2008

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Your file Votre référence
ISBN: 978-0-494-46125-9
Our file Notre référence
ISBN: 978-0-494-46125-9

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by

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M.Sc. Candidate, Wilfrid Laurier University, 2008

THESIS

Submitted to Kinesiology and Physical Education, Faculty of Science

in partial fulfilment of the requirements for

Master of Science

Wilfrid Laurier University

Abstract

Exercise induced arterial hypoxemia (EIAH), expiratory flow limitation (EFL) and exercise induced diaphragm fatigue (EIDF) are examples of how the pulmonary system limits endurance exercise. The differences in the female anatomy and hormone fluctuations have been reported to cause differences in the occurrence of EIAH and EFL in males and females. EIDF has been reported to occur in males, but to date no investigations have reported the occurrence of EIDF in females. Therefore the purpose of this study was to determine the occurrence of exercise induced diaphragm fatigue (EIDF) in females identified by significant reductions in transdiaphramatic pressure (P_{di}) post exercise in response to supramaximal bilateral phrenic nerve stimulation.

Ten females 20 (± 0.37) years old completed exercise at 75 and 95% $V0_2max$ to volitional fatigue on 2 separate days, on approximately day 7 (± 2 days) of their menstrual cycle. Bilateral phrenic nerve stimulation in combination with balloon tipped catheters, used to measure esophageal and gastric pressure, were used to provide an objective measurement of trans-diaphragmatic pressure. BPNS was performed at 1, 10, 20, 50, and 100 Hz prior to exercise, immediately after exercise, 30, and 60 minutes after exercise.

Post exercise there were no significant reductions in P_{di} after the 75% $V0_2max$ exercise condition; however, in the 95% $V0_2max$ exercise condition P_{di} was significantly reduced with 1 and 50 Hz stimulation. Recovery from fatigue occurred within approximately 30 minutes after exercise. This study objectively reported the occurrence of EIDF in females exercising at 95% $V0_2max$.

Acknowledgements

This is quite possibly the hardest part of the thesis to write. It is difficult for me to express my gratitude enough to all that have contributed to my success, but I will try my best.

First of all, this thesis would not have been possible without the assistance of my supervisor Dr. Mark Babcock. Your patience and support have been much appreciated. Throughout this project I have grown and matured and I owe that all to you. I would also like to Dr. Peter Tiidus, Dr. Jill Tracy, and my external examiner Dr. Michael Wilkie for their careful reading and critique of my thesis.

Having people to provide support and encouragement is essential for success. All the faculty and staff in the KPE department at Wilfrid Laurier University have been very helpful to me, willing to answer any question regardless of the situation. I would like to especially thank Dr. Renee MacPhee for all her help and support. All of the KPE graduate students have been more than willing to offer support throughout this difficult process. I would like to particularly mention Sobia Iqbal, who in her own special way, gave me the motivation I needed.

My family has always tried to instil in me the importance of hard work. I now completely understand that hard work results in success, and that is something I will never forget. I would also like to thank Michael Fischer and Colleen Genova who are the two most generous people in the entire world. You two will never know how much you have helped me throughout the years.

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Chapter I

Introduction

Statement of the Problem

The structural and hormonal composition of the female anatomy is thought to produce differences in the response of the respiratory system to exercise. Therefore the purpose of this study was to determine the influence of exercise intensity and duration, on exercise induced diaphragm fatigue in normal healthy females with a broad range of fitness levels.

Introduction

The body of knowledge pertaining to female exercise physiology, specifically respiratory physiology, is very limited. A majority of exercise physiology studies have been conducted on young healthy men, which indicates that our understanding of the human body and its response to exercise is based on male physiology. Over the past 40 years, the popularity of women's sport have risen, demonstrated by the inclusion of the women's 1,500 meter run in the 1972 Olympics, the first female marathon in the 1984 Olympics and the creation of the woman's national basketball association (WNBA) in 1996.

