EXERCISE-INDUCED DIAPHRAGM FATIGUE WITH SUPERIMPOSED HYPOXIA

by

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The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, a thesis entitled:

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Abstract

The mechanisms and sites that contribute to skeletal muscle fatigue vary depending on the specifications of the task, oxygen delivery, and fibre type composition. Due to anatomic and physiologic differences, men and women can have altered responses to a similar muscular fatiguing stimulus. The aerobic diaphragm muscle may exhibit neural protection under cases of potentially high fatigue, with a higher inhibition at the periphery. Hypoxia exacerbates key factors in diaphragm fatigue development, that may vary on the basis of sex. **PURPOSE:** The purpose of this thesis is to compare diaphragm fatigue between men and women under conditions of high-intensity exercise in normoxia and acute hypoxia. **METHODS:** Twenty healthy participants (n=10 men) came to the lab on three occasions, the first day included pulmonary characterization and a maximal graded exercise test. The final two trial days consisted of randomized exercise in a normoxic or a hypoxic condition (F_IO_2 =0.21, 0.15, respectively). Exercise consisted of cycling at ~85% $\dot{V}O_{2max}$ until exhaustion; diaphragm force production was assessed via cervical magnetic stimulation pre- and post-exercise, and into recovery. **RESULTS:** There were no significant differences in the degree of diaphragm fatigue regardless of sex or F_IO_2. Time-to-failure was significantly shorter in hypoxia for both groups (men: $p = 0.016$, women: $p < 0.05$). A lowered F_IO_2 decreased diaphragm force production up to 60 minutes into recovery for the female diaphragm more so than their male counterparts. **CONCLUSION:** The authors conclude that decreasing F_IO_2 during whole-body cycling exercise has little effect on the degree of diaphragm fatigue achieved. This result was found in both men and women. However, women had impaired recovery during hypoxia, whereas men have a similar recovery pattern in both conditions.

Lay Summary

Skeletal muscle fatigue is an inability for muscles to produce enough force for a given exercise, and these inhibitions can occur at many points between the brain and the muscle. The diaphragm is the main muscle of inspiration, and its loss of force production capability can have whole-body effects. Depending on the factors including the type of task, amount of oxygen delivered, the physiologic makeup of the muscle of interest, fatigue can develop differently in men and women. The purpose of this thesis is to compare diaphragm muscle fatigue between men and women, and how changing inspired oxygen affects it. The authors found that the amount of diaphragm fatigue is similar between both sexes, in both a lowered- and normal-oxygen exercise condition. Despite this, a lowered inspired oxygen decreased exercise time by ~1/3 in both groups.

Preface

This research study was designed by myself, Paige A. Reinhard, with the help of my committee, (Dr. Bill Sheel, Dr. Mike Koehle, and Dr. Jordan Guenette), and members of the Health and Integrative Physiology Lab at the University of British Columbia. The scheduling, testing, analysis, and writing was performed by myself. Dr. Bruno Archiza, Jenna Benbaruj and various other members of the lab assisted me greatly in the testing protocols. Interpretation was completed by myself with assistance from the Health and Integrative Physiology Lab students. All methods executed in this thesis was approved by The University of British Columbia's Research Ethics Board (H18-02674).

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List of Abbreviations

ANOVA Analysis of Variance

B*f* Breathing Frequency

BPNS Bilateral Phrenic Nerve Stimulation

CMAP Compound Muscle Action Potential

CMS Cervical Magnetic Stimulation

CNS Central Nervous System

CV Coefficient of Variation

DLCO Diffusion Capacity of the Lung

EELV End Expiratory Lung Volume

EMG_{SCM} Electromyography of the Sternocleidomastoid

EMG_{VL} Electromyography of the Vastus Lateralis

$FEV_{1.0}$ Fraction of Expired Volume in 1-second

F_IO_2 Fraction of Inspired Oxygen

FRC Functional Residual Capacity

FVC Full Vital Capacity

HFF High Frequency Fatigue

IC Inspiratory Capacity (Maneuver)

LFF Low Frequency Fatigue

MIV Maximal Isocapnic Ventilation

MVC Maximal Voluntary Contraction

P_{eso} Esophageal Pressure

P_{di} Diaphragm Pressure

$P_{di,tw}$ Diaphragm Twitch Pressure

P_{ga} Gastric Pressure

P_i Inorganic Phosphate

PAV Proportional Assist Ventilator

PET_{CO2} Pressure of End-Tidal CO_2

PCO_2 Partial Pressure of CO_2

PO_2 Partial Pressure of O_2

PTP Pressure Time Product

PTP_{eso} Pressure Time Product of Esophagus

PTP_{di} Pressure Time Product of Diaphragm

PEF Peak Expiratory Flow

RER Respiratory Exchange Ratio

Q Cardiac Output

RMS Root Mean Squared

RMS_{scm} Root Mean Squared of Sternocleidomastoid

RMS_{vl} Root Mean Square of Vastus Lateralis

S_pO_2 Peripheral Oxygen Saturation

TLC Total Lung Capacity

TTE Time to Exhaustion

V_E Minute Ventilation

V_T Tidal Volume

VCO_2 Volume of CO_2 Output

V ̇O2 Volume of O_2 Consumed

$\dot{\mathbf{V}}\mathbf{O}_{\mathbf{2max}}$ Maximal Volume of O_2 Consumed

$\mathbf{W}\boldsymbol{b}$ Work of Breathing

$\mathbf{W}\boldsymbol{b}_{\mathbf{I,res}}$ Inspiratory Resistive Work of Breathing

$\mathbf{WR}_{\mathbf{max}}$ Maximal Work Rate

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The support and encouragement of Dr. Sheel is unparalleled to any other advisory role, and I express my deepest gratitude for his patience with me along this wild ride. He believed in me when I couldn't feel it myself and gave me the confidence to persevere. The skills I've learned from him and all of the other students in the Health and Integrative Physiology have developed a different person than who entered the program. Each person has helped shaped this thesis and the researcher I aspire to be.

For anyone who has stopped to listen and engage, I am grateful for every encounter and every piece of advice. I would not be here without the help of Bruno Archiza for aiding me and providing a voice of reason. Jenna Benbaruj stepped up during the final push and was an integral part to testing.

Lastly, I'd like to acknowledge NSERC for providing funding for this thesis and the rest of my committee for the continued support and guidance, helping me to navigate a complex topic.

Dedication

To my chosen family. You know who you are.

Chapter 1: Introduction

Skeletal muscle fatigue is defined as a state in which there is a loss in the capacity for developing force and/or velocity of a muscle, resulting from muscle activity under load and which is reversible by rest (NHLBI, 1990). There are several sites along the neuromuscular chain where a decrease in force production could occur. Fittz (1994) has described a model of fatigue based on the site(s) of occurrence - either central or peripheral. Central fatigue can be defined as fatigue occurring at any point along the neural network from excitatory stimuli to higher brain centers to neuromuscular transmission. Peripheral fatigue can be described as an impairment of the contractile properties of the muscle, distal to the neuromuscular junction (from the excitability of the sarcolemma down the neuromuscular chain to the substrate availability for muscular contraction).

It has been reported that women may have a resistance to skeletal muscle fatigue, primarily during dynamic contractions of the small muscle mass, when participants were matched for grip strength, and working at the same relative intensity during the test (Hicks, 2001). A number of key differences have been identified between the sexes with respect to skeletal muscle fatigue. For example, males tend to rely more on glycolysis, whereas females rely more on oxidative pathways for ATP generation (Tarnopolsky, 1999). The differences in substrate utilization can lead to a higher metabolite response in men, decreasing muscular force production. Whole muscle fibre type composition may also explain sex-based differences in muscular fatigue (Hicks, 2001). Men tend to have a larger percentage of type II muscle fibres which are prone to fatigue and have a higher reliance of glycolysis as a substrate for force production (Hunter, 2014). Lastly, men and women generally differ with respect to skeletal muscle mass, with men having higher volume of muscle. During sustained muscular

contractions, a higher volume of muscle would occlude blood flow to the working muscle to a greater degree (Hicks, 2001). Hunter (2009) matched men and women for maximal resting force output of elbow flexors and measured fatigue by time to failure. During an isometric exercise, men and women demonstrated similar time to task failure. However, during dynamic contractions of the same intensity, women had a significantly longer time to task failure relative to men. These findings suggest that when corrected for muscle mass (i.e., estimated by maximal force output), women are resistant to fatigue during dynamic contractions and skeletal muscle fatigue will vary depending on the task performed.

The diaphragm, as the major muscle of inspiration, is primarily comprised of 55% aerobic type I muscle fibres that are capable of consistent rhythmic contraction without fatigue under resting conditions (Polla et al., 2004). During high-intensity dynamic exercise, there is a substantial increase in ventilation, with an accompanying increase in the work of breathing (W*b*) (Milic-Emili & Orzalesi, 1998). If heavy exercise (> 85% maximal workload) is performed to volitional failure, diaphragm fatigue has been shown to occur in healthy males (Johnson et al., 1993). To address the possibility of sex-differences in exercise-induced diaphragm fatigue, Guenette et al. (2010) evaluated the diaphragmatic response to high-intensity, constant work rate cycling exercise. The findings of Guenette et al. (2010) support women having an increased resistance to diaphragm fatigue, similar to that seen preciously in peripheral muscles (Hicks 2001; Hill et al., 2018; Welch et al., 2018).

Lowering the inspired O_2 fraction can hasten the development of both respiratory and locomotor muscle fatigue (Fregosi et al., 1986; Bigland-Ritchie & Vollestad, 1988). For example, Babcock et al. (1995a) investigated exercise-induced diaphragm fatigue with acute hypoxia. Compared to normoxic conditions, exercise time to exhaustion was reduced by 37%

with hypoxia and yet the recovery time increased despite a similar degree of diaphragm fatigue. The participants that Babcock et al. (1995) studied were eight males and three females and sex-based comparisons were not made. In conclusion, based on the available literature, women appear to be more resistant to exercise-induced diaphragm fatigue in normoxia relative to men. Hypoxia exacerbates exercise-induced diaphragm fatigue in men. It is unknown if this is the case in women. This thesis examines the inter-relationships between biological sex, hypoxia and exercise-induced diaphragm fatigue.

1.1 REVIEW OF LITERATURE

Skeletal muscle fatigue is defined as a state in which there is a loss in the capacity for developing force and/or velocity of a muscle, resulting from muscle activity under load and which is reversible by rest (NHLBI, 1990). Fatigue of skeletal muscles is seen in both dynamic and static contractions. For example, fatigue can be assessed using small mass exercise such as a hand ergometer which can measure force output with either rhythmic muscular contractions or isometric holds, targeting the flexor digitorum superficialis. After either type of activity, fatigue is seen in the muscle of interest by a time to failure trial at a set contractions-per-minute or measuring force output at the beginning of exercise and comparing to end-exercise values, respectively (Clark, 1962).

Fatigue and task-failure are not synonymous; fatigue implies that the muscle is unable to withstand the imposed load and is an impairment in the maximal force generating capacity of a muscle (Hunter, Duchateau, & Enoka, 2004). Fatigue should be considered on a continuum, rather than a threshold approach. When measuring fatigue, it can only be correctly quantified by serial measurements over time indicating a fall in the contractile properties of the muscle, as compared to a previous measurement (ATS Respiratory Muscle Fatigue, 2002). Task failure

during muscular contraction is due to overpowering inhibitory supraspinal neural signals that prevent further muscular contractions at the desired load and can vary with the type of intervention performed (Hunter, Duchateau, & Enoka, 2004). The causal mechanisms of fatigue are still unknown, but based on previous studies (Enoka & Stuart, 1992; Enoka, 1995; Fitts, 1994; Gandevia, Allen, & McKenzie, 1995; Westerblad, Allen, & Lannergren, 2002) a framework of the key factors including motivation, circulating neurotransmitters, group III/IV afferents, excitation-contraction couple inhibition, acidosis, and substrate depletion that when combined, induce skeletal muscle fatigue.

The purpose of this review is to summarize the current state of knowledge on sex-based differences in respiratory muscle fatigue in healthy humans. How hypoxia impacts the development of fatigue and if sex-differences are present will also be considered. For the purposes of this review, those studies that included laboratory-based manipulations of inspired O_2 to study fatigue were considered. High altitude studies were excluded due to potential psychological/motivational confounds.

1.1.1 Classification of Fatigue

Skeletal muscles fatigue by different mechanisms and at different rates based on the muscle fibre composition, the work rate percentage at which an individual is working, and the available oxygen (Enoka & Stuart, 1992). Based on the operational definition of fatigue, it is understood that adequate recovery time post-intervention is an important factor in differentiating fatigue from weakness or injury. Recovery with rest separates muscular fatigue from muscular weakness/injury, where muscular function will not return to baseline levels with adequate rest based on the exercise prescription (NHLBI, 1990). Fatigue can elicit a change at all levels of the muscle or neural network, and the potential sites for fatigue include: (a) excitatory input to

higher motor centres, (b) excitatory drive to lower motor neurons, (c) motor neuron excitability, (d) neuromuscular transmission, (e) sarcolemma excitability, (f) excitation-contraction coupling, (g) contractile mechanism, and (h) metabolic energy supply (Fittz, 1994). The list of potential sites of fatigue is divided into central (a-d) and peripheral fatigue (e-h) and each is discussed below. Before discussing causal mechanisms of fatigue at the individual sites, a short introduction on measurement of fatigue is required to understand how sites can be identified by how they are measured.

1.1.1.1 Measurement of Fatigue

There are many ways that fatigue can be both induced and quantified, creating a challenge for direct comparison between studies which include nerve stimulation (electrical or magnetic) or measures of volitional effort (Vollestad, 1997). Electrical stimulation has the ability to stimulate the muscle of interest supramaximally but takes longer to find the correct position when stimulating the muscle. When performing post-intervention stimulations, increased time between the cessation of the intervention and the initiation of stimulations is a limitation, allowing the muscle of interest to begin recovery and results may be underestimated (Johnson et al., 1993). Similowski et al. (1989), compared the standard electrical stimulation with magnetic stimulation at the cervical vertebrae, termed cervical magnetic stimulation (CMS) of the phrenic nerve, and found the results were highly reproducible by both methods. CMS as compared to electric stimulation: a) less cutaneously painful for participants and b) does not require multiple stimulation to ensure the trial is optimal (Wragg et al., 1994). A limitation with magnetic stimulation is that while the coil depolarizes, a large area and there is a high probability of stimulating the nerve of interest, it will also stimulate the surrounding structures. Additionally, researchers may not be able to stimulate the muscle of interest supramaximally when using

magnetic stimulation, a maneuver often used when examining muscular fatigue to ensure that changes in the force output of a muscle are due to peripheral fatigue and not to central limitations (i.e. stimulation intensity) (Similowski et al., 1989).

A less objective measure of muscular force production is performing maximal voluntary contractions before and after an intervention and comparing force output. Using voluntary contractions is limited by the inability to differentiate between neuromuscular impairments and motivation to perform (ATS/ERS Respiratory Muscle Testing, 2002).

1.1.1.2 Central Fatigue

Central fatigue is seen at the higher levels of motor input and transmission, activation of lower motoneurons, initiation of the action potential and encompassing up to the propagation of the signal to the neuromuscular junction (Vollestad, 1997). Central fatigue is also based on subjective volitional effort to a maximal task and creating/propagating action potentials to fulfill the task (Gandevia, Allen, & McKenzie, 1995). To measure central fatigue a common method is to apply an external stimulus to a muscle during a maximal voluntary contraction (MVC) – termed interpolated twitches. If, on application of the stimulus, the twitch force output of the muscle of interest increases, there is an inhibitory effect from a fatiguing process higher than the peripherally stimulated nerve (Hunter, Duchateau & Enoka, 2004).

1.1.1.2.(a) Excitatory Input to Higher Centres

Using transcranial magnetic stimulation, there is direct evidence of a lowered central motor drive after fatiguing exercise. Specifically, the theory behind the lowered central motor drive is a depletion of key neurotransmitters such as acetylcholine, and an accumulation of neurotransmitters such as serotonin (known for inducing lethargic behaviors) that can accentuate fatigue (Davis & Bailey, 1997). There is some evidence supporting the Serotonin Hypothesis of

Central Fatigue, which illustrates the idea of increased concentrations of circulating tryptophan acting as a precursor for serotonin (and other key neurotransmitters) produced in the brain but that cannot cross the blood brain barrier. As brain serotonergic activity increases, it may lead to decreased drive to perform and increased lethargy (Meeusen et al., 2012).

