in the activity of type III (mechanical) afferent nerve fibers (24). Also, trained limbs have a reduced sympathetic response compared to the untrained limb at a given pH (62). Furthermore, training results in a decrease in mechanically sensitive muscle afferent nerve fiber discharge (60). These adaptations would explain the attenuation in MAP seen in the experimental group. In addition, when metabolites are above a certain threshold, there seems to have an additive effect on the sympathetic response (increase in HR, MAP) when mechanoreceptors are stimulated (61). Therefore, with changes that result in less metabolites being produced, it will decrease the activity of both the mechanically (type III) and metabolically (type IV) stimulated afferents.

There was an increase in time to exhaustion in the experimental group after 5 weeks of training (Fig.13), which was expected as strength training resulted in an increase in time to exhaustion (56). After the 5 weeks of detraining, time to exhaustion was not different between post training and post detraining, however it is trending toward returning to pre-training levels. There was no relationship between the change in MAP and the change in time to exhaustion, indicating that the increase of time to exhaustion is not likely due to changes in the respiratory metaboreflex (Appendix 5). The lack of a relationship between MAP and time to exhaustion may suggest that other factors may play a role in this increase in time to exhaustion of the resistive breathing task from pre-training (first time) to post training (second time) in the experimental group. One factor being the familiarization effect. Just a single familiarization session with the certain mode of exercise will change/improve the participant's approach to the exercise seen in time trials (10, 42). However, in the current thesis there was no difference in time to exhaustion throughout the 3 testing days (pre-training, post training and post detraining) in the control group, suggesting that the effect of familiarization is minimal (Fig. 13). Another factor is the improvement of strength. The improvement from strength training improves the anaerobic capacity, which also leads to the improvements of time to exhaustions (56). The improvement of time to exhaustion is likely a result of the familiarization effect as these anaerobic capacity improvements were seen after 8 weeks of training and the changes of muscle fiber type occurs after 6 weeks (47, 56). Additionally, the strength of the MIPs remained elevated after detraining (Fig. 7) however, the time to exhaustion was already trending towards back to pre-training levels (Fig.13), supporting that the strength likely played a minimal role in the time to exhaustion.

6.5 Technical considerations and limitations

There are a few limitations with the usage of the photoelectric plethysmography to measure beat-by beat blood pressure since the measurements are taken during a resistive breathing task, and the large inspiratory pressures generated can affect blood flow to the fingers. Normally, the photoelectric plethysmography requires a minimal automatic calibration after every 70 beats, which in an exercise environment (i.e., resistive breathing task), results in more calibrations during the data collection. Another limitation of photoelectric plethysmography is that any contraction of the hand/fingers with the photoelectric plethysmography can affect the measurements measured at that time. Various precautions were done regarding the photoelectric plethysmography to ensure proper measurements were collected, which include the height correction factor, "zero" the values, reminding the participant to keep their hand relaxed and warming up the participants fingers if needed.

To ensure the respiratory muscles were in a similar metabolic state, the work performed during the resistive breathing task was matched between each trial for each participant (Fig. 9). By matching the work completed, this ensured that the comparison of the measurements (i.e., HR and MAP) are done with the participant experiencing the same magnitude of the stressor. Additionally, the MEPs for all participants were not different between all three visits. This is important for the training group as it shows that the IMT was isolated to the inspiratory muscles and our intervention was not a learning effect. However, the addition of a familiarization visit would be beneficial for future studies as it seems to have a potential effect on the MIPs seen in the controls group improving when comparing pre-training values to post-detraining values (Fig. 7). Another limitation of this study was the were no direct measure of sympathetic activity. However, it is well established that changes in HR and MAP are associated with changes in sympathetic activity

(70).The participants recruited in this study comes from a relatively small homogenous group, thus these findings may not be directly applicable to other populations such as the athletes, elderly population, a clinical population, or females due to sex differences. In this current study, both males and females were recruited, but females had to be on monophasic birth control, which results in their metaboreflex response to be more comparable with the response of males (44). However, females have a blunted respiratory muscle metaboreflex compared to males (63, 73). Therefore, the findings in this study are only applicable to the small percentage of females on monophasic birth control. Only 5 weeks of duration for training and detraining were used in the protocol in this study. A longer protocol would have been more ideal as then we could see how long the strength and attenuation of the metaboreflex will remain after training and if they will be similar. In addition, a metaboreflex test was done for the TA would provide another comparison between the respiratory muscles and the TA. This allows for a more complete comparison as it includes the strength and cardiovascular response from training for each muscle.