When you consider a normal healthy person at rest breathes on average 15 times a minute, every minute of every day, it is obvious that the respiratory muscles are built for endurance. The human diaphragm muscle fibre type composition consists of approximately 55% slow twitch oxidative (Type I), which are highly fatigue resistant (Rochester, 1985). Approximately 25% of the muscle fibre type is fast twitch oxidative

(Type IIa), which are also relatively fatigue resistant; the remaining 20% of the muscle fibre type is fast twitch glycolytic (Type IIb), which are the most susceptible to fatigue of the three fibre types (Rochester, 1985). The small proportion of fast twitch muscle fibres in the diaphragm are important for high velocity functions such as coughing or sneezing, while the majority of the slow twitch muscle fibres are important for the phasic activity of breathing and sustained activity involved in maintaining posture (Polla *et al.*, 2004).

The diaphragm receives its blood supply from a complex network of arteries which consists of the internal mammary, the intercostals , and the inferior phrenic arteries (Supinski, 1988). Studies on mongrel dogs have shown an anastomotic connection between the three vessels and which is believed to occur in all mammals (Comtois *et al.*, 1987). The network of arteries is an important feature of the diaphragm which allows blood flow to remain constant if there is an occlusion in any of the three vessels. In fact, blood flow through non-occluded vessels will increase when any one of the blood vessels is occluded (Lockhat *et al.*, 1985). The diaphragm is designed for optimal oxygen delivery. The muscle fibres in the diaphragm are smaller in diameter than other skeletal muscles, this reduces the diffusion distance and makes oxygen transport more efficient (Mizuno, 1991). These types of features are important for muscles involved in prolonged activity to be able to sustain activity.

Exercise induced diaphragm fatigue has been shown to occur in males with a variety of fitness levels in whole body endurance exercise at an intensity of 85% $V0_2$ max or greater (Johnson *et al.*, 1993). Whole body endurance training alone does not cause sufficient structural alteration in the diaphragm to resist diaphragm fatigue in endurance exercise. Evidence has shown that "high fit" individuals (average $V0_2$max 69 ml/kg/min

or greater) are not more resistant to diaphragm fatigue compared to "fit" individuals (average $V0_2$max 50.4 ml/kg/min or less) (Babcock *et al.*, 1996). Any increase in diaphragm muscle strength in high fit individuals due to whole body endurance exercise typically does not improve performance because of the increased demand placed on the pulmonary system due to exercising at higher intensities. Incorporating an additional inspiratory muscle strength training protocol can improve the performance of the inspiratory muscles in exercise. Romer et al. (2002) has shown that focusing specifically on inspiratory muscle training at 50% of maximal mouth pressure reduced recovery time between high intensity sprint activity by 6.2% compared to the control group. Additionally, Romer et al. (2002) has shown that specific inspiratory muscle training reduced 20 and 40 KM time trials by 65 and 114 seconds respectively.

There is also an epidemiological importance for studying inspiratory muscles. Inspiratory muscle training has become a fundamental rehabilitation practice for many pulmonary diseases to increase exercise tolerance. Chronic obstructive pulmonary disease (COPD) is an umbrella term that consists of such diseases as bronchitis, asthma, or emphysema that cause airway flow limitations. A majority of the research has linked inspiratory muscle training with relieving the feeling of dyspnea by improving diaphragm muscle endurance in patients with COPD (Ramirez-Sarmiento *et al.*, 2002;Huang *et al.*, 2003) . The belief is that relieving dyspnea will allow COPD patients to exercise for longer durations. In actuality, patients with COPD typically lose the elasticity of the lungs which causes the lung to hyperinflate which leads to impairment of respiratory muscle function (Ferguson, 2006). Adaptations such as an increase in type I fibres, shortening of the diaphragm muscle fibres (Ramirez-Sarmiento *et al.*, 2002), and the loss

of muscle mass result in a reduction of the force generating capacity of the diaphragm (Gosker *et al.*, 2000). This is proposed to cause diaphragm fatigue earlier in COPD patients compared to healthy individuals.

Patients recovering from chronic heart failure (CHF) benefit from regular exercise to help with rehabilitation and reduce the occurrence of any further cardiovascular diseases. A study by Hughes et al. (1999) has confirmed that patients with CHF have a larger proportion of type I muscle fibres which results in a lower peak inspiratory generating capacity. The increase in the proportion of type I muscle fibres was thought to occur because of an increased respiratory workload. By increasing the proportion of type I muscle fibres the onset of fatigue can be delayed; however, the ability to generate larger forces was lost due to the reduction type II muscle fibres. The reduction in respiratory muscle strength was minor but caused an increase in difficulty of breathing (dyspnea) to occur which discouraged prolonged exercise. Inspiratory muscle training makes the diaphragm more fatigue resistant, allowing for CHF patients to exercise at higher intensities, which was beneficial in the rehabilitation process.