Motivation is a factor at the higher cortical levels and includes the ability to withstand pain or discomfort. Bigland-Ritchie et al. (1986) required subjects to perform voluntary timed submaximal rhythmic muscular contractions. During the rest interval, single or train stimulations were sent to the muscle, and the decreases in submaximal voluntary contractions were compared with the maximal contraction via stimulation. Sometimes voluntary force output and stimulation output paralleled each other and other times, voluntary force output decreased before stimulation output decreased, labelled low motivation.

1.1.1.2.(b) Excitatory Drive to Lower Neurons

Bigland-Ritchie et al., (1992) show support for motor neuron firing rates and muscular contraction speed being independent of each other, rather they theorized that type III/IV free nerve endings are linked with motor neuron firing rate, instead of contraction speed. There is support for the afferent nerve endings having a negative impact on interneurons in the spinal cord with an increased sensitivity to muscle metabolites produced during fatiguing contractions. Group III/IV afferent signals may also have an impact on the excitatory input to higher centres (Davis & Bailey, 1997).

1.1.1.2.(c) Motor Neuron Excitability

As muscular contractions increase force output, electrical activity of motor pathways in the brain are increased, as compared to when the muscle is relaxed – termed motor evoked potentials (Legatt 2014). The motor evoked potentials continue to increase during exercise until

firing of the muscle spindle mechanoreceptor afferents declines (due to muscular contractions surpassing a fatiguing threshold) and decreases the rate of motor firing rate (Taylor et al., 1996).

Motor unit firing rate may also be affected by the descending drive sending conflicting inhibitory and excitatory signals, the relative contributions of each of the signals depending on the task performed (Hunter, Duchateau & Enoka, 2004). The researchers examined the electromyographic (EMG) output, heart rate, and mean arterial pressure between two fatiguing tasks – requiring the same level of torque and muscles groups used; one task required maintaining an objects position in space (position task) and one task required the same net force but to just exert the force against an immovable object (force task). The position task elicited a higher heart rate and mean arterial pressure as compared to the force task, and had a similar %EMG activation as a percent of MVC, but also had an increased degree of fatigue, which the researchers conclude is from the spinal cord receiving more excitatory and inhibitory signals so that the EMG output not significantly difference from once another (Hunter, Duchateau & Enoka, 2004).

1.1.1.2.(d) Neuromuscular Transmission

As mentioned, a decrease in cholergenic activity during fatiguing exercise slows synaptic transmission of motor evoked potentials, resulting in a slowing of the muscular transmission across the neuromuscular junction (Davis & Bailey, 1997). Not only does the signal need to reach the muscle, the central command must be able to send an appropriate amount of motor output as to sustain the contraction, and to economize the muscle activation. Based on the force needed for contraction, varying amounts of motor units may be recruited. The behaviour of the central nervous system to enlist the motor units in an efficient manner has a large effect on fatigue of those units, and ultimately the whole muscle (Enoka, 1995).

1.1.1.3 Peripheral Fatigue

Peripheral fatigue is defined as impairment of contractile force or velocity distal to the neuromuscular junction and inhibition of the excitation-contraction coupling process (ATS/ERS Respiratory Muscle Testing, 2002). The relative contributions of both central and peripheral fatigue are dependent on the task performed, the available oxygen, muscle fibre composition, and many other factors (Enoka & Stuart, 1992).

1.1.1.3.(a) Sarcolemma Excitability

The sarcolemma depolarizes from action potentials at the neuromuscular junction, and is transmitted rapidly along the muscle membrane, and at a lower speed through the T-tubules. The ability of the sarcolemma to be electrically excited is due to opening of sequential voltage gates Na^+ channels, propagating the action potential along the membrane (Kent-Braun, Fitts, and Christie, 2012). When muscles contract rhythmically over a period of time, the chronic depolarization interferes with action potential propagation because of a slowing of the Na^+ channels and an inactivation of the T-tubule voltage sensing protein. The previous factors induce a net K^+ efflux, lowering the resting membrane potential, which in turn creates larger threshold needed for depolarization and will decrease the compound muscle action potential (CMAP) wave produced from stimulation (Allen et al., 2008). A previously proposed theory of fatigue hypothesizes a decrease in intracellular K^+ concentration and increases in Na^+ and $Ca^{2+,}$ and water which contributes to the idea that the Na^+/K^+ pump in unable to withstand the challenge posed with exercise to continue to maintain the electrochemical gradient with a rapidly changing environment. The Na^+/K^+ pump inefficiency theory has only been substantiated with in vitro research, in consequently not generally accepted for in vivo studies (Fittz, 1994).

1.1.1.3.(b) Excitation-Contraction Coupling

Following the excitation of the sarcolemma, Ca^{2+} is released, which binds to troponin, causing the filaments to move to a favourable position for cross-bridge formation (Vollestad, 1997). Increases in H^+ and inorganic phosphate (P_i) decrease Ca^{2+} sensitivity in the sarcolemma, both of which are bi-products of high-force muscular contractions and with low concentrations of Ca^{2+}, the effects of increased H^+ and P_i concentrations are exacerbated (Kent-Braun, Fitts, & Christie, 2012). Inorganic phosphate and free Ca^{2+} in the sarcoplasmic reticulum combine to create a precipitate, limiting the free Ca^{2+} available for release, with evidence for a precipitate forming and not for a leak in the sarcoplasmic reticulum (Allen et al., 2008). With a decrease of Ca^{2+} in the sarcolemma, the proteins troponin and tropomyosin are not activated, both of which regulate muscular contraction, and a decrease in force output and shortening velocity is seen.

1.1.1.3.(c) Contractile Mechanism

Recent studies have focused on P_i as a potential contributing factor to fatigue. The evidence suggests that increased myoplasmic P_i may have an effect on cross-bridge formation, aids in a potential decrease of myofibrillar sensitivity to Ca^{2+}, and direct effect of Ca^{2+} influx into the sarcoplasmic reticulum as mentioned previously (Westerblad, Allen, & Lannergren, 2002). As the power stroke is one of the limiting factors in the shortening velocity of a muscle and is directly impacted by the hydrolysis of ATP by myosin, affected by both [H^+] and [P_i]. The exact mechanism is unknown. In addition to the breakdown and energy release of ATP causing an increased [P_i], as phosphocreatine is broken down for rephosphoylation of adenosine-diphosphate (ADP), restoring ATP (Fittz, 1994).

1.1.1.3.(d) Metabolic Energy Supply

Cross-bridge formation is one of the rate-limiting steps in muscular contraction, and the other is the dissociation of ADP which primarily contributes to muscle shortening velocity. With high ADP concentrations, there is an increased affinity of the filaments for ADP, and muscle shortening velocity decreases substantially (Kent-Braun, Fitts, & Christie, 2012).

1.1.1.4 Fatigue Frequencies

Peripheral muscular fatigue can further be broken down into low-frequency fatigue (LFF) and high-frequency fatigue (HFF). Metzger & Fitz 1986, defined HFF as an impairment of action potential transmissions, potentially due to (1) and increased extracellular K^+ concentration, or (2) an extracellular deficit of Na^+ and is detected at high stimulation frequencies. LFF can be described as an excitation-contraction inequality, resulting in a loss of force for a longer period of time, and the impairment in force produced was detected at lower frequencies (Jones, 1996). Along with the frequencies at which fatigue is detected, HFF and LFF are separated based on the speed of recovery to initial force production. HFF recovers more quickly (approximately one-minute) and LFF recovers more slowly (approximately forty-five minutes) (Fittz, 1994; Metzger & Fittz, 1987). Generally, LFF is used when studying humans due to its' feasibility and its ability to be measured on a magnetic stimulator, whereas HFF requires an electrical stimulator.

1.1.2 Anatomy and Physiology of the Respiratory System

1.1.2.1 Anatomy and Mechanics

The major muscle of inspiration is the diaphragm, originating at the inferior edge of the ribs and the sternum and attaching to the central tendon, flattens upon contraction and assumes a dome shaped structure upon relaxation (Seeley, Stephens, & Tate, 2003). During quiet breathing, inspiration requires active contraction of the respiratory muscles whereas, expiration is a passive.

As ventilation increases (ie. exercise, hypoxia), expiration becomes active and requires abdominal contraction and the intercostal muscles to increase tidal volume (V_T) and regulate end-expiratory lung volume (EELV).

During inspiration, the flattening of the diaphragm increases the thoracic space by changing the orientation of the abdominal cavity downwards and forwards. Along with diaphragm contraction, the rib cage is forced upward by contraction of the external intercostals and scalenes (Shier, Butler, & Lewis, 1996). The coordination of the inspiratory muscles increases the volume of the thoracic cavity, creating a negative pressure system and air is forced in to fill the void. Intrapleural pressures range from -2 (expiration) to -6 (inspiration) cm_{H2O} during quiet breathing. Pressures fall upon inspiration because of the increase in volume of the thorax and atmospheric air is forced in to fill it. Due to the elastic properties of the chest wall and the lung recoil tendencies, a slight negative pressure is maintained intrathoracically to balance these two opposing structures (West, 2012). The small range of pleural pressures create a large change in the volume of the lung, making the organ compliant at low-moderate lung volumes. At high lung volumes, compliance is low as the lung is already stretched, and changing pressures won't elicit any more change in volume. Large changes in the pressures (and therefore the volume) of the lung, which are seen during exercise, can compromise the length-tension muscular relationship of the diaphragm for maximal force production, putting it in a compromising position and more susceptible to fatigue.

1.1.2.2 Cardio-Respiratory Changes During Exercise

Dynamic whole-body exercise causes: (a) increases in oxygen utilization of the working muscles, (b) a rise in mixed venous CO_2 content, (c) ventilation increases as to match the cellular respiration of the muscular requirements for O_2, but to minimize work of breathing (W*b*), and (d)

an increased blood flow to both locomotor and respiratory working muscles (Sheel, MacNutt, & Querido, 2010). Quickly after whole-body aerobic exercise begins, minute ventilation increases rapidly, and at maximal work rates can range up to 20-fold from resting values. The two main variables responsible for increasing minute ventilation (V_E) are: V_T and frequency of breathing (B*f*). Tidal volume is increased first, to decrease the dead space fraction, and if the Respiratory Centre of the brain is still unsatisfied by the P_{CO2} and P_{O2} levels of the blood, fb will increase. Respiratory rate increases to prevent V_T growing excessively and reducing compliance (West, 2012). Cardiac output (Q) also increases up to 6-fold to match the demand of the working muscles for oxygen. Strenuous whole-body aerobic exercise is associated with both peripheral muscle fatigue and respiratory muscle fatigue.

1.1.2.3 Work of Breathing

In response to increasing minute ventilation by increasing B*f* and V_T, W*b* also increases. The W*b* is a measure of mechanical efficiency of the respiratory system and provides an estimate of the energetic cost of breathing at varying ventilations (Milic-Emili & Orzalesi, 1998). Mechanical work can be calculated as:

$$W = F\Delta d$$

Equation 1: W = mechanical work, F = force, d = distance

with no work being performed without a change in distance. When referring to the respiratory system, force is estimated by pressure generated by the respiratory muscles and distance is estimated by changes in lung volume, affecting the length at which the muscle fibres contract.

The W*b* can be separated into three areas of work: inspiratory-resistive ($Wb_{I,res}$), inspiratory-elastic, and total expiratory (expiratory work can be further subdivided into resistive and elastic, but not for the purpose of this document). Under resting conditions expiration is

passive and Wb_E is low, and only when the abdominals are activated under forceful exhalation will the Wb_E increase. Resistive work comes from efforts the respiratory system has to expend to overcome the resistance from airway diameter, demonstrated by Poiseuille's Law:

$$Q = \frac{\pi \mathrm{Pr}^4}{8\eta l}$$

Eq 1. Q = flow, P = driving pressure, r = radius of vessel, n = viscosity of liquid/gas flowing, l = length of the vessel

where radius of the airway is the largest contributing factor to resistance. The air around the walls has a lower flow rate than the air closer to the middle of the airway (Otis, 1954). Elastic work of breathing is the work required to overcome the elastic forces of the lung with a tendency to collapse inwards, to inflate it fully (Guenette et al., 2009).

1.1.3 Respiratory Muscle Fatigue Models

The diaphragm is capable of functioning without fatigue while contracting with low force output intermittently throughout an individual's entire life, but fatigue will develop when the muscle is stressed beyond a threshold. A study by Archiza et al. (2017) demonstrated exercise-induced diaphragm fatigue development in men with whole-body exercise after as little as six minutes, increasing until volitional failure. The diaphragm is more likely to exhibit fatigue with work rates over 85% of an individual's maximal oxygen consumption ($\dot{V}O_{2max}$) (Johnson et al., 1993). As minute ventilation increases (thus increasing Wb), the oxygen cost of breathing increases curvilinearly, increasing up to 15% of Q in highly trained male subjects (Aaron et al., 1992). Later, when represented as a fraction of whole-body $\dot{V}O_2$, the oxygen cost of breathing in women was higher than in men (Dominelli et al., 2014). The repercussions of a high cost of breathing include decreased exercise performance, decreased peripheral blood flow, and increasing inhibition feedback to the CNS (Aaron et al., 1992; Dominelli et al., 2017).

Differentiating between central and peripheral diaphragm fatigue is achieved by delivering phrenic nerve stimulations superimposed on MVCs and observing if there is an increase in the output of the diaphragm. Bellemare & Bigland-Ritchie (1987) used interpolated twitches to distinguish between central and peripheral diaphragm fatigue. Using a set relative intensity of diaphragm force output, MVC's and bilateral phrenic nerve stimulation (BPNS) performed during the test, the researchers were able to see a decrease in the subjects' voluntary maximal diaphragm output, compared to that of an external stimulus. They concluded that diaphragm fatigue after an isolated, small muscle fatiguing task is primarily limited by voluntary motor drive (a component of central fatigue) when attempting to maximally contract the diaphragm at task failure (Bellemare & Bigland-Ritchie, 1987).

Diaphragm fatigue can be assessed by inserting two balloon catheters, one that is placed in the esophagus (P_{eso}) and one that is placed in the stomach (P_{ga}). The balloons are filled with a known amount of air to measure the pressures at the respective sites (P_{eso} and P_{ga}). P_{eso} is used as an estimate of pleural pressure due to the relatively close physical locations of the structures and the flaccid nature of the esophagus; it will mimic pressures by the nearby lungs (Milic-Emili, J. et al., 1964). The difference between the two pressures is an estimate of the pressure generated across the diaphragm, or transdiaphragmatic pressure (P_{di}). Using CMS, and stimulating the phrenic nerve, electrical activity of the muscle is measured by EMG surface electrodes and a CMAP is created to ensure maximal stimulation of the diaphragm, and corresponds with a spike in P_{di}, measured by the balloons. Fatigue is measured based on how P_{di} changes with an intervention (ATS/ERS Respiratory Muscle Testing, 2002).

Respiratory muscle fatigue can be partitioned into inspiratory and expiratory muscle components. Expiratory muscle fatigue occurs primarily in the abdominals as they increase

expiratory airflow and increase V_T by reducing EELV. To measure expiratory muscle fatigue, the same magnetic stimulation protocol can be used with stimulating between T_8 and T_{11} to target the thoracoabdominal nerves, and to measure the electrical activity of the rectus abdominus and external oblique by EMG. Fatigue was measured by changes in P_{ga}. (Taylor at al., 2006). But, based on a comparison of Johnson et al. (1993) and Taylor et al. (2006) both expiratory and inspiratory muscles fatigue at similar rates and by comparable deviations from baseline P_{di} values (using CMS) after intensive whole-body exercise.

The general principle when conducting fatigue studies is: the higher the load and timing of contraction, the shorter the time to exhaustion. Researchers must also clearly define what classifies as task failure vs. muscular fatigue. McKenzie et al. (1997) evaluated diaphragm fatigue during various inspiratory loads while at rest to target the respiratory muscles. Using the same relative work (as a percent of maximal inspiratory pressure), subjects were asked to continue at the set inspiratory resistance until failure. The authors found little evidence of diaphragm fatigue even at task failure, they cite CO_2 retention rather than fatigue, was the limiting factor in performance. Later, when Rohrbach et al. (2003) investigated diaphragmatic fatigue via a similar technique (threshold loading) and accounted for the CO_2 retention, P_{di} was found to be significantly lower post volitional failure. The authors found using this method, P_{eso} was significantly lower after the intervention, but P_{ga} was not, while still an indicator of diaphragm fatigue, this method may have not targeted the diaphragm as specifically as the researchers hypothesized. From previous work, the partial pressure of end-tidal CO_2 (PET_{CO2}) was monitored to make sure the subject was not retaining CO_2, and arterial saturation was maintained, both stated as reasons for potential task failure from McKenzie et al., (1997).