6.6 Future directions

One future direction from the results of the current thesis would be investigating a longer detraining period. Based on the result from this thesis, the attenuation of the respiratory muscle metaboreflex persists after 5 weeks of detraining (Fig. 11). However, it is unknown how long it will persist for. It is known that respiratory strength remains elevated up to 9 weeks, but it is unknown if the attenuation of the respiratory muscle metaboreflex will be similar (53). Another future study could investigate the potential maintenance regime required to maintain the attenuation of the respiratory muscle metaboreflex. Both these investigations would help structure training programs for athletes and COPD patients, as this attenuation of the respiratory muscle

metaboreflex would lead to a reduction in the competition of blood flow during exercise (33). Ultimately, other working locomotor muscle could receive more blood flow reducing muscle fatigue.

Another direction would be to investigate the effects of the ventilator on the diaphragm as the diaphragm will atrophy at a higher rate than other skeletal muscles. The finding of the current study suggest that the temporal response should be the same however, with a ventilator all stressors are removed from the diaphragm (i.e., ventilation) whereas in the detraining phase in this study we are only removing the stressor of the training and there is still a consistent stressor the diaphragm experiences from normal ventilation, which may be the reason of the difference in rate of atrophy.

Another future direction could be looking to examine if there are any different responses of the respiratory muscle metaboreflex to another similar muscle's metaboreflex, which would be the TA's metaboreflex based on the reasoning mentioned above. There is some evidence suggesting that blood flow to the respiratory muscle (i.e., diaphragm) is prioritized over other locomotor muscles (58). This comparison would help add to the understanding of this potential phenomenon.

Another idea would be a similar study to this current thesis but introduce the training of the expiratory muscles in addition to the inspiratory muscles. During the resistive breathing task, it would include resistive breathing for expiration and inspiration. It has been shown that expiratory muscle fatigue can exacerbate exercise tolerance and locomotor muscle fatigue (68). It is also known that IMT training results in a decrease locomotor muscle fatigue, likely due to the inspiratory muscle not competing as much for blood flow (15, 33). The addition of expiratory muscle training would demonstrate whether if the training of inspiratory and expiratory would have a proportional additive effect on the attenuation of the respiratory muscle metaboreflex.

7.0 CONCLUSION

In conclusion, 5 weeks of RMT and TA strength training resulted in strength increases and the strength remained elevated after 5 weeks of detraining. As a result, the respiratory muscles and the TA have similar temporal strength responses to training and detraining. Five weeks of RMT also attenuates the respiratory metaboreflex and the attenuation persist after 5 weeks of detraining, which may have other benefits (i.e., reduce the competition of blood with other muscles during exercise).

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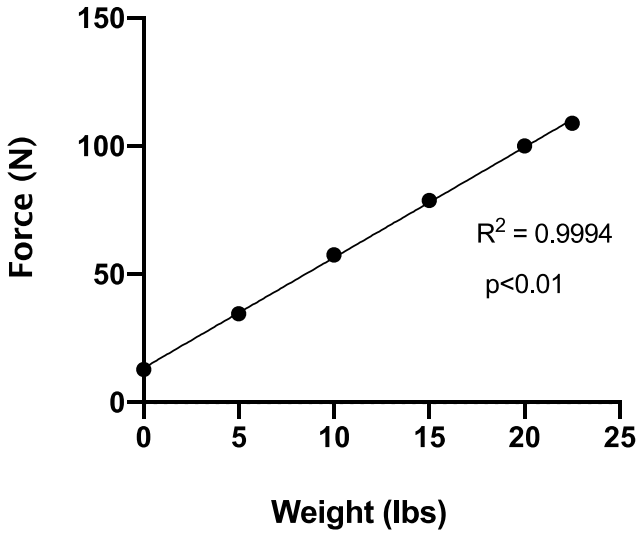
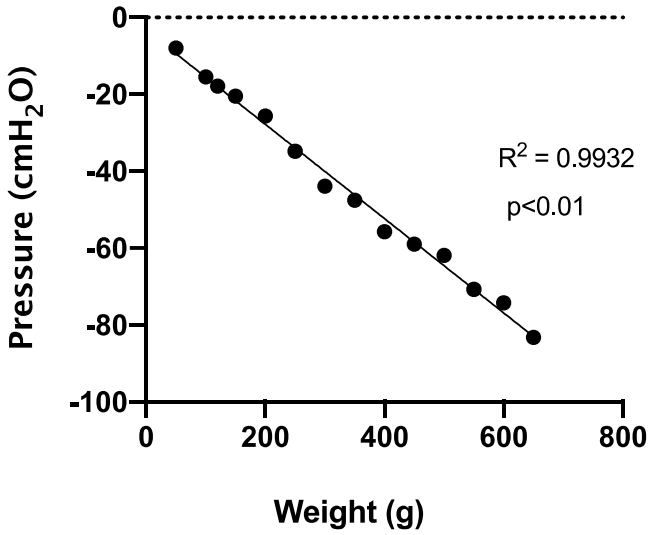
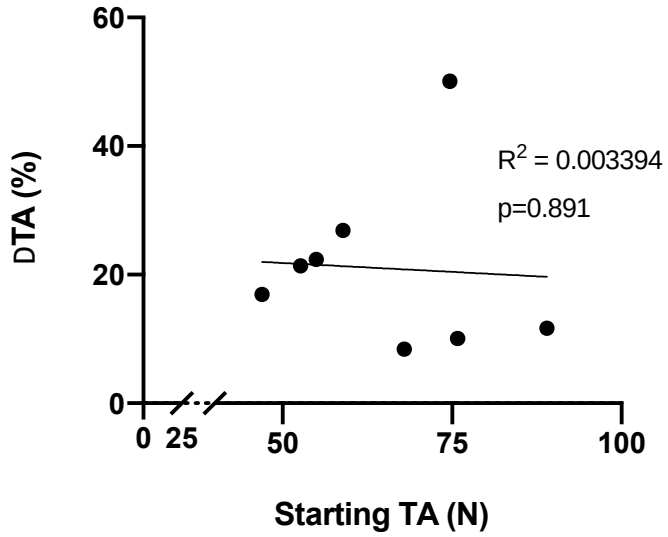
APPENDIX