Diaphragm studies have shown that exercise induced diaphragm fatigue can be a limitation for males in whole body endurance exercise(Romer *et al.*, 2006;Harms *et al.*, 2000b). The notable differences between the male and female respiratory system encourages the investigation of how the female physiological response to exercise is different compared to males. Knowledge of these differences could change training and competition strategies. To date there have been no studies that have investigated the unique characteristics of exercise induced diaphragm fatigue strictly in females. Generalization of the male data on diaphragm fatigue has been applied to females. The

function of the respiratory muscles in males was all that was available and did not provide a sufficient model of function of the healthy respiratory muscles in females. Studying diaphragm fatigue in females would help to identify any differences in the function of the respiratory system in exercise between the two genders. Most importantly, this would also add to the already limited body of knowledge on female respiratory and exercise physiology.

Chapter II

Literature Review

Adaptations of the Respiratory System to Endurance Exercise

The response of the respiratory system to physical activity was to maximize oxygen intake to ensure adequate oxygen transport to the working muscles. In the normal healthy non-trained human the anatomical make up of the lung was more than adequate to meet the demands placed on it by whole body exercise. The pulmonary system responds by matching alveolar ventilation to metabolic demand. Changes also occur that would optimize the transfer of oxygen from alveolar gas to arterial blood, for example, adequate red cell transit time through pulmonary capillary bed, maintenance of minimal alveolar capillary diffusion distance and ventilation to perfusion ratios that are high and uniformly distributed at maximal exercise (Secher *et al.*, 1984).

Endurance exercise training was a stimulus for the body to improve the efficiency of oxygen transport and metabolism. This was very important as the body's demand for oxygen must be met in order to prevent fatigue. The four main systems responsible for oxygen transport are the lungs, heart, blood and muscles; working together in a coordinated fashion these systems move oxygen throughout the body. In a similar fashion to how resistance training increases muscle size, it would be thought that endurance training would increase functional lung size; however endurance exercise was not a stimulus for lung growth (Wagner, 2005). The only stimuli that has been shown to increase functional lung size were prolonged exposure to environment hypoxia and the removal of a substantial portion (50%) of the lung (Wagner, 2005). The benefit of increasing functional lung size would be to increase the capacity to inspire air and to

provide a larger diffusion surface area for oxygen and carbon dioxide exchange. The increase in the body's capacity to transport oxygen with endurance training was dependent on adaptations of the heart, blood, and muscle to transport oxygen. Theoretically, if endurance training was able to increase functional lung volume this would help to increase the limit of alveolar ventilation and delay the onset of fatigue.

The primary muscle involved in inspiration was the diaphragm. The external intercostals, the scalenes, and the sternocleidomastoid muscles are referred to as the accessory inspiratory muscles and were recruited as the ventilatory demand increased. Collectively, all of these muscles are referred to as the inspiratory muscles. The diaphragm, being the major muscle used in inspiration performs the bulk of the respiratory workload during endurance exercise. Whole body endurance training was thought to induce adaptations that would allow the diaphragm to handle a larger ventilatory demand. This was shown by Babcock et al. (1996a) where exercise induced diaphragm fatigue was compared between fit and high-fit male subjects (Babcock *et al.*, 1996). The high-fit group produced greater diaphragm pressure generation for the first 60% of exercise compared to the fit group, after this point the diaphragm pressure generation was not significantly different until the end of exercise between the two groups. Despite the fact the high-fit group produced more diaphragm pressure generation; there was no difference in the amount and duration of low-frequency fatigue between the two groups at the end of exercise. The increase in fatigue resistance of the high-fit group was believed to be from an increase in the aerobic capacity of the respiratory muscles with endurance training. Endurance trained rats have shown an increase in citrate synthase activity in type I muscle fibres in the diaphragm as well as a

decrease in type II muscle fibre distribution (Green *et al.*, 1989;Vincent *et al.*, 2002). The data from rats coincide with results of whole body training studies on inspiratory muscle performance where inspiratory muscle endurance was shown to improve (Eastwood *et al.*, 2001;Robinson & Kjeldgaard, 1982).