Threshold loaded breathing is one method of fatiguing the respiratory system but, it does not follow spontaneous breathing patterns. Generally, threshold breathing has a low frequency of breathing (~15 breaths per minute), and a high load to each breath, causing fatigue whereas, spontaneous breathing during exercise creates fatigue with high frequency of breathing (~60 breaths per minute) and low load to each breath, as seen with hyperpnea (Verges, Bachasson, & Wuyam, 2010). There has been support shown by Hershenson et al. (1989) that inspiratory loaded breathing doesn't target the diaphragm for fatigue but shares the load more evenly among the inspiratory muscles, and maximal ventilation tests targets the diaphragm more selectively. Maximal isocapnic ventilation (MIV) has been shown to produce LFF from the diaphragm from Hamnegard et al. (1996) when they asked subjects to attempt a trial of holding MIV for two minutes and used CMS to assess fatigue of the diaphragm. Ventilation declined substantially immediately (deviating from target ventilation) but plateaued below target MIV around one minute into the trial, and a significant difference between pre- and post-hyperpnea twitches was still achieved [pre: 32.9 (6.9) to post: 25.2 (5.9) cmH_20]. The quick fall in ventilation during the trial was correlated with a fall in the pressure-time-product of both the inspiratory and expiratory muscles, leading to LFF of the diaphragm.

Voluntary hyperpnea (as seen in the MIV trials) achieves the high frequency of breathing and low load of spontaneous breathing seen during exercise but does not simulate the peripheral muscle fatigue and blood flow changes that occur during maximal exercise. A key factor in the development of fatigue is blood flow availability for the working muscles; when respiratory muscles and locomotor muscles are both working at maximal capacity, there is competition for the finite cardiac output because of the metaboreflex (Dempsey et al., 2006). Babcock et al., (1995b) directly compared the force output of the diaphragm during exercise and used a

subsequent day to mimic the V_T, B*f*, average P_{di}, and duty cycle to apply the same load during both trials, just without the supplemental locomotor muscle work. Pre- and post- CMS was used as an assessment of diaphragm fatigue for both trials and following the time to exhaustion (TTE) trial, P_{di} dropped an average of 26% (2.9%) and during the matched hyperpnea trial there was no significant change in P_{di} post trial. Johnson et al. (1993) had subjects perform an initial maximal exercise test and on separate occasions had the participants perform time to exhaustion trials at both 80-85% and 90-95% of maximal oxygen uptake ($\dot{V}O_{2max}$), with pre- and post-exercise CMS twitches. At 95% $\dot{V}O_{2max}$, the average P_{di} was 20% (3%) less than baseline values. and at 85% $\dot{V}O_{2max}$, there was an average decrease in P_{di} by 15% (5%), indicating fatigue, as compared to baseline values.

Locomotor muscle fatigue is not the only type of fatigue that can inhibit exercise performance; respiratory muscle fatigue leads to changes in peripheral vascular conductance and blood flow to working muscles (Dempsey et al., 2006). The blood flow redistribution theory termed 'metaboreflex' is due to high work of the respiratory muscles and type III (metaboreceptors) and IV (mechanoreceptors) afferents detect changes from the respiratory muscles, along with other metabolic by-products from muscular contractions. These signals are sent to the central nervous system and a sympathetic outflow to vasoconstrict at the periphery is emitted (Dempsey et al., 2006). To increase blood flow to the fatiguing respiratory muscles, it is theorized that locomotor muscle blood flow is decreased and therefore peripheral fatigue and perception of effort increase (Romer & Polkey, 2007; Harms et al., 1997; Harms et al., 1998). Consequences of changing W*b* (with direct effects on blood flow) is also demonstrated when subjects perform exercise trials on a proportional assist ventilatory (PAV) which alleviated their W*b*, respiratory muscle blood flow was decreased and limb blood flow increases. During trials

where W*b* was increased, the opposite holds true (Dominelli et al., 2017). There are 3 main methods of eliciting fatigue from the respiratory muscles: threshold loading, voluntary or induced hyperpnea, or whole-body exercise.

As mentioned, there is a finite Q available during maximal exercise which causes competition between the working muscles and the respiratory muscles for blood flow, cause vasoconstriction and potentially small vessel blunting at the periphery (Sheel, Boushel, & Dempsey, 2018). Due to the metaboreflex, the human body inherently places the respiratory muscles in higher priority than locomotor muscles and during high ventilatory work will redirect blood flow from the peripheral muscles to the respiratory muscles (up to 16% of total Q) (Dempsey et al., 2006). Unloading the respiratory muscles using a proportional assist ventilator, absolute Q can decrease due to both a decrease in intrathoracic pressure (a key component in W*b*) and metabolic requirements of the respiratory muscles (Harms et al., 1998). Diaphragm fatigue is dependent on two main variables: blood flow and W*b*. The variables for diaphragm fatigue are supported by Babcock et al. (2002) when a PAV was used to alleviate the work done by the diaphragm, and it was shown that exercise times doubled when subjects used the PAV. The competition for blood flow is demonstrated when Dominelli et al. (2017) used a PAV to have trials of both increased and decreased respiratory muscle work, while measuring blood flow to both locomotor limb and respiratory muscle used (sternocleidomastoid). When the W*b* was alleviated, the 'extra' blood flow was redirected to the peripheral muscles and ventilation increased (by 114 +/- 19% of control). When the participant inspired through a resistor, increasing Wb, ventilation decreased (by 86 +/- 9% of control) and locomotor limb blood flow decreased. There are several indications that high ventilatory work impairs exercise performance by inhibiting blood flow to the peripheral muscles as ventilation and W*b* increase proportionally.

1.1.4 Effects of Hypoxia on Fatigue Development

Respiratory muscle fatigue occurs under normoxia, but the human lungs do not always operate under normoxia. The control of ventilation is tightly regulated with constant monitoring and feedback from both the active limbs and the respiratory system. Chemoreceptors (both peripheral and central) monitor blood partial pressure of CO_2 and O_2, (PCO_2 and PO_2, respectively) and [H^+]. Arterial PCO_2 is the most important factor in controlling ventilation, with highly sensitized receptors that allow minimal change in the arterial PCO_2 (West, 2012). Hypoxia increases ventilation by decreasing the fraction of inspired oxygen (F_IO_2) (lowering arterial PO_2) and PCO_2 both being sensed by the peripheral chemoreceptors.

Hypoxia exaggerates respiratory muscle fatigue by decreasing the oxygen available to the working muscles, while the working muscles are continually increasing their oxygen needs, creating an exponentially increasing disparity (Babcock et al., 1995a). Sheel, MacNutt, & Querido (2009) characterize three initial responses of the body to hypoxia is increased ventilation to make up for the decrease in F_IO_2. The increase in ventilation has three major consequences: firstly, dyspnea increases proportionally with ventilation which has been known to decrease exercise tolerance. Secondly, ventilation and W*b* are inherently linked and W*b* will increase the likelihood of fatigue development. Finally, as flow rate increases, there in an increase in water evaporation of the airways (Sheel, MacNutt, & Querido, 2009). Consequently, hypoxia not only increases ventilation, but changes ventilatory patterns and increases perceptions of breathing of the subject during exercise.

Of the potential sites where fatigue may be present, hypoxia may contribute to both central and peripheral fatigue. With regards to central fatigue, the type III/IV afferents are

increased as compared to the same work in normoxia due to increased metabolites from working muscles sensed in the CNS (site "a- excitability of high motor centres"), increasing the neural drive to breathe (Dempsey, 2006; O'Donnell et al., 1999). Increases in exercise metabolic by-products such as H+ impair many mechanisms of peripheral fatigue, primarily site "f-excitation-contraction coupling" and "g-contractile mechanisms" which are both susceptible to sensitivity changes based on muscle [H^+] (Kent-Braun, Fitts, & Christie, 2012; Babcock et al., 1995a).

Normobaric hypoxia can range from mild changes in F_IO_2 (~0.19) to severe decreases in F_IO_2 (~0.12). The severity of hypoxic intervention can have a detrimental effect on driving pressure of atmospheric air, across the blood-gas barrier. Driving pressure is one of the key components of how effectively oxygen can bind to haemoglobin, by Fick's Law of diffusion (West, 2012). A mix of central and peripheral factors affect fatigue and perceptions of discomfort.

The severity of hypoxemia a participant experiences, changes where fatigue occurs along the neuromuscular chain. This concept is shown by Amann et al. (2007a), when participants exercise to volitional failure with differing F_IO_2 levels, and at task failure they receive a hyperoxic inspirate. The authors support and increased degree of peripheral fatigue under a normoxic or a moderately-hypoxic inspirate, due to no change in exercise performance with an oxygen saturation (S_pO_2) increase at failure. Whereas, more central limitation occurred breathing a severely hypoxic inspirate, as seen with an extension of exercise when S_pO_2 was increased (Amann et al., 2007a). Moderate hypoxia is defined as an $F_IO_2 = 0.15$, severe hypoxia as 0.10, and used a hyperoxic inspirate of 0.30 at end exercise.

Babcock et al. (1995a) outline three main reasons why hypoxia specifically changes exercise-induced diaphragm fatigue: (1) with increases in ventilation EELV and expiratory flow

limitation increase (including Wb increases), (2) there is a decreased delivery of oxygen to the diaphragm, and (3) an increase of circulating metabolites from locomotor muscles could be taken up by the diaphragm. In their study, using an $F_IO_2 = 0.15$, subjects were unable to generate a higher flow (i.e. expiratory flow limited) at the end of a hypoxic exercise trial by up to 37%, and in the normoxic exercise, only up to 25% flow limited. The exercise time with hypoxia was approximately one-third of the time with normoxic air, but the diaphragm exhibited a similar degree of fatigue during both trials [normoxia -5.8 (+/- 2.1), hypoxia -5.8 (+/- 1.6) $_{cmH20}$]. A difference between the trials is seen in the length of recovery; during the hypoxic trial (with a decreased TTE) it took significantly longer to return to resting levels, compared to normoxia. Verges, Bachasson, & Wuyam (2010) investigated which variable is more consequential for diaphragm fatigue using hyperpnea as their model. Hyperpnea-induced diaphragm fatigue was significantly increased under hypoxic conditions ($S_pO_2 = 80\%$) as shown by a significant decrease in P_{di} twitch post-hyperpnea test, compared to the normoxia trial, but in the hyperoxia ($F_IO_2 = 0.60$) had no significant differences to respiratory muscle fatigue. It was concluded that hypoxia affects hyperpnea-induced respiratory muscle fatigue and implies that its effect is independent of hypoxic effects on skeletal muscle work (both respiratory and locomotor) during exercise.

1.1.5 Sex-Based Anatomical Differences Changing Physiology

1.1.5.1 Sex-Differences in Fatigue of Skeletal Muscle

Previously, many studies that investigated skeletal muscle fatigue used a male-only, or mixed sex sample and the results were generalized to both sexes. These studies incorporated muscles from the upper limb, lower limb, and respiratory system (Babcock et al., 1995; McKenzie et al., 1997; Taylor et al., 1997; Babcock et al., 2002; Dominelli et al., 2017).

Recently, studies have illustrated sex-based differences in some skeletal muscle fatigue resistance (Guenette et al., 2010; Fulco et al., 1999; Hunter 2009). Hicks, Kent-Braun, & Ditor (2001) outline three main areas in which men and women may differ in respect to fatigue resistance: (a) muscle mass, (b) substrate utilization, and (c) muscle morphology. Generally, women have a lower volume of a given muscle, which corresponds to a lower absolute force output when working at the same relative work rate as a male subject. With a larger volume, it was theorized that there was a higher percentage of blood flow was occluded from the muscle during contraction and may decrease oxygen delivery in men (Miller et al., 1993). The idea of muscle mass differences between the sexes as a mechanism of explaining differences in fatigue resistance was shown by Fluco et al. (1999) by matching male and females for absolute force and quantified fatigue development. During dynamic contractions of the adductor pollicis at the same matched work rate, women still had an increase in fatigue resistance as indicated by an increased time to failure and higher force output for MVC's during the protocol.

Hunter (2009) illustrates the importance of task specificity when researching sex-differences in fatigue. Task specificity refers to the specific requirements from the nervous and muscular system that vary depending on how a task is performed. Depending on how a task is performed, it can stress different fatiguing systems, and decrease the sex-difference (Hunter, 2009).When performing isometric contractions of the adductor pollicis, men and women were matched for force output of the muscle (an estimate of volume) and had a similar rate of fatigue (as indicated by time to failure). When dynamic contractions where the measure, women were able to sustain the target voluntary contraction longer than men (Hunter, 2009).

When biopsied, sex-differences muscle glycogen levels were negligible but, there is support for males relying more heavily on the glycogenic pathway, and females more on fat

oxidation for energy generation. Females, on average, have a 4-5% lower respiratory exchange ratio during submaximal endurance efforts when compared to men, supporting a heavier reliance on oxidative mechanisms for energy production (Tarnopolsky, 1990). Along with the idea of differences in substrate use is differences in the actual muscle fibres, therefore requiring different substrates for force production. Men generally have a higher percentage of type II anaerobic muscle fibres that have a higher force output, but are also more susceptible to fatigue (Hunter, 2014).

The importance of oxygen delivery to fatigue development was investigated by Russ & Kent-Braun (2003) who controlled the amount of blood to the ankle dorsiflexors during a fatiguing task (free blood flow or ischemic conditions). An iso-time test was used with MVC's performed during the trial, and pre-post magnetic stimulations of the peroneal nerve to quantify muscular fatigue. In the post-exercise stimulations for the ischemic condition, the fatigue resistance sex-difference was attenuated as compared to the free blood flow trial. The theory is women tend to rely upon oxidative phosphorylation and have an advantageous oxygen delivery system, and when this system is hindered, greater reliance is placed upon the glycolytic pathways that have more fatigue inducing by-products, as to mimic that of men (Tarnopolsky, 1990).

1.1.5.2 Sympathetic Activation Differences

Along with substrate utilization and fibre composition as potential systems that may contribute to sex-based differences of skeletal muscle fatigue, Hunter (2009) contributed the idea of sex-based differences in sympathetic activation of some skeletal muscles. A study was performed to analyze the changes in time to failure of a fatiguing task (maintain constant elbow angle-isometric) under control (low visual load) and when participants were also shown visual "cognitive load," (Mottram et al., 2006). During the control conditions, women had a longer time

to failure than men during a submaximal isometric elbow flexion task, but the sex differences were abolished during the stressful dual task, with the physiological responses mimicking those of the men. The average female TTE decreased 27% between the low and high cognitive load trials. The researchers attributed the time to failure in women due to increases in heart rate and mean arterial pressure, and changes in motor outflow, and in men were associated with changes in EMG activity and motor output.

These findings led the researchers to the conclusion that men and women may differ in the mechanisms for which task failure and fatigue occur, and the mechanisms additionally change based on many internal and external factors.

1.1.5.3 Anatomic Differences

While similarities aid in the understanding of respiratory mechanics, sex-based differences still occur on many levels of the respiratory system. As one of the major factors in fatigue development, men and women increase ventilation in different patterns; there are reports that males increase ventilation proportional to increases in work, and with women they increase ventilation more quickly to their maximum ventilation and plateau, so maximum ventilation is seen even at submaximal efforts. When height and lung sized matched, women tend to have smaller diameter airways, increasing resistance of air flow (Sheel et al., 2009). With the anatomic sex-differences in the respiratory system such as smaller diameter airways, and generally lower capacity for flow generation, women also generally have a higher W*b* for a given ventilation, specifically resistive W*b* due to the strong influence of airway radius on resistance to flow (Guenette et al., 2007).