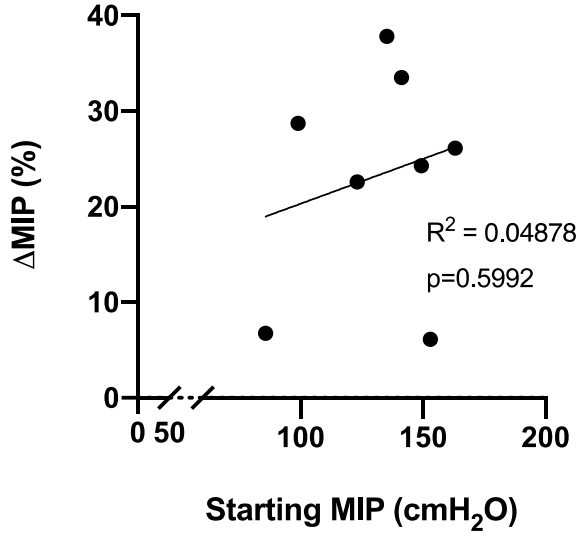
Appendix 1. Relationship between the force generated with various standard weights.



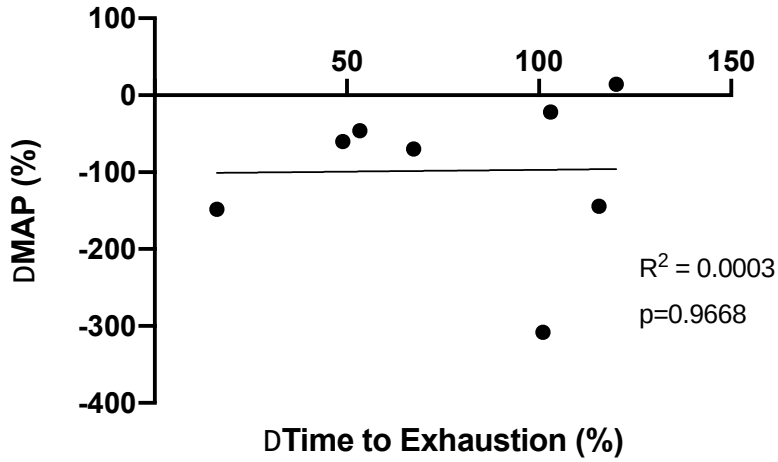
Appendix 2. Relationship between the pressure generated with various standard weights at a fixed volume.



Appendix 3. Relationship between the change in maximal tibialis anterior strength (TA) and starting TA strength from pre-training to post training. No relationship between the percent change in MIP and the starting MIP of the experimental group from pre-training to post training (p=0.891).



Appendix 4. Relationship between the change in maximal inspiratory pressure (MIP) and starting MIP from pre-training to post training. No relationship between the percent change in MIP and the starting MIP of the experimental group from pre-training to post training (p=0.5992).



Appendix 5. Relationship between the change in mean arterial blood pressure and time to exhaustion during the resistive breathing task from pre-training to post training. No relationship between the percent change in arterial blood pressure and the change in time to exhaustion of the experimental group from pre-training to post training (p=0.9668).