The minor adaptations of the inspiratory muscles due to whole body endurance training have little to no effect on the pulmonary system's ability to improve endurance exercise. The respiratory muscles are the only component of the pulmonary system that adapt to whole body endurance exercise. Reducing the work done by the diaphragm has been shown to reduce the amount of energy required by the respiratory muscles and also to reduce the amount of diaphragm fatigue after endurance exercise (Babcock *et al.*, 2002).

Limitations to Endurance Exercise

Continuous endurance exercise requires oxygen to fuel the body's metabolic processes. Once the body's demand for oxygen exceeds the capacity for oxygen uptake, fatigue occurs which eventually leads to exercise termination. So any situation where optimal oxygen delivery was prevented in the face of increasing oxygen demand was considered a limitation of endurance exercise. There are situations where the pulmonary system is the source of limitations for endurance exercise. The three examples of where the pulmonary system fails to meet the demands placed on it by endurance exercise are: 1) exercise induced arterial hypoxemia; 2) expiratory flow limitation; and 3) exercise induced diaphragm fatigue.

Exercise Induced Arterial Hypoxemia

Defining Exercise Induced Arterial Hypoxemia

The alveolar to arterial oxygen difference (A-a $D0_2$) was a measure of adequate alveolar ventilation and perfusion of the lungs. In moderately trained athletes it was expected to see a widening of the A-a $D0_2$ of about 15-20 mmHg without presenting a significant impact on oxygen delivery. However in highly trained athletes one might expect a difference of 35 mmHg or greater due to a combination of factors such as diffusion limitation and decreased blood transit time. This results in a reduction of the partial pressure of oxygen in the blood ($Pa0_2$) and inhibits the removal of carbon dioxide (Dempsey & Wagner, 1999). Metabolic acidosis and increased temperature during exercise results in a reduction in the arterial oxygen saturation ($Sa0_2$). Exercise induced arterial hypoxemia (EIAH) was a condition that typically occurred in a percentage of highly trained athletes exercising over 80% of their maximum intensity (Dempsey & Wagner, 1999). EIAH was thought to occur when oxygen transport was decreased to a point that was insufficient to meet the needs of the working muscles. Dempsey et al. (1984) have reported that 8 of 16 subjects tested ($V0_2$ max 72 ± 2 ml/kg/min) exhibited a reduction in $Pa0_2$ below 75 mmHg, which is in agreement with other studies that report EIAH occurred in about 50% of highly trained subjects (Dempsey *et al.*, 1984;Powers *et al.*, 1988). In the literature a number of different parameters have been used to define EIAH, for example, when the $Sa0_2$ falls below 88% (Dempsey & Wagner, 1999), or a reduction in $Pa0_2$ of 7.5 mmHg (Prefaut *et al.*, 2000). But the most commonly used definition was the one proposed by Dempsey and Wagner which stated; mild EIAH as a $Sa0_2$ between 93 – 95%, moderate EIAH as a $Sa0_2$ between 88 - 93%, and severe EIAH as a $Sa0_2$ below 88% (Dempsey & Wagner, 1999).

Consequences of Exercise Induced Arterial Hypoxemia

There are a number of proposed causes of EIAH which include: inadequate hyperventilatory response, increases in A-a $D0_2$, inequalities in ventilation and perfusion in the lung, diffusion limitation at the level of the alveolus, and increased capacity of the cardiovascular and metabolic systems (Dempsey & Wagner, 1999).