1.1.5.4 Sex-Differences in Diaphragm Fatigue

With an increased work of breathing, it was hypothesized that women generally would also be more prone to diaphragm fatigue. Guenette et al. (2010) used a time to exhaustion trial with pre- and post-exercise magnetic stimulations, with fatigue defined as a P_{di} twitch decrement of 15% or greater than baseline values. The study supports that women's diaphragms generally were more resistant to fatigue than their male counterparts, as show with a frequency of 58% of males with diaphragm fatigue and 42% of females exhibiting fatigue after exercise at the same percentage of the individuals $\dot{V}O_{2max}$. This phenomenon is also seen in non-respiratory skeletal muscles with (1) lower absolute muscle mass, (2) utilization of lipids more predominantly than males, and (3) potential fibre composition differences as the key reasons for these differences (Hicks, Kent-Braun, & Ditor, 2001). In regard to muscle fibre differences, the diaphragm is primarily composed of type I muscles fibres and currently there is no research on sex-based differences of the muscle fibre composition of the diaphragm; using muscle biopsy samples to confirm or deny if other skeletal muscle fibre composition differences extend to the diaphragm. Female and male respiratory systems have many anatomic differences that equate to large changes in the physiology both at rest and exacerbated with exercise.

1.1.6 Conclusion

Recently, there has been increased interest to study men's and women's respiratory systems separately because their differences in structures change their respiratory patterns, and their potential differences in skeletal muscle fatigue resistance. Fatigue has many precursors at various points along the neural and muscular chain of response that allow different types of fatigue to be present. Respiratory muscle fatigue affects not only an individual's sensation during exercise but limits peripheral muscle exercise tolerance and impedes performance. Using hypoxia in respiratory muscle fatigue studies changes the ventilatory patterns and in vivo

chemistry, causing changes in fatigue development and recovery. These consequences of high Wb and fatigue negatively affect structures all over the body during exercise and further research in this area will aid in understanding the limits of human performance.

1.2 PURPOSE

The purpose of this thesis is to compare diaphragm fatigue between men and women under conditions of high-intensity exercise in normoxia and acute hypoxia.

1.3 HYPOTHESIS

It is hypothesized that men and women will develop a similar degree of diaphragm fatigue following a bout of high intensity cycling exercise in hypoxia.

Chapter 2: Into Practice

2.1 METHODS

2.1.1 Subjects

Using a previously reported effect size (0.49; Guenette et al., 2010), the calculated number of participants needed to achieve a statistical power of 0.8 is 20 participants (10 M and 10 W) (Guenette et al., 2010). All subjects provided written informed consent prior to beginning of testing and al experimental procedures received institutional ethical approval [H18-02674] and conformed to the *Declaration of Helsinki*. Subjects were excluded from participation if presented with; contraindications to exercise (as assessed by PARQ+ and medical history), abnormal spirometry or pulmonary diffusion capacity [as defined as outside 80-120% of predicted values (Quanjer et al., 2012) and a ratio of fraction of expired volume in 1-second to forced vital capacity ($FEV_{1.0}$/FVC) below 0.7, as assessed on the first testing visit.

Females that volunteered all experienced normal menstrual cycles as determined by self-evaluation and recorded on a Menstrual Cycle Questionnaire (Appendix 2) and were tested randomly throughout their cycle. Visits 2 and 3 were scheduled, at most, 96 hours apart to keep the subjects in the same (or close to) phase of their cycle for both testing sessions.

2.1.2 Experimental Overview

All testing took place at the University of British Columbia. Participants were asked to come into the lab for testing on three separate occasions, each separated by at least 48 hours. Every visit, participants were asked to refrain from exercise, caffeine, and food for 24, 12, and 2 hours, respectively, before each visit (food was prohibited only for testing visits 2/3).

Day 1 Resting pulmonary function was assessed and then after 5 minutes of resting breathing subjects performed an incremental graded exercise test to exhaustion on an electronically braked

cycle ergometer (Velotron, RacerMate; Seattle, WA, USA) to determine $\dot{V}O_{2max}$. *Day 2 and 3* Both of the testing days (visits 2 and 3) were time to exhaustion (TTE) tests on the same cycle ergometer. Both were conducted the same, except for a different fraction of inspired oxygen (F_IO_2) (normoxia: $F_IO_2 = 0.21$, hypoxia = $F_IO_2 = 0.15$).

We used a hypoxic inspirate considered "moderate," (Johnson et al., 1993) for the following reasons. First, it would facilitate comparisons with previous work (Babcock et al., 1995a). Second, to reduce potential medical risks associated with "severe" hypoxia. To ensure that excessive hypoxemia was not present, we used an S_pO_2 value of 75% for termination of the exercise test. Subjects whose S_pO_2 fell below 75% were removed from exercise and subsequent data analysis. One participant was excluded from analysis and this thesis from this criterion.

2.1.3 Procedures

2.1.3.1 Pulmonary Function

Forced vital capacity (FVC), forced expired volume in one-second ($FEV_{1.0}$), ratio of $FEV_{1.0}$/FVC, total lung capacity (TLC), peak expiratory flow (PEF) and diffusion capacity of the lungs for carbon monoxide (DLCO) were measured using a commercially available operating assessment system (Vmax Encore 229, V62J Autobox; CareFusion, Yorba Linda, CA) according to the recommended guidelines (Kastelik et al., 2002).

2.1.3.2 Graded Exercise Test

Subjects performed an incremental cycle exercise test to exhaustion with women beginning at 80 watts and men at 120 watts, both increasing 20 watts every 2 minutes. Participants had no constraints on their cadence while cycling, but test termination occurred when the participants' cadence dropped below 60 rpm.

2.1.3.3 Time to Exhaustion Test

Using the data from the graded exercise test, ventilatory thresholds were determined by graphical inspection when plotting ventilatory equivalents and $\dot{V}O_2$ against VCO_2 for each subject. The 60% Delta method (McLellan, 2011), factors in ventilatory threshold and $\dot{V}O_{2max}$, using the value 60% between them. Subjects could choose their own cadence but were informed that when their cadence dropped below 60 rpm, the test would be terminated.

2.1.4 Measurements

2.1.4.1 Flow, Volume, and Pressure

Inspiratory and expiratory flow were measured using two calibrated and heated pneumotachometer (model 3813, Hans Rudolph, Kansas City, MO) attached to a two-way-non-rebreathing valve (model 2700, Hans Rudolph, Kansas City, MO). Inspiratory and expiratory volumes were calculated by integration of the respective flow channels. Mixed expired gases were measured by a port at the end of the mixing chamber (ML 206; ADInstruments, Dunedin, New Zealand). Esophageal and gastric pressures (P_{eso} and P_{ga}) were measured by two-balloon tipped catheters (no. 47-9005, Ackrad Laboratory, Cranford, NJ) which were individually placed transnasally after application of a local anesthetic to minimize discomfort (Viscous Lidocaine 2%) upon insertion. One balloon (P_{eso}) was positioned in the lower third of the esophagus by use of a negative inflection in the pressure output during sharp inspirations, once observed, the balloon was retracted a further 10 cm. Air was removed from the balloons, and the P_{eso} balloon was filled with 1 ml of air and the P_{ga} was filled with 2 ml of air as per the manufacturer specifications. Transdiaphragmatic pressure (P_{di}) was calculated as the difference between P_{ga} and P_{eso}. Mouth pressure (P_{mo}) was collected and sampled through a custom port in the mouthpiece. Pressures were measures using Validyne Pressure Transducers (model DP15-34,

Validyne Engineering; Northridge, CA, USA) and were calibrated using a digital pressure manometer (2021P, Digitron; Torquay, UK).

2.1.4.2 Diaphragm Fatigue

Using the dual balloon catheter system explained above to estimate P_{di}, we also employed CMS of the phrenic nerves, innervating the diaphragm (Similowski et al., 1989). The BPNS was performed using a 90 mm circular coil, powered by a magnetic stimulator (Magstim 200 Mono Pulse, MagStim, Whitland, Wales). The subject was seated in a chair, neck in flexion, and using the spinous process of the individuals' C7 as a landmark, the coil was placed on the back of the neck. Where P_{di} and the CMAP was largest, was marked on the individual to ensure a low variation in stimulation locations. For every stimulation performed with the magnetic stimulator, as lung volume plays a key role in the diaphragm muscle length and therefore twitch amplitude, all stimulations were delivered at functional residual capacity (FRC), or EELV. The lung volume was verified by continual P_{eso}, P_{ga}, and P_{di} monitoring during stimulations. The stimulation was delivered when Peso was stable and less negative (as compared to inspiration).

To ensure supramaximal diaphragm activation, we performed three stimulations at increasing intensities (three at each 60%, 70%, 80%, 90%, 95%, and 100% of the 2 Tesla max stimulator output). Between each stimulation, there was a 30-second gap to avoid potentiation of the twitches, potentially leading to a falsely higher twitch amplitude. As the intensity of the stimulator increases near and at maximal, and if no increase in P_{di} output is seen with a higher stimulator intensity we can support supramaximal stimulation of the diaphragm. The average P_{di} output for each stimulator intensity was compared to the maximal P_{di} values (100% stimulator output) and significance was determined.

Surface EMG of the right and left hemidiaphragm were measured using self-adhesive Ag/AgCl electrodes (H59P, Kendall-LTP, MA, USA). These were placed between the sixth and eighth intercostal space along the anterior axillary line and the ground placed on the acromial process of the scapula on each respective side. In addition to hemidiaphragm EMG recordings, surface EMG of the sternocleidomastoid (EMG_{SCM}) and of the vastus lateralis (EMG_{VL}), which were placed on the muscle belly of the target muscle (Merletti et al., 2001), with the grounds as the left clavicle and left patella, respectively, which produced the least noise after experimenter trials. EMG signals were amplified, band-pass filtered (0.1 Hz to 3 kHz; P511 Series, Grass Instruments; Warwick, RI, USA), with all of the signals continuously recorded using PowerLab data software (LabChart v8.1.17, ADInstruments, Colorado Springs, CO). Analysis for EMG_{SCM} was performed using the baseline maximal inspiratory pressure or inspiratory capacity maneuvers, whichever had the highest values, and compared during the exercise intervention. Compound muscle action potential analysis was performed for both hemidiaphragm sides to analyze peak-to-peak twitch amplitude, onset latency, duration, and total rectified area.

The specific protocol for the measurement of baseline and post-exercise P_{di} comprised a series of 7 potentiated twitches, all at the maximal stimulator output (100%), the first 3 of which are discarded, and the latter 4 are used for analysis.The potentiated twitches involved the subject performing a maximal inspiration for approximately 5 seconds against an occluded mouthpiece. Participants were then instructed to take one normal breath, and at the end of the next exhale a stimulus was delivered, at FRC. Subjects and experimenters had visual feedback of the maximal inspiratory efforts, with coaching from experimenters on diaphragmatic activation during the maneuvers and with graphical feedback from the pressure measurements. The change in twitch force output of the diaphragm ($P_{di,tw}$) based on sex and condition is the main outcome variable for

this study. The threshold for if an individual experiences diaphragm fatigue was a decrease in $P_{di,tw} \geq 15\%$ post exercise relative to pre exercise values. This indicator of fatigue is synonymous with Guenette et al., 2010 & Kufel et al., 2002 and was selected due to it being 2-3 times larger than the average coefficient of variation in $P_{di,tw}$, ensuring measurement of true physiologic function.

2.1.4.3 Handgrip

Before and after exercise on TTE days, participants performed a handgrip MVC with a force transducer (LabChart v8.1.17, ADInstruments, Colorado Springs, CO). Using their dominant hand, participants were asked to hold the dynamometer, with the elbow in 90-degrees of flexion in the frontal plane and to perform three or more MVC maneuvers. Each MVC lasted 4-5 seconds and was separated by 30 seconds. The handgrip protocol was used to quantify an estimate of central fatigue. If a difference is seen in the handgrip values pre-post intervention, there may be higher centres of the brain affected by fatigue. The forearm muscles are not a dominant muscle group when determining cycling performance, and post exercise should have little peripheral muscular impairments that would impact force production.

2.1.4.4 Heart Rate

Heart rate was measured continuously on LabChart (LabChart v8.1.17, ADInstruments, Colorado Springs, CO) by a commercially available heart rate monitor strap (T34, Polar, Electro, Kempele, Finland).

2.1.4.5 Saturation

During rest and throughout exercise, participants wore a finger-clip connected to an oximeter (9600 Pulse Oximeter, Nonin, Minnesora, USA). The device converted the S_pO_2 signal to analog and with a custom BNC cable, connected to LabChart.

2.1.5 Data Analysis

The work of breathing was calculated by averaging eight representative breaths (absent of any physiological artifact that would skew the results – i.e., cough, swallow) prior to the IC maneuver and integrating the degree of P_{eso} swings and the volume of air flowing to the lungs based on the pressure generated in methods described by Dominelli and Sheel (2012). Pressure-time products (PTP) is used as an estimate of respiratory muscle oxygen consumption, only calculated during inspiration, by integrating the area under the pressure curves (P_{eso}, P_{gas}, and P_{di}) for the individual PTP of each (PTP_{eso}, PTP_{gas}, and PTP_{di}). The ratio of PTP_{di}:PTP_{eso} estimates the contribution of the diaphragm to total respiratory pressure generation.

2.1.5.1 Statistics

A two (sex - male/female) by two (F_IO_2 - 0.21/0.15) by five (time – presented as %TTE in 20% increments) repeated measures analysis of variance (ANOVA) was used to assess changes over time during the test for most variables (cardiorespiratory variables, EMG, calculated variables), or into recovery for diaphragm twitch analyses. Post-hoc Tukey tests were used to determine specific significance. Transdiaphragmatic pressure was calculated and monitored continuously during trials (LabChart v8.1.17). Coefficient of variation (CV) for $P_{di,tw}$ was calculated for each block of potentiated stimulations to control for within-subject variability. Prior to the baseline stimulation block, between-session variability was assessed by the RAMP procedures. Slope analyses of PTP_{di}:PTP_{eso} was used to calculate the relationship between how pressure generated by the diaphragm contributed to overall pressure generated by the respiratory muscles. Independent t-tests were run for the participant characteristics, maximal exercise data, and CMAP analysis. Significance was set at $p \leq 0.05$ for all tests. Statistical analyses were run using the 0.12.1 Version of JASP.

Note on Data Set. Due to the presence of the COVID-19 pandemic, testing was suspended with fifteen participants having completed all of the testing days (nine women and six men), and the remainder of the future participants will not be included in this thesis.

2.1 RESULTS

2.2.1 Subject Characteristics and Pulmonary Function

Subject characteristics are presented in Table 2.1. Participants were similar for age (p = 0.25), height (p = 0.44) and mass (p = 0.41). Men had higher values for absolute $FEV_{1.0}$ (p = 0.02), PEF (p = 0.007), hemoglobin concentration (p = 0.01) and DLCO ($p < 0.001$), but all the relative predicted values for pulmonary function are comparable with women as estimated by Quanjer et al., 2012.

2.2.2 Cardiorespiratory Response to Maximal Graded Exercise Test (Day 1)

Table 2.2 summarizes maximal cardiovascular and respiratory responses. At end exercise, men had significantly larger V_T, V_E, and carbon dioxide output ($\dot{V}CO_2$) (p = 0.008; p = 0.028; p = 0.044, respectively). Oxygen uptake ($\dot{V}O_2$) and maximal work rate (WR_{max}) tended to be higher in men but these differences were not significantly different (p = 0.08; p = 0.06, respectively).

2.2.3 Diaphragm Function and Fatigue

2.2.3.1 Supramaximal Stimulation

With increasing the intensity of the magnetic stimulator, a plateau in $P_{di,tw}$ was observed, on average, at 85-90% of maximal stimulator output as shown in Figure 1. A plateau was not seen in two women and one man. The coefficients of variation for men and women between

testing days was 6.6% and 6.1%, respectively. There was no change in the CMAP variables between pre-post exercise in both conditions.

2.2.3.2 Diaphragm Fatigue in Normoxia

For the purposes of this study, diaphragm fatigue was defined as a reduction of >15% from baseline in $P_{di,tw}$. This definition is based on the premise that the typical CV for assessing diaphragm fatigue is approximately 7%, and by doubling the CV we are better able to make conclusions about fatigue and the contractile properties of the diaphragm (Guenette et al., 2010). Figure 2A illustrates the degree of diaphragm fatigue achieved directly post exercise, and at 10-, 30-, and 60-minutes post recovery expressed as a percent of baseline. The average reduction in $P_{di,tw}$ in women compared to baseline for each time block when diaphragm, fatigue was assessed (n=9) was -21.5 +/- 6.9, -14.2, +/- 5.9, -14.6 +/- 5.7, and -12.2 +/- 5.4% for post exercise and into recovery, respectively and the average reduction in $P_{di,tw}$ in men (n=6) was -18.8 +/- 5.7, -11.7 +/- 6.4, -10.7 +/- 6.8, and -8.5 +/- 5.7%, respectively. When using absolute values of $P_{di,tw}$ in cmH_2O, the baseline measurements were on average 27.0 +/- 10.8 cmH_2O for men and 24.0 +/- 6.1 cmH_2O for women, determined to be non-significantly different during post-hoc analysis ($p = 1.00$). The average absolute $P_{di,tw}$ values for men are 21.9 +/- 10.0, 23.9 +/- 11.6, 24.2 +/-12.5, and 24.9 +/-10.2 cmH_2O and for women are 18.2 +/- 5.4, 20.7 +/- 4.9, 20.7 +/- 6.0, and 20.8 +/- 5.4 cmH_2O, as seen in Figure 2C. As seen in both sexes, directly post exercise (T = 0) is the only block of twitches that exhibits a $P_{di,tw}$ that is significantly different from baseline in normoxia (women: $p < 0.001$, men: $p = 0.026$), and at every other point the degree of fatigue is non-significant from baseline, seen in both terms of absolute $P_{di,tw}$ and as a percent of baseline.

Both men and women had a similar absolute drop in cmH_2O pre-post exercise, -5.1 and -5.8 cmH_2O. There were no sex-based differences in the drop of diaphragm twitch pressure post

exercise or into recovery. At the end of high intensity normoxic cycling to exhaustion, 6 women and 4 men exhibited diaphragm fatigue (66% rate of occurrence for both) immediately post-exercise.

2.2.3.3 Diaphragm Fatigue in Hypoxia

Diaphragm fatigue in hypoxia as shown in Figure 2B, illustrates a non-significant difference between the degree of fatigue in hypoxia for both men (-18.5 +/- 10.9, -17.1 +/- 11.7, -7.8 +/- 13.1, and -5.4 +/- 7.1%) and women (-29.9 +/- 7.4, -21.3 +/- 10, -19.2 +/- 14.3, and -14.8 +/- 13.7%), compared to that in normoxia. The degree of fatigue in men and women directly post-exercise as a percent of baseline is not significantly different between the sexes ($p = 1.00$). The absolute $P_{di,tw}$ values for men for each measurement point are (baseline, post-exercise, 10-, 30-, and 60-minutes into recovery): 28.2 +/- 7.6, 23.0 +/- 8.1, 23.8 +/- 7.9, 26.4 +/- 9.9, and 27.1 +/- 8.1 cmH_2O, respectively, women presented with the following absolute $P_{di,tw}$ values: 25.7 +/- 6.6, 18.3 +/- 4.3, 20.0 +/- 4.5, 20.2 +/- 3.9, and 21.3 +/- 3.7 cmH_2O, respectively, both of which can be seen in Figure 2D.

The only time point when men exhibit significantly different $P_{di,tw}$ response as a percent of baseline is directly post exercise ($p = 0.023$), mirroring the pattern of recovery in normoxia. In addition, women do not exhibit the same pattern of recovery in hypoxia as they do in normoxia; $P_{di,tw}$ is significantly different from baseline at post exercise, 10-, and 30-minutes into recovery ($p < 0.001$, $p < 0.001$, and $p < 0.001$, respectively) and with slight differences at 60-minutes into recovery ($p = 0.061$) (Figure 2B). Expressed as a percent of baseline, in hypoxia neither men nor women have a significant difference in the degree of diaphragm fatigue, as compared to the values seen in normoxia [men: ($p = 1.00$) for all measurements, women: ($p = 0.56$) post-exercise, and ($p = 1.00$) for all subsequent measurements]. The absolute values of female $P_{di,tw}$ up

until 60-minutes into recovery are significantly different than the baseline absolute value ($p < 0.001$, $p < 0.001$, $p < 0.001$, $p = 0.023$, respectively) (Figure 2D) but the men only have a significantly lower $P_{di,tw}$ post exercise in hypoxia ($p = 0.038$). As in normoxia, men have slightly higher absolute $P_{di,tw}$ values than women, but not significantly. The absolute $P_{di,tw}$ drop pre-post exercise is -5.1 cmH_2O for men and -7.5 cmH_2O for women.

2.2.4 Time to Exhaustion (TTE) Day 2/3

2.2.4.1 Cycling Time

In normoxia men cycled an average of 12 minutes and 30 seconds (+/- 2 min 25 seconds) and women 15 minutes and 15 seconds (+/- 6 minutes 8 seconds), which are not significantly different ($p = 0.6$) (Appendix Figure A). In hypoxia but the cycling time to exhaustion for both was significantly lower than the times in normoxia [men: 6 minutes 27 seconds (+/- 3 minutes 27 seconds) ($p = 0.016$) and women: 7 minutes 3 seconds (+/- 2 minutes 50 seconds) ($p < 0.001$)] but not significantly different from each other.

2.2.4.2 Cardiorespiratory and Metabolic Responses

The respiratory and cardiovascular response are shown in Table 2.3, which shows variables expressed at a percent time to exhaustion (in 20% increments). Heart rate was similar between women and men throughout normoxic and hypoxic exercise. Overall, hypoxia had a significant effect on heart rate ($p = 0.003$), with slightly lower heart rates observed with the lower F_IO_2. Sex had a significant effect on V_T ($p = 0.004$), with men having larger volumes than women in both conditions. Men had no change in their V_T over time, this resiliency seen in both F_IO_2 conditions (normoxia: $p = 0.987$, hypoxia: $p = 0.919$). In normoxia, women have significantly lower tidal volumes at end exercise, as compared to at the beginning of the exercise ($p = 0.005$), with this trend emerging at 80%TTE ($p = 0.09$). However, in hypoxia by the end of

exercise, the average tidal volume was not significantly different compared to the beginning of exercise ($p = 0.15$). Breathing frequency (F*b*) increased significantly with time ($p < 0.001$), in the absence of a sex difference. Women trended to have higher F*b*, irrespective of F_IO_2. There is a significant effect of time ($p < 0.001$) and condition ($p = 0.031$) on minute ventilation. In hypoxia, both men and women increase ventilation more upon the initiation of exercise as compared to in normoxia. After 40% TTE, men have on average higher ventilations in hypoxia and by end exercise have higher ventilations, but not significantly. Women's ventilation is similar in both conditions and does not increase in hypoxia as much as the men's does. Oxygen uptake is significantly lower in hypoxia ($p = 0.003$) for all participants as seen in Figure 3E, with men having higher $\dot{V}O_2$ values than women during both conditions, but no significant effect of sex ($p = 0.61$) was found. Men experienced a significant increase in their $\dot{V}O_2$ with hypoxia, as compared to the beginning of exercise (trends emerging at 60%TTE), where in normoxia there is a constant $\dot{V}O_2$ during the course of the trial. Respiratory exchange ratio is significantly affected by condition ($p = 0.011$), both men and women had higher respiratory exchange ratio (RER) values with a lower F_IO_2.

Saturation was estimated for all participants as shown in Figure 5. For women in hypoxia, by 80% TTE, the saturation was significantly lower than in the beginning ($p < 0.001$) and continued to decrease or was significantly lower through end exercise ($p < 0.001$), and this the only condition which exhibits a significant change over time [a %TTE x F_IO_2 x sex interaction ($p = 0.007$)]. The saturation response of both sexes in normoxia and men in hypoxia stays even over the course of the trial. At every point of the trial, for both sexes, the saturation in normoxia was significantly higher than in hypoxia ($p < 0.001$).

The baseline, mid exercise, and end exercise values for finger lactate concentration are presented in Table 4. There was no effect of sex on lactate values, with an overall effect of condition ($p = 0.003$). In hypoxia, both men and women had significantly different lactate values from baseline by 2-minutes into exercise ($p < 0.001$, both), which happens at 4-min for both in normoxia ($p < 0.001$, both).

2.2.4.3 Breathing Mechanics

Cumulatively, there were no significant sex differences in the total mechanical W*b* in either normoxia or hypoxia as described in Figure 3. Yet, in both men and women, the cumulative values are significantly lower in hypoxia compared to normoxia at end exercise ($p = 0.048$ and $p < 0.001$, respectively) with a significant effect of condition on cumulative total W*b* ($p < 0.001$) across all time points. Differences between the conditions in women are seen at 80% TTE where the cumulative W*b* is lower than that in normoxia (p = 0.003). Figure 4B illustrates the W*b* throughout the cycling trials. Women have similar total W*b* in both F_IO_2 conditions at all time points (also with little change in $\dot{V}_E$ between the trials). At 80% of the total exercise time in hypoxia the instant total W*b* for men increase

and by the end of exercise the values continue to diverge, but not to significance with total Wb ($p = 0.11$). As total W*b* is the sum of Wb_E and $Wb_{I(res+ela),}$ this rise of total W*b* stems from significant increase of $Wb_{I,res}$ for men only in the hypoxic condition at end exercise ($p = 0.02$) (Appendix Figure B).

The absolute-pressure-time products for the diaphragm and esophagus, and the ratio of PTP_{di}:PTP_{eso} are shown in Figure 5A-E. The esophageal pressure-time product (Figure 4A), increases significantly by end exercise in both conditions for both sexes ($p < 0.001$, both). In hypoxia, there is a steeper increase of the PTP_{eso}, and this becomes significantly different from

the beginning of exercise at an earlier point than in normoxia. The cumulative PTP_{eso} was not significantly different between the sexes in either conditions but considerably more PTP_{eso} with the lowered F_IO_2 ($p = 0.008$; $p < 0.001$, respectively), which fits with the significantly lower time exercising in hypoxia (Appendix Figure A). The absolute and cumulative PTP_{di} are both significantly affected by sex ($p = 0.011$; $p = 0.035$, respectively) and as shown in Figures 4C and 4D. Men tend to have higher PTP_{di} in normoxia than women by end exercise ($p = 0.067$) and also have a significantly higher cumulative PTP_{di} as compared to women ($p = 0.027$). In hypoxia, the trend continues with men having slightly higher end exercise values of PTP_{di} than women ($p = 0.86$), but do not have significantly higher values of cumulative PTP_{di} than women ($p = 0.54$). PTP_{di}:PTP_{eso} was variable between subjects but not between testing days, particularly in the women, and Figure 4E demonstrates the relationship of diaphragm contribution to overall pressure generation. In both FIO2 conditions, men began exercise with a very high PTP_{di}:PTP_{eso} and it decreased throughout the trial, giving the male PTP_{di}:PTP_{eso} a negative slope (N: m = -0.12 +/- 0.03; H: m = -0.20 +/- 0.15). A negative slope may indicate the diaphragmatic contribution declining with exercise time progression. Women in normoxia have a slope m = 0.057 +/- 0.10 (i.e., diaphragm activation may increase slightly or remain relatively stable over time) and is significantly different from the slope of the male PTP_{di}:PTP_{eso} ($p < 0.001$), whereas in the hypoxic condition the slope becomes negative (m = -0.10 +/- 0.21) and is not significantly different from the slope of the men in hypoxia ($p = 0.42$).

2.2.4.4 Electromyography

Using root mean squared (RMS) analysis to integrate the total amplitude of the EMG_{scm} (RMS_{scm}) throughout exercise, having a lowered F_IO_2 had a trending effect on the activity of the SCM ($p = 0.053$) and a significant effect of time ($p = 0.002$). Men had slightly higher values of

RM_{scm} overall, but not significantly ($p = 0.11$), but by end exercise in the hypoxic condition. It is the only point that men and women are significantly different ($p = 0.016$) and is significantly different than the same men in normoxia ($p = 0.009$). The mean RMS_{scm} values are presented in Figure 6A and are expressed as a percentage of the maximal RMS_{scm} values obtained during baseline measurements. The root mean squared of the EMG_{vl} (RMS_{vl}), using a maximal isometric contraction, the participants attempted to extend the leg from a seated hip- and knee-90-degree flexion position. Expressed as a percent of the baseline MVC, women had little change in the RMS_{vl} based on their FIO2, but men had an increase of RMS_{vl} between conditions. In normoxia, on average the men had lower RMS_{vl} than women, but in hypoxia, they have a similar RMS_{vl} to women as illustrated in Figure 6B.

Chapter 3: Conclusion

3.1 DISCUSSION

This study evaluated exercise-induced diaphragm fatigue under normoxic and hypoxic conditions in healthy men and women. Subjects performed high intensity cycling exercise to exhaustion and fatigue of the diaphragm was assessed using phrenic nerve stimulation. We hypothesized that men and women would develop a similar degree of diaphragm fatigue with hypoxia. The main findings were: 1) the average time to exhaustion was significantly lower for both sexes in the hypoxic condition compared with the normoxic condition, 2) the degree of diaphragm fatigue did not change significantly for either sex based on the F_IO_2 (0.21 vs. 0.15), and 3) the degree of female diaphragm fatigue in hypoxia was similar to that seen in normoxia, but the capacity of the muscle was impaired into recovery significantly longer in hypoxia. They exhibited significant differences from baseline up to 30 minutes into recovery, with men exhibiting a similar pattern of recovery in both conditions. Collectively, the findings of this study are that diaphragm force production at exhaustion is similar with both a normoxic and a moderately hypoxic inspirate, for both men and women. However, the lowered F_IO_2 prolonged fatigue and extended the recovery of the female diaphragm as compared to normoxia.

3.1.1 Diaphragm Fatigue and CMS

3.1.1.1 Diaphragm Fatigue

The current study evaluated diaphragm fatigue by CMS pre-exercise, directly post exercise (within 3 minutes of exercise termination), and during recovery (10-, 30-, and 60-minutes post exercise). The post exercise measurement in normoxia shows similar levels of fatigue for both healthy men and women (-18.8 +/- 5.7 and -21.5 +/- 6.9 % baseline $P_{di,tw}$, respectively). The participants were not intentionally matched but were similar in height, mass, age, and average

fitness level [as a percent of predicted $\dot{V}O_{2max}$ (Jones 1997) (Table 2.3)]. The results of the current thesis are contrast to Guenette et al., (2010), who found significant sex differences in diaphragm fatigue with highly trained athletes after cycling exercise. With Guenette et al. (2010), the researchers found 10 minutes post-exercise, women had an increased resistance to exercise-induced diaphragm fatigue with cycling exercise. Differences between the exercise prescription, time to failure, and diaphragm use may account for the difference in result. As compared to the women in the current study, those in Guenette et al. (2010) cycled on average for a shorter time than those in the current study, and the TTE work rate intensity was higher than in the current study (90% maximal work rate vs. 83.6% $\dot{V}O_{2max}$). They also had a higher diaphragmatic contribution to overall respiration as seen in average lower PTP_{di}/PTP_{eso} values for both men and women in the current work in comparison.

At higher intensities, sex differences in skeletal muscle fatigue become more pronounced, in part due to general differences in the muscle fibre composition, with men having a higher proportion of Type II muscles, capable of higher force production, but more susceptible to fatigue. As the intensity of the exercise lowers, sex differences in muscular fatigue may become less pronounced, with both sexes using a high proportion of Type I fibres for propulsion (Hunter, 2014). In comparison of Guenette et al. (2010), our subjects were on average less fit ($\dot{V}O_{2max}$ men: 64.0 +/- 1.6 and women: 57.1 +/- 1.5 ml/kg/min vs. men: 54.8 +/- 11.8 and women: 46.5 +/- 10.7 ml/kg/min, respectively) with the sexes having a similar discrepancy between $\dot{V}O_{2max}$ values for both studies. Babcock et al. (1996) investigated the effect of fitness on the degree of diaphragm fatigue and found when participants exercised at 95% $\dot{V}O_{2max}$ until exhaustion, there was a similar magnitude of fatigue exhibited by both a highly fit group and a fit group. The limitation of this study is the researchers use a mix sample of sexes and fail to disclose the

distribution of sex into their categories of fitness. They emphasize the importance not on the capabilities of the diaphragm muscle, but its contribution to respiration; more fit individuals had a higher force output of the diaphragm during the first half of exercise, with no differences between diaphragm force output during the latter half of exercise between the groups.