Numerous studies have shown EIAH to be a limiting factor of $V0_2$ max (Powers *et al.*, 1989;Aaron *et al.*, 1992;Stewart & Pickering, 2006;Grataloup *et al.*, 2005). This was done by adding oxygen to the inspirate gas to maintain $Sa0_2$ at resting levels. Interestingly enough, only the highly trained individuals who had exhibited EIAH showed an improvement in $V0_2$ max in these studies (Powers *et al.*, 1989). Powers et al. (1989) reported two very substantial findings; 1) in subjects with moderately severe EIAH ($Sa0_2 < 92\%$), every 1% decrement in $Sa0_2$ equals approximately a 1% reduction in $V0_2$ max; and 2) $V0_2$ max increased only in highly fit subjects ($V0_2$ = 70 ml/kg/min) compared to fit subjects ($V0_2$ = 56 ml/kg/min). These data provided evidence to suggest that the respiratory system contributes significantly to the limitation of $V0_2$ max rather than the oxidative capacity of the working skeletal muscles (Powers *et al.*, 1989). Grataloup et al. (2005) demonstrated the effects of preventing arterial desaturation on $V0_2$max in athletes that do and do not present symptoms of EIAH. The non-EIAH group had a $Sa0_2 \geq 95\%$ and the EIAH group had a $Sa0_2 < 95\%$ at maximal exercise. The two groups were similar across all other categories including $V0_2$ max. Each group exercised in normoxia (20.9% 0_2) and hyperoxia (30% 0_2). The results showed there were no differences in peak heart rate, ventilation, respiratory exchange ratio, blood lactate concentration and haemoglobin concentration at $V0_2$max between both groups in hyperoxic conditions, however, $V0_2$max was significantly higher in the EIAH group (4.9

L/min) in hyperoxic conditions compared to the non-EIAH group (4.5 L/min) (Grataloup *et al.*, 2005). This provides evidence that EIAH was a limiting factor in exercise performance in highly trained athletes. When EIAH was removed and arterial saturation was restored, these athletes had the ability to work at a higher $V0_2max$ than athletes that do not exhibit EIAH symptoms.

Convincing evidence has shown that EIAH negatively impacts $V0_2$ max and endurance exercise performance; however, there are still questions as to how this occurs. Romer and Dempsey (Romer & Dempsey, 2006) were concerned whether EIAH induced mechanisms affected peripheral muscle fatigue. To determine this, they investigated the effects of arterial desaturation on quadricep muscle fatigue after cycling trials. When $Sa0_2$ was kept at 98% using hyperoxia (F_I0_2 0.28) during exercise supramaximal magnetic stimulation of the femoral nerve showed a 16% reduction in quadriceps muscle force, versus control (F_I0_2 0.21) conditions where a 33% reduction in quadriceps muscle force was recorded in reaction to supramaximal stimulation (Romer & Dempsey, 2006). Prevention of arterial desaturation also reduced the rate and the amount of lactate produced as well as limb discomfort and dyspnoea (Romer & Dempsey, 2006). This demonstrated that muscle force reduction can be significantly attributed to EIAH preventing adequate oxygen transport to the working muscles.

Expiratory Flow Limitation

The action of inspiration at rest was mainly coordinated by the contraction of the diaphragm and expiration at rest was a passive process with the relaxation of the diaphragm and recoil of the lungs and chest wall. With the transition to exercise the tidal volume (V_T) increased from approximately 1 L to 3 L to allow more oxygen to enter

circulation. The increase in V_T was a result of coordinated recruitment of the respiratory muscles during exercise to reduce the amount of air remaining in the lungs after expiration; known as the end expiratory lung volume (EELV), and increased the amount of air in the lungs at the end of inspiration; known as end inspiratory lung volume (EILV). EILV in healthy subjects can increase to as high as 75 – 90% of total lung capacity (TLC) (Johnson *et al.*, 1999). The expiratory muscles are recruited to reduce EELV below functional residual capacity (FRC). As a result, at the end of expiration the diaphragm was at a shorter muscle length which was optimal for generating force. So in preparation for the next inspiration the diaphragm was able to produce more force more easily.

Defining Expiratory Flow Limitation

The pulmonary system provides mechanical constraints that limit the flow rate of air during inspiration and expiration in healthy humans. Increased tidal volume during exercise would eventually reach a level where the transpulmonary pressure during expiration exceeds the minimal required pressure to produce maximal expiratory flow. A progressive increase in transpulmonary pressure as minute ventilation reached maximal voluntary ventilation lead to the occurrence of expiratory flow limitation (EFL) (Johnson *et al.*, 1999). Expiratory flow limitation was typically a concern for highly trained individuals or those that could reach a rate of ventilation (> 120 L/min) that encroaches on the maximum expiratory boundary(Johnson *et al.*, 1992). The extent of the EFL was varied based on the individual due to such factors as airway resistance, as seen in diseased individuals (Stevenson *et al.*, 2005), and the amount of transpulmonary pressure generated by the expiratory muscles (Pellegrino *et al.*, 1993a). The most commonly used

technique to identify EFL requires patients to perform maximal inspiratory and expiratory maneuvers to produce the maximal flow volume loop between total lung capacity (TLC) and residual volume (RV) to develop a reference for the maximal flow volume envelope (Johnson *et al.*, 1999). The extent of EFL could be calculated by studying flow volume loops during exercise; the percentage of the expiratory tidal volume that impinges on the maximal expiratory flow volume envelope would determine the degree of limitation that was present (Johnson *et al.*, 1999).