When cycling in acute hypoxia, the degree of diaphragm fatigue was similar to that in normoxia, but the lowered F_IO_2 accelerated the development of fatigue, reaching similar levels to that in normoxia in a shorter amount of time. Our findings are similar to those of Babcock et al., (1995a). The degree of diaphragm fatigue at exhaustion does not appear to be affected by F_IO_2 at exhaustion, only on the time spent exercising. In both cases, the exercise time to exhaustion was significantly shorter in the hypoxic trial and the degree of diaphragm fatigue was similar. Having a lowered F_IO_2 may exacerbate the metaboreflex so the respiratory muscles may have certain protection under hypoxic conditions, to prevent possible whole-body tissue hypoxemia. This can increase vasoconstriction and sympathetic activation to the limbs, and decreasing time to failure (Dempsey 1986; Bigland-Ritchie & Vollestad, 1988).

Many previous papers control for the time performing the task, having iso-time working in both a normoxic and hypoxic condition, which leads to a greater degree of skeletal muscle fatigue seen under hypoxia conditions. In the current study, we used volitional failure as the termination of exercise criterion and observed changes in the time to failure instead of changes in the respiratory muscle contractile properties (Amann et al., 2006; Verges, Bachusson, & Wuyam 2010; Vogiatzis et al., 2007).

3.1.1.2 Diaphragmatic Load

The absolute load on the diaphragm (when quantified by calculating the W*b*) was similar in normoxia between the sexes. As the work W*b* is correlated with ventilation, the groups also had

similar V_E. Figure 4B shows the W*b* over time with little differences between men and women in normoxia, but in hypoxia by 80% through the trial, men have an increase in the W*b*. As total W*b* is the sum of the both inspiratory and expiratory, divided further into elastic and resistive components during the cycle of the breath, the increase in the total W*b* increase seen in men only in the hypoxia condition comes from an increase in the inspiratory resistive component of W*b*.

In normoxia, women and men have similar cumulative values of PTP_{eso}, representing the total pressure time product for the respiratory system. Yet, men have significantly higher cumulative PTP_{di} than women, which supports previous work citing men having a higher reliance on their diaphragm for ventilation increases (Guenette et al., 2010), even when matched for cumulative PTP_{eso}. The instantaneous values for PTP_{di} were significantly different between the sexes ($p = 0.02$), whereas PTP_{eso} or PTP_{ga} were not. Bellemare, Jeanneret, & Couture (2003) found men having an increased reliance on the diaphragm for pressure generation of the respiratory system, where women rely more on accessory muscle recruitment, which is primarily due to differences in ribcage dimensions; the shape of the thorax assists in dictating the length of the muscles, and how they're recruited as ventilation increases. In hypoxia, men and women both had significantly lower cumulative PTP_{eso}, but were still not different from each other, yet the PTP_{di} of men was now non-significantly different from that of women, different to what was seen in normoxia. The difference seen between sexes in cumulative PTP_{di} in normoxia is diminished in hypoxia which could be an indication of either a) men have a lowered diaphragm activation or b) women have an increased diaphragm activation.

3.1.1.3 The Role of Hypoxia in Diaphragm Fatigue

All participants cycled for less time in hypoxia than in normoxia, yet on average reached the same level of diaphragm fatigue. Minute ventilation was not significantly different based on

condition, for either sex, or consequently the total W*b* not significantly different for either sex with a reduced F_IO_2. The role of arterial saturation may have direct effects on diaphragm oxygen delivery in hypoxia, accelerating the development of fatigue; shown here in Figure 6, both men and women have significantly lower SpO_2 levels during the hypoxia trial. Vogiatzis et al. (2007) purposely matched ventilations and PTP_{di} during various F_IO_2 inspirate exercise trials. With lowered F_IO_2, there was exaggerated exercise-induced diaphragm fatigue, increased oxygen needs of the diaphragm in hypoxia (which may be not adequately met by the potential vasodilatory effects from hypoxia), and increased blood flow to the diaphragm hastening the development of fatigue. This can cause the diaphragm to produce more metabolic by-products (lactate, H^+, etc.) which have been suggested to create an acidotic environment for the diaphragm and contributing to low frequency peripheral fatigue.

In the current study, we used a moderately hypoxic inspirate ($F_IO_2 = 0.15$) and had a cap on the S_pO_2 of the exercising participants at 75% O_2 during exercise which according to previous work is not a severe enough drop in oxygenation to elicit central fatigue without the presence of peripheral fatigue (Johnson et al, 1993; Millet at al., 2012). The researchers in the current thesis applied a handgrip MVC pre- and post-exercise and found no differences in force output for either sex or condition ($p < 0.05$). Severe hypoxia ($S_pO_2 < 75\%$ O_2) has been shown to decrease cerebral oxygenation levels (prefrontal, premotor, and motor) and can decreased cortical voluntary action to muscles not directly impacted by the exercising intervention (Rasmussen et al., 2010), even without the influence of group III/IV afferents on motor excitability (Millet et al., 2012). With performing an MVC of the forearm muscles pre- and post- cycling exercise and can show similarities between sex and condition, we are able to support an increase in peripheral diaphragm fatigue in our participants.

In the hypoxic condition, all participants had lower $\dot{V}O_2$ values throughout exercise, and similar minute ventilations, indicating an increased hypoxic drive to breath (in excess to the oxygen demand) to match the metabolic rate of muscular contraction. The increased ventilation strains the respiratory system, increasing the metaboreflex inhibition of the periphery (Dempsey 1986; Bigland-Ritchie & Vollestad, 1988). When oxygen is limited, the $\dot{V}O_{2max}$ of an individual is lowered, but the respiratory system is not compromised. During whole body exercise in hypoxia, the inhibition to the CNS from the respiratory system overpowers those from exercising limb muscles, leading to an increased blood flow to the respiratory muscles through peripheral excitation and vasoconstriction, as a protective measure for oxygen delivery. The CNS vigilantly monitors the available oxygen and will increase motor drive accordingly so that the metabolic needs of the exercising muscle are precisely met, and the remainder is redirected towards respiration, as to protect global tissues for deoxygenation. Peripheral muscles can experience high deoxygenation without harm to other muscles and systems, the same cannot be said about the respiratory muscles. Bigland-Ritchie & Vollestad (1988) hypothesize that diaphragm function and protection may be increased during hypoxic exercise, as compared to the limb muscles.

3.1.1.4 Diaphragm Recovery

In both F_IO_2 conditions, women and men experienced diaphragm fatigue to a similar degree, but in hypoxia it was apparent, particularly with women, that recovery is impaired with hypoxia. When measured as a percent of baseline, in hypoxia men and women both have significantly different $P_{di,tw}$ than at baseline (this trend also is seen in the normoxia condition), but women continue to have significantly different values from baseline 10- and 30-minutes post exercise, and trending at the end of the recovery period (60 minutes). Using absolute $P_{di,tw}$ values,

women have significantly different values from baseline in hypoxia all the way to 60-minutes into recovery. Men in normoxia and hypoxia exhibit a similar recovery pattern, with the only significantly different $P_{di,tw}$ measurement from baseline in the post exercise stimulations.

3.1.1.4 Measure Reliability

For diaphragm fatigue measurements, we used a magnetic stimulator, activating the phrenic nerve, for a maximal involuntary assessment of the excitation-contractile properties of the diaphragm. This method has been used previously to investigate diaphragm fatigue with reliable results (Similowski et al., 1989; Guenette et al., 2010; Welch et al., 2018; Geary et al., 2019). As shown in Figure 1, we were able to see a plateau of diaphragm force output at the higher intensities for all conditions, indicating a maximal stimulation of the muscle. To quantify diaphragm fatigue, we stimulate the phrenic nerve directly post-MVC (Mueller Maneuver, cite) as to ensure complete motor unit recruitment. Kufel and collogues (2001) used an MVC prior to stimulation to increase circulating Ca^{2+} in the sarcoplasm, temporarily increasing the force production potential of the muscle of interest and these potentiated twitches have in increased capacity to detect fatigue.

3.1.2 Diaphragm Activation and Accessory Muscle Recruitment

In normoxia, women have a lower diaphragm contribution to overall respiration, and the slope of PTP_{di}:PTP_{eso} is close to zero, showing little change in diaphragm contribution over time. Men have a higher diaphragm activation at the beginning of the trial and the slope is negative, indicating an increase of diaphragm contribution over time and the female and male slopes are significantly different ($p < 0.001$). Guenette et al., (2010) observed a similar phenomenon in highly trained female and male cyclists; whereby women had a consistent diaphragm activation over time and men had a negative slope which was attributed to lower diaphragm contribution

over time. This decrease is associated with a higher accessory muscle recruitment in the later stages of exercise. In hypoxia, the slope of PTP_{di}:PTP_{eso} for women becomes negative, and the male slope becomes increasingly negative and the slopes become non-significantly different (p = 0.42). Lowering the F_IO_2 does not have a significant effect on V_E but changes the diaphragm contribution to respiratory pressure over time. As diaphragm contribution decreases through the course of exercise, accessory muscles recruitment increases to supplement the diaphragm as seen by EMG_{SCM} in Figure 7A, countering the effects of maintaining ventilation and decreasing diaphragm activation. Hypoxia increases the EMG_{SCM}, indicating an increase in electrical neural signals to activate the muscle, with men having significantly increased sternocleidomastoid activity by end exercise so they are higher than in normoxia, and significantly different from the women in hypoxia. This may be a protective mechanism to minimize diaphragm fatigue in men, when oxygen is in short supply. Bellemare, Jeanneret, & Couture (2003) investigated sex differences in respiratory muscle recruitment at rest and they concluded women may have a higher reliance on extra-diaphragmatic muscles, and men (due to a larger resting P_{ga} and therefore slightly larger P_{di}) have a higher reliance on diaphragmatic breathing at rest. We hypothesize in hypoxia women have an increased diaphragm contribution to overall respiration, as supported with an inhibition of force up to 60-minutes post exercise. A slowed recovery was not seen in men during hypoxia, potentially due to increased reliance on extra-diaphragmatic muscles by end of exercise as supported by significant increases in EMG_{scm} for men only. Further research should be completed on respiratory muscle recruitment pattern with changing F_IO_2.

3.1.3 Consequences of Diaphragm Fatigue

As the diaphragm muscle fatigues [which can begin as early at 6-minutes into exercise (Archiza et al., 2017)] group III/IV afferents signal for increases in blood flow and redistribution of the finite Q can be altered to favour the respiratory muscles (Sheel et al., 2018), increasing blood flow up to 10-16% of Q, depending on training status (Harms et al., 1997; Dominelli et al., 2013). High ventilations can also cause chest wall deformation, placing the diaphragm at an unfavourable angle for efficient contraction, this increasing the W*b,* exercise metabolic products, and placing excess strain on the extra-diaphragmatic muscles leading to an increase in blood flow to the thorax (Harms et al., 2000). The current study supports these findings, with the participants' EMG_{SCM} recordings increasing significantly in the hypoxic condition, compared to that in normoxia.

The previous respiratory physiological changes that occur as diaphragm fatigue develops during exercise are accompanied with changes in cardiac output available for peripheral muscles. In men, increasing the W*b* induces increased limb vascular resistance and norepinephrine spillover indicating sympathetic activation of the peripheral vasculature (Harms et al., 1997). And the converse is also true in both men and women, decreasing W*b* increases locomotor muscle blood flow (Dominelli et al., 2017).

In a study by Amann et al. (2007b), the researchers manipulated the W*b* in cycling participants with both a normoxic and a moderately hypoxic ($F_IO_2 = 0.15$) inspirate. Decreasing the work done by the respiratory system in normoxia had little effect on peripheral fatigue yet, in hypoxia, when the W*b* was decreased by ~70%, peripheral fatigue attenuated by ~40%. This led to the conclusion that work performed by the respiratory muscles is more influential to exercise performance effects in hypoxia compared with normoxia. In the current study, on average the

participants cycled for significantly less time in the hypoxia condition compared to the normoxia conditions, but the average total work of breathing increased slightly, due to a significant increase in the $Wb_{I,res}$ ($p < 0.05$). Small changes in the W*b* in hypoxia induce exacerbated downstream effects for limiting exercise performance. The increase in $Wb_{I,res}$ was seen primarily in men, which may contribute to their task failure, without the presence of increased diaphragm fatigue.

3.1.4 Limitations

Prior to exercise initiation, participants were asked to perform graded FVC trials and IC maneuvers, and the IC measurements continued into exercise. Post exercise we instructed participants to perform potentiated twitches and the handgrip exercise and decided the timing of the twitches outweighed post-exercise graded FVC maneuvers. We are unable to construct accurate maximal flow-volume loops without correcting for gas density.

Using magnetic stimulation to excite the phrenic nerve has limitations in the strength of the stimulator intensity. The same two females and one male did not show a plateau of diaphragm force output in either condition during the recruitment protocols. Previous research by Verges et al. (2006) indicate that when comparing diaphragm force output at 94% and 100% of stimulator intensity, only 4 out of 11 participants experiences a plateau of $P_{di,tw}$. Yet, a plateau is seen in 8 out of 11 participants when the stimulator intensity when the $P_{di,tw}$ values are compared between 98 and 100% of stimulator intensity. The protocol of the current thesis had larger increments for stimulator intensity changes than those in the previous study. In addition, to keep the stimulations constant, we marked the point on the neck clearly where the coil is placed for the highest P_{di} output and CMAP.

The current study did not standardize for menstrual cycle phase in women and tested women randomly throughout their cycle. When obtaining informed consent, women completed a Menstrual Cycle Questionnaire (Appendix 2) and reported normal menstrual cycles. For Day 2 and 3, all participants had the days scheduled maximum 4 days apart, as to decrease between-day variation of hormone fluctuations, with the intention of having the testing days in the same phase of the cycle for each female. Yet, previous work has shown female hormones may have an effect on ventilatory drive (Schoene et al., 1981) As we tested women randomly throughout their cycle, the randomization may confound the effects of hormone fluctuations. Further research is required into any potential differences in diaphragm fatigue based on hormone changes during the menstrual cycle.

3.1.5 Conclusion

The magnitude of diaphragm fatigue developed in men and women do not appear to be impacted by external F_IO_2 changes. Yet, the recovery of diaphragm force after exercise in acute moderate hypoxia was decreased in females as compared with recovery in normoxia. The diaphragm may have increased protection from fatigue during hypoxia, limiting exercise performance and retaining the force production capabilities of the diaphragm.

In summary, any skeletal muscle (limb or respiratory), when enough inhibition is present from either the central or peripheral systems, fatigue will develop. Decreasing the F_IO_2 can change the mechanisms of fatigue that limit exercise performance and may favour the respiratory system as to prevent possible global hypoxemia or harm to other tissues.

Table 1: Summary of inspiratory muscle fatigue studies

Study	Intervention	Effect
McKenzie et al., 1997	Threshold Loading/Normoxia	No sig ΔPdi, CO_2 retention limiting factor
Rohrbach et al., 2003	Threshold Loading/Normoxia	Significant decrease in ΔP_{di}, based on pre-post MIPs
Hannegard et al., 1996	MIV/Normoxia	Sig decreases in pre-post CMS stimulations in ΔPdi
Johnson et al., 1993	TTE trials 80-5% + 90-5% $\dot{V}O_{2max}$/Normoxia	Sig decreases in pre-post CMS, 20% decrease in 85% trial and 15% in 95% trial
Babcock et al., 2002	PAV/Normoxia	No sig ΔP_{di} post PAV, used CMS
Dominelli et al., 2017	PAV + Resistor/Normoxia	PAV: higher Q% to VL/VM, resistor: higher Q% to SCM

CMS = cervical magnetic stimulation, MIP = maximal inspiratory pressure, MIV = maximal isocapnic ventilation, PAV = proportional assist ventilator, SCM = sternocleidomastoid, TTE = time to exhaustion, VM = vastus medius, VL = vastus lateralis, Q = cardiac output

Table 2: Subject Characteristics

	Men (n = 6)	**Women** (n = 9)
Age (years)	26 +/- 4	24 +/- 3
Height (cm)	178 +/- 6	175 +/- 7
Mass (kg)	73 +/- 6	70 +/- 10
TLC (l) (% predicted)	7.3 +/- 0.7 88.6 +/- 10.5	6.4 +/- 1.0 96.0 +/- 9.3
FVC (l) (% predicted)	5.8 +/- 0.9 107 +/- 11	4.9 +/- 1.0 109 +/- 18
$FEV_{1.0}$((l) (% predicted)	4.8 +/- 0.6 103 +/- 10	3.9 +/- 0.6 ***** 101 +/- 14
$FEV_{1.0}$/FVC (% predicted)	81 +/- 5 98 +/- 7	82 +/- 7 93 +/- 8
PEF ($l \cdot s^{-1}$) (% predicted)	12.0 +/- 3.1 117 +/- 18	8.5 +/- 1.1 ***** 110 +/- 15
$PEF_{25-75\%}$ ($l \cdot s^{-1}$) (% predicted)	4.8 +/- 1.2 95 +/- 16	4.0 +/- 0.6 96 +/- 18
Hb ($g \cdot dl^{-1}$)	14.4 +/- 1.2	13.1 +/- 0.6 *****
DLCO ($ml \cdot mg^{-1} \cdot mmHg^{-1}$) (% predicted)	39.2 +/- 3.3 98 +/- 8	29.5 +/- 4.7 ***** 98 +/- 15

Abbreviations: TLC = total lung capacity, FVC = forced vital capacity, $FEV_{1.0}$ = forced expired volume in one second, PEF = peak expiratory flow, $PEF_{25-27\%}$ = peak expiratory flow at 25-75% of FVC, Hb = hemoglobin, DLCO = diffusion capacity of the lung for carbon monoxide.

***** Significantly different from men ($p < 0.05$)

Table 3: Maximal Cardiorespiratory Variables (Day 1)

	Men (n = 6)	**Women** (n = 9)
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	54.8 +/- 1.8	46.5 +/- 10.7
(% predicted†)	103.2 +/- 16.4	96.4 +/- 19.8
$\dot{V}O_2$ ($l \cdot min^{-1}$)	4.0 +/- 0.9	3.2 +/- 0.7
$\dot{V}CO_2$ ($l \cdot min^{-1}$)	4.3 +/- 0.9	3.4 +/- 0.7 *
RER	1.08 +/- 0.05	1.08 +/- 0.05
V_E ($l \cdot min^{-1}$)	162.0 +/- 45.9	114.2 +/- 29.5 *
F*b* ($breaths \cdot min^{-1}$)	56.0 +/- 11.5	56.4 +/- 8.8
V*t* (l)	2.8 +/- 0.4	2.1 +/- 0.4 *
Heart Rate (bpm)	178 +/- 4	186 +/- 13
WR_{max} (watts)	313 +/- 70	251 +/- 49
$PEtCO_2$ (mmHg)	25.6 +/- 5.7	25.3 +/- 2.7
$V_E/\dot{V}O_2$	40.5 +/- 8.1	35.7 +/- 4.6
$V_E/\dot{V}CO_2$	37.3 +/- 6.9	33.9 +/- 5.1

Abbreviations: $\dot{V}O_2$= oxygen uptake, $\dot{V}CO_2$ = carbon dioxide output, RER = respiratory exchange ratio, V_E = minute ventilation, F*b* = frequency of breathing, Vt = tidal volume, WR_{max} = maximal work rate, $EtCO_2$ = end tidal CO_2, $V_E/\dot{V}O_2$ = ventilatory equivalent for oxygen, $V_E/\dot{V}CO_2$ = ventilatory equivalent for carbon dioxide.

* Significantly different from men ($p < 0.05$)
† $\dot{V}O_{2max}$ predicted values from Jones, 1997

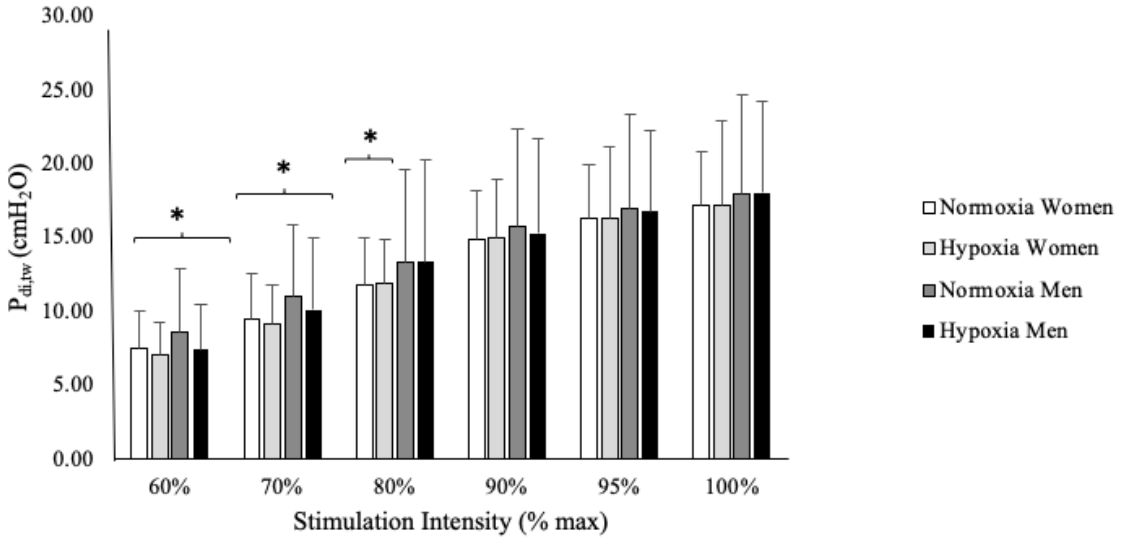

Figure 1: Absolute trans-diaphragmatic pressure output in response to increasing magnetic stimulator intensities for all conditions. Values are mean +/- SD. * Significantly different from the 100% twitch response in the respective condition.

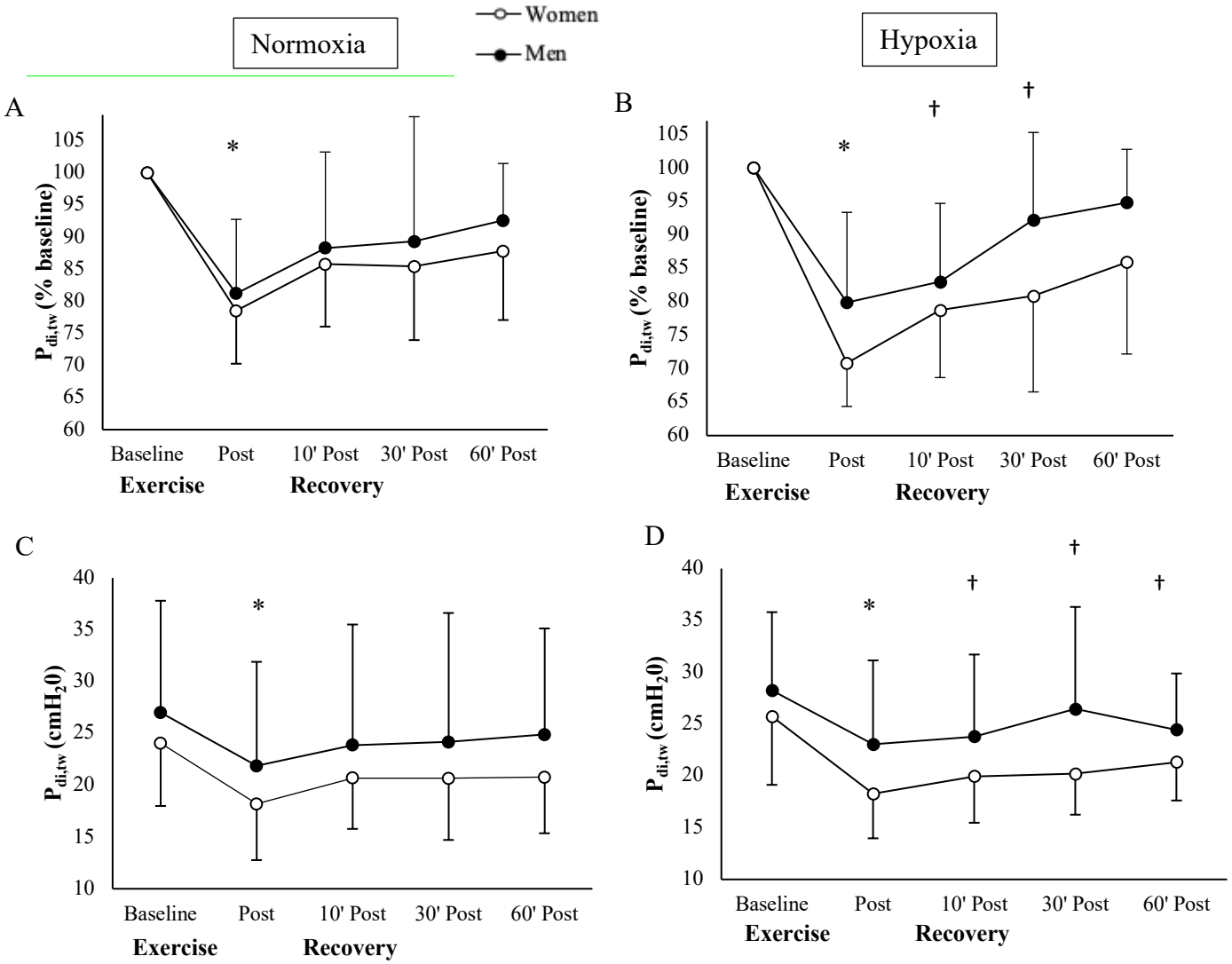

Figure 2(A-D): A + B: Diaphragm fatigue expressed as a percent of baseline for all participants. C + D: Diaphragm fatigue expressed at absolute $P_{di,tw}$ averaged for all participants. Figures A+C show the results in normoxia, and Figures B+D shows the results in hypoxia. Values are expressed as average +/- SD. * Significantly different from baseline for both sexes from respective values † Significantly different for only women from respective baseline

Table 4: Cardiovascular and Respiratory Variables during isowork, TTE cycling exercise in normoxia and hypoxia. Values are presented mean +/- SD. * Significantly different from beginning exercise (20% TTE) values in the respective group and condition ($p < 0.05$). No significant sex differences found.

		NORMOXIA (%TTE)					HYPOXIA (%TTE)				
		20	40	60	80	100	20	40	60	80	100
MEN	HR (bpm)	157 +/- 6	166 +/- 6*	172 +/- 5*	176 +/- 5*	176 +/- 5*	147 +/- 9	154 +/- 9	161 +/- 6*	165 +/- 5*	168 +/- 6*
	V_T (l)	2.8 +/- 0.5	2.9 +/- 0.5	2.8 +/- 0.3	2.8 +/- 0.4	2.6 +/- 0.3	2.8 +/- 0.5	2.9 +/- 0.5	2.9 +/- 0.5	2.7 +/- 0.5	2.6 +/- 0.3
	B*f* (bpm)	37 +/- 6	40 +/- 8	43 +/- 9	46 +/- 8	50 +/- 11*	34 +/- 5	39 +/- 9	45 +/- 9	50 +/- 10*	55 +/- 11*
	V_E (l/min)	108 +/- 37	122 +/- 38	125 +/- 40	136 +/- 32*	138 +/- 39*	103 +/- 25	119 +/- 45*	139 +/- 50*	148 +/- 52*	153 +/- 47*
	$\dot{V}O_2$ (l/min)	3.6 +/- 1.0	3.9 +/- 1.1	3.7 +/- 0.9	3.8 +/- 0.9	3.8 +/- 0.9	3.0 +/- 0.6	3.2 +/- 0.5	3.4 +/- 0.5	3.4 +/- 0.4*	3.5 +/- 0.5*
	RER	1.06 +/- 0.06	1.03 +/- 0.04	1.02 +/- 0.04	1.02 +/- 0.06	1.02 +/- 0.06	1.09 +/- 0.09	1.14 +/- 0.12	1.14 +/- 0.14	1.11 +/- 0.14	1.09 +/- 0.13
WOMEN	HR (bpm)	157 +/- 12	164 +/- 12	170 +/- 11*	170 +/- 10*	172 +/- 9	155 +/- 11	162 +/- 10*	163 +/- 14*	168 +/- 12*	171 +/- 13*
	V_T (l)	2.1 +/- 0.5	2.0 +/- 0.4	2.0 +/- 0.3	1.9 +/- 0.5	1.8 +/- 0.4*	2.2 +/- 0.5	2.1 +/- 0.5	2.0 +/- 0.4	2.0 +/- 0.5	2.0 +/- 0.4
	B*f* (bpm)	38 +/- 8	49 +/- 8	50 +/- 10	52 +/- 10*	60 +/- 12*	40 +/- 5	46 +/- 9	52 +/- 9*	53 +/- 8*	56 +/- 9*
	V_E (l/min)	84 +/- 27	92 +/- 25	98 +/- 28	102 +/- 32*	108 +/- 36*	87 +/- 25	96 +/- 25	105 +/- 28*	109 +/- 28*	112 +/- 28*
	$\dot{V}O_2$ (l/min)	2.8 +/- 0.8	2.8 +/- 0.8	2.9 +/- 0.8	3.0 +/- 0.9	3.1 +/- 1.1	2.5 +/- 0.6	2.5 +/- 0.6	2.6 +/- 0.6	2.7 +/- 0.6	2.7 +/- 0.6
	RER	1.01 +/- 0.06	1.00 +/- 0.06	0.98 +/- 0.06	0.96 +/- 0.06	0.96 +/- 0.06	1.09 +/- 0.07	1.1 +/- 0.05	1.09 +/- 0.05	1.05 +/- 0.07	1.05 +/- 0.06

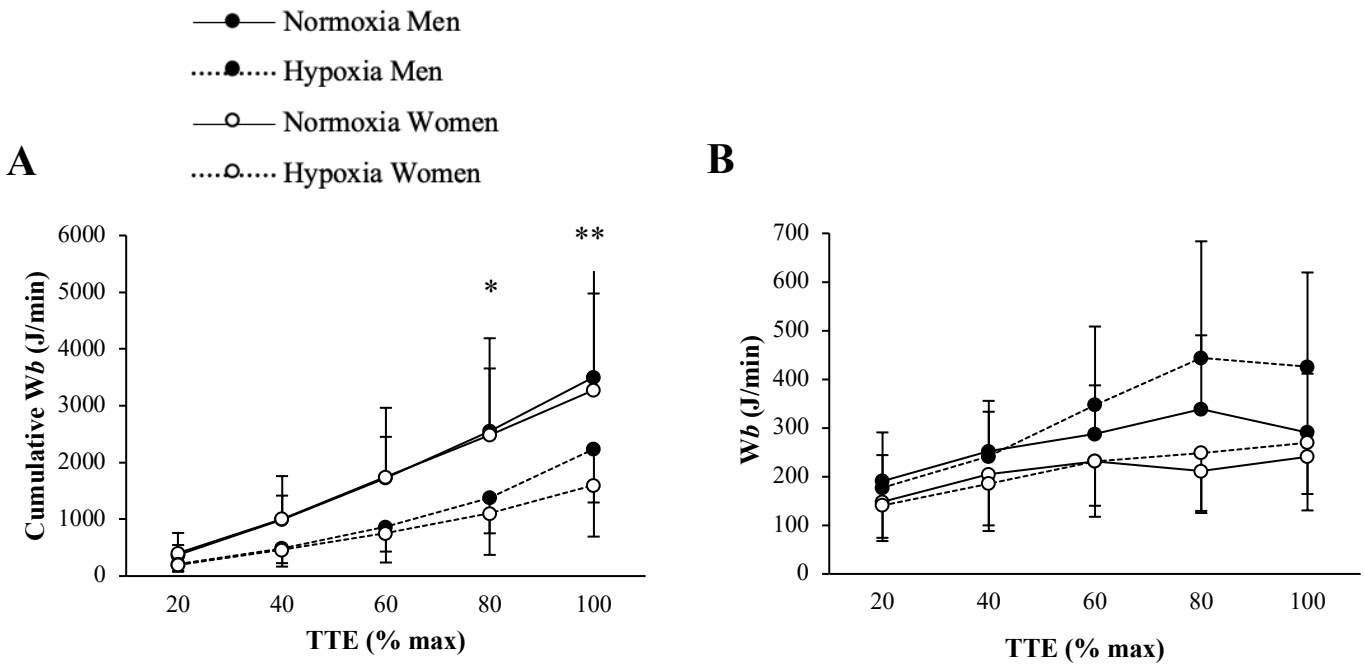

Figure 3(A + B): Cumulative and instantaneous total W*b* for both men and women with both F_IO_2 conditions. Values are mean +/- SD. * Significantly different based on F_IO_2 for <u>women only</u> ($p < 0.05$). ** Significantly differently based on F_IO_2 in <u>both sexes</u> ($p < 0.05$).

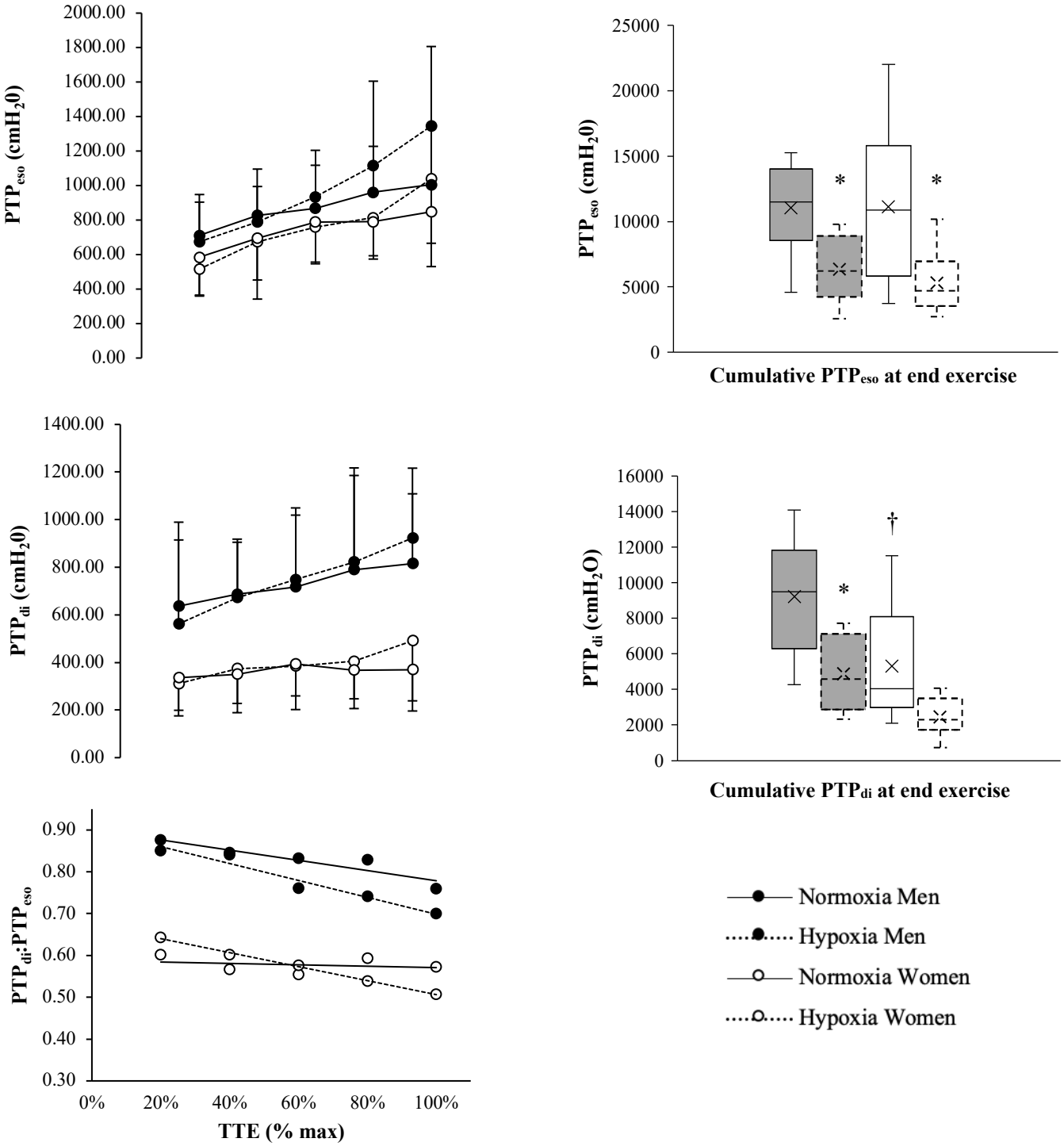

Figure 4(A-E): The absolute- and cumulative-pressure-time products, esophageal and diaphragmatic, and their relationship over time. Values are mean +/- SD. † Significant effect of sex ($p < 0.05$). * Significant effect of condition ($p < 0.05$).

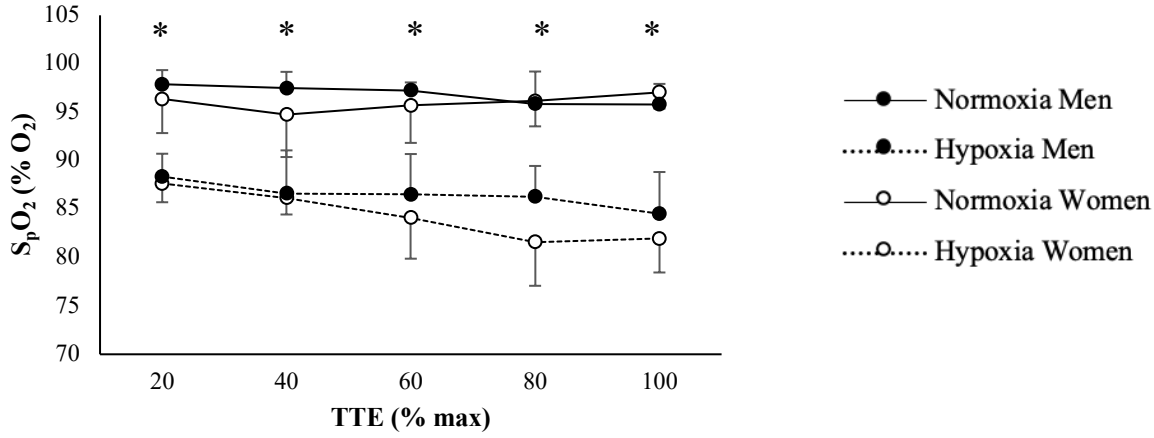

Figure 5: Saturation estimation by finger-clip oximeter. * Significantly different between conditions for <u>both sexes</u> ($p < 0.05$). Values are mean +/- SD.

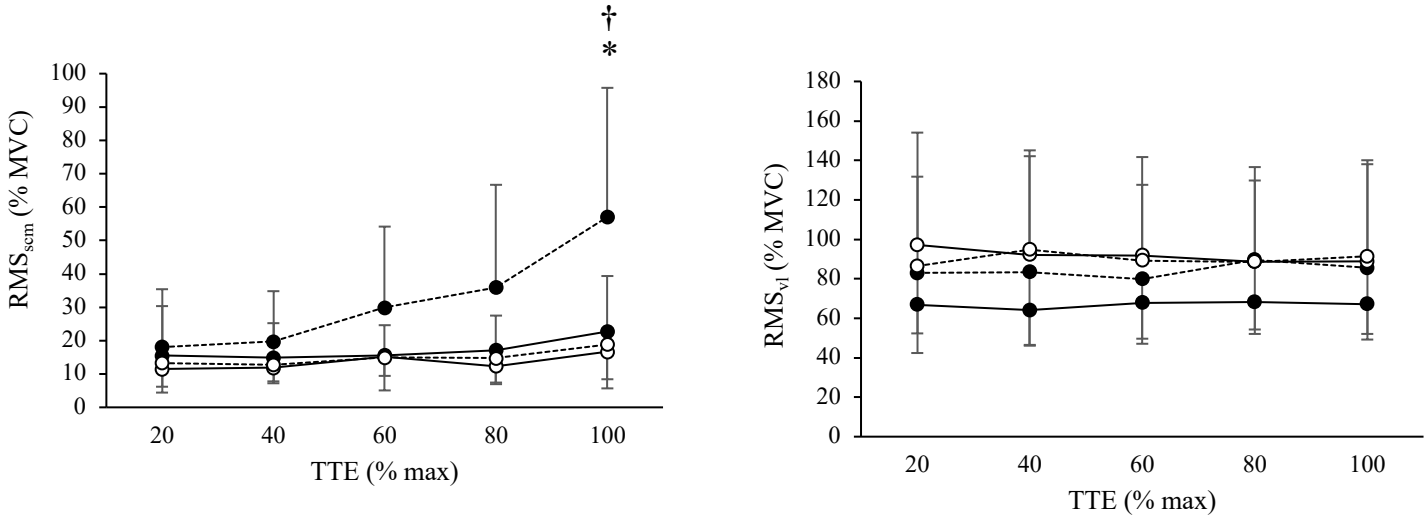

Figure 6(A + B): Electromyographic response of the left sternocleidomastoid (A) and left vastus lateralis with normoxic and hypoxic inspired FIO2.Values are mean +/- SD. * Significantly different <u>between men and women</u>. †Significantly different <u>between F_IO_2</u>.

Table 5: Absolute and developing levels of lactate as measured at the finger, during exercise. Values are mean +/- SD. * Significantly different from baseline. † Significantly different between normoxia and hypoxia.

	Baseline Lactate (mmol)	[La] - 2 minutes (mmol)	[La] – 4 minutes (mmol)	End Exercise Lactate (mmol)
Normoxia - Men	1.5 +/- 0.7	3.4 +/- 1.7	6.8 +/- 2.2 *	10.9 +/- 4.5 *
Normoxia - Women	1.8 +/- 0.6	4.5 +/- 0.5	6.8 +/- 1.7 *	10.1 +/- 2.6 *
Hypoxia - Men	1.1 +/- 0.5	5.9 +/- 1.7 *	9.1 +/- 4.4 *	12.1 +/- 4.7 *
Hypoxia - Women	1.6 +/- 0.5	5.5 +/- 0.9 *	8.7 +/- 1.6 *	12.0 +/- 2.5 *

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Appendix 1

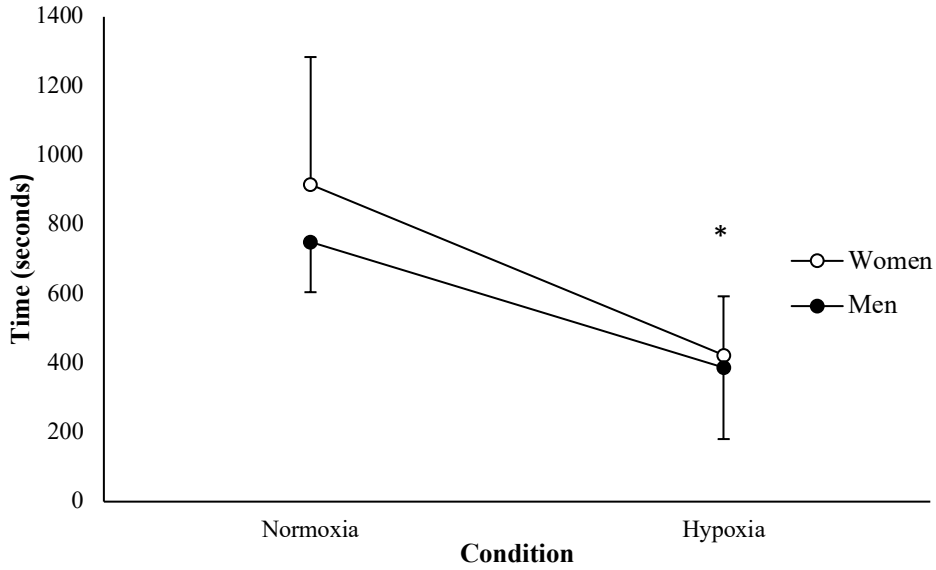

Figure A: Time to exhaustion in seconds for Day 2 and 3 (randomized). Values are mean +/- SD. * Significant difference from normoxic condition for both sexes ($p < 0.05$).

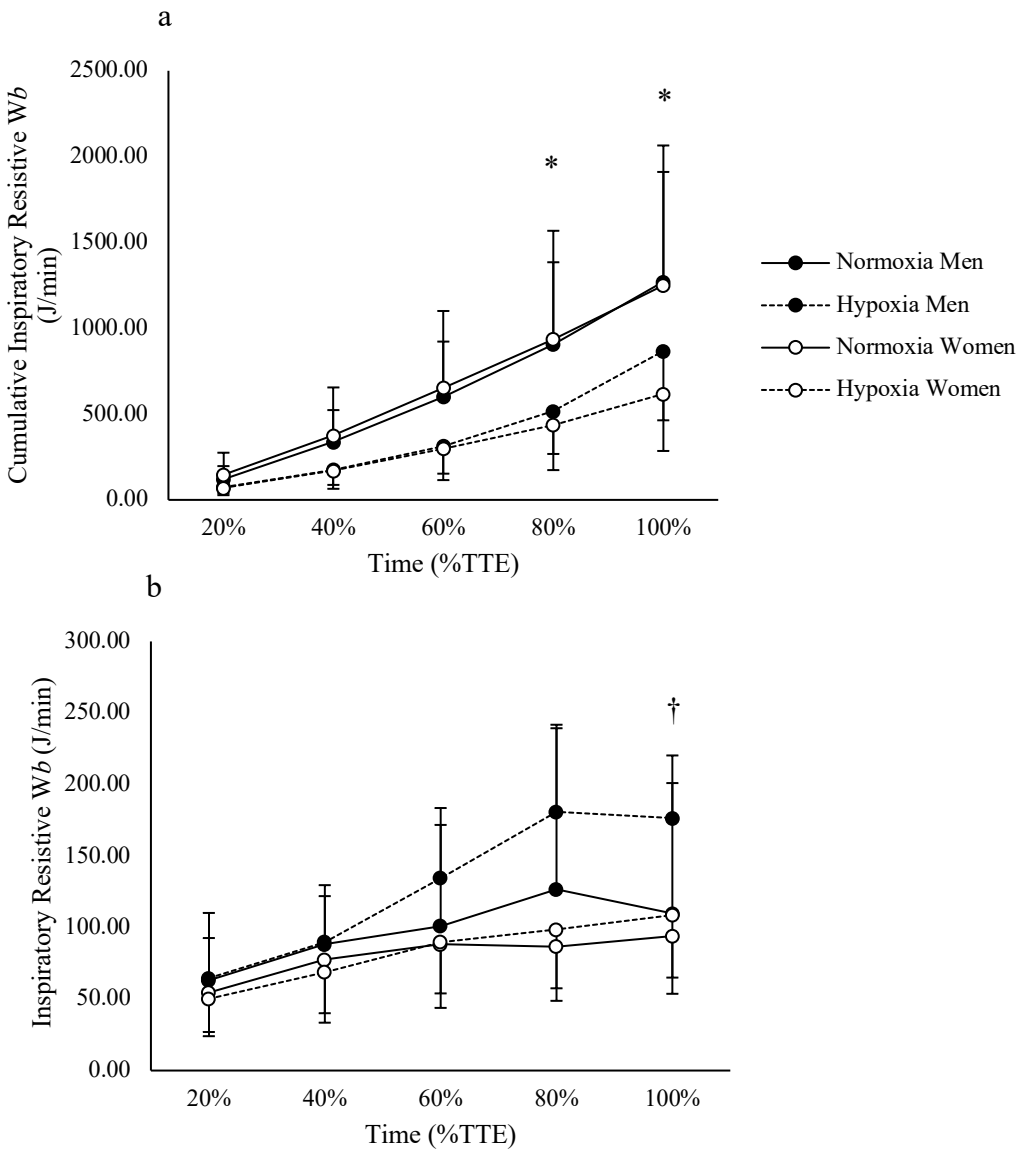

Figure B (a+b): Inspiratory resistive work of breathing, both cumulatively (a) and instantaneously (b). Values are means +/- SD. * Significantly different based on F_IO_2 for <u>women only</u> ($p < 0.05$). † Significantly different based on F_IO_2 for men only ($p < 0.05$).

Appendix 2

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Menstrual History Questionnaire

1. Are you having regular periods? YES/NO
2. How long is your cycle length? _____days
3. How many days long is your flow? _____days
4. Can you usually tell, by the way you feel, that your period is coming? YES/NO
5. Do you usually experience the following symptoms?

Breast tenderness	YES/NO
Appetite changes	YES/NO
Mood changes	YES/NO
Fluid retention	YES/NO

6. How many times did you menstruate in the past year? _____
7. How many periods did you miss in the last five years? _____
8. Are you currently taking oral contraceptives? YES/NO
    a. If yes, for how long? _____
    b. What is the name of the oral contraceptive that you are taking?

    _____
9. When was the last start of your period (Day 1)? _____