Consequences of Expiratory Flow Limitations

Hyperinflation, which is defined as an increase of functional residual capacity (FRC) (Koulouris *et al.*, 1995), occurred as a result of premature closure of the airways during expiration. The large pressure generated during peak expiratory flow would cause dynamic compression of the small airways and would reduce the expiration time; as a result EELV would increase (Pellegrino *et al.*, 1993b). Increasing lung volumes have been shown to decrease the ability to maintain breathing tasks against resistive loads (Roussos *et al.*, 1979;Tzelepis *et al.*, 1988). The increase in EELV as seen in hyperinflation eventually leads to a decrease in diaphragm muscle length and as a result of the length tension relationship of skeletal muscles the efficiency of the inspiratory muscles would be reduced. Decreasing diaphragm muscle length reduces the velocity of shortening and the ability to generate inspiratory pressure (Johnson *et al.*, 1992). In addition, due to the increased amount of excitation that was required to produce the same amount of sub-maximal tension, more type II muscle fibres may be recruited which are less fatigue resistant (Tzelepis *et al.*, 1988). In studies on subjects that are flow limited, it has been shown that the increase in minute ventilation was due primarily to the increase

in breathing frequency rather than the increase in tidal volume (Johnson *et al.*, 1992). The increased breathing frequency raised a concern for an increased energy demand on the respiratory muscles due to the addition of the required work of breathing.

Exercise Induced Diaphragm Fatigue

Diaphragm Muscle Fatigue

The measure of diaphragm fatigue was determined by changes in trans-diaphragmatic pressure (P_{di}) which represent the pressure developed across the diaphragm. Trans-diaphragmatic pressure was determined by the addition of esophageal pressure and gastric pressure. As ventilation increased, the diaphragm developed more pressure which raised gastric pressure and allowed P_{di} to increase (Bye *et al.*, 1984). This suggested continued increase in P_{di} did not completely coincide with the idea of diaphragm fatigue. Other studies have indicated that P_{di} levels off with endurance exercise (Johnson *et al.*, 1993). In this scenario the accessory muscles increased their contribution to pressure generation as indicated by an increase in esophageal pressure, as the gastric pressure declines.

Given the importance and high aerobic capacity of the diaphragm, it has been found to fatigue during intense whole body endurance exercise; however the diaphragm has not been found to reach task failure during whole body endurance exercise. With this in mind an appropriate definition has been developed to describe respiratory muscle fatigue which is "a loss in the capacity of the muscle for developing force and/or velocity of a muscle shortening, resulting from muscle activity under load and which was reversible by rest" (NHLBI Workshop, 1990). Johnson et al. (1993) have shown diaphragm fatigue to occur after 14 – 30 minutes of intense whole body endurance

exercise, in male subjects of a variety of fitness levels who exercised above 85% $V0_2$ max with recovery occurring after 70 minutes. In comparison, EFL and EIAH only occurred in highly trained individuals; therefore diaphragm fatigue affects a larger proportion of the population. Diaphragm fatigue was displayed by a substantial reduction in the diaphragm's response to a supramaximal electrical stimulation. Babcock et al. (1995) have shown that at rest, voluntary mimic of the exercise ventilation does not result in significant diaphragm fatigue. These results seem to indicate that other factors besides the mechanical work of the diaphragm alone contributed to the appearance of diaphragm fatigue. The proposed theory stated that during whole body endurance exercise there was competition for circulating blood between the diaphragm and the working muscles which created a situation where the diaphragm was susceptible to fatigue (Babcock *et al.*, 2002)

Implications on Performance

We are concerned with diaphragm fatigue because of the consequences of the cardiovascular response to endurance exercise. It has been determined that at maximal work rates, the respiratory muscle work used 15% of the total $V0_2$ (Aaron *et al.*, 1992) and 14 – 16% of the cardiac output (Harms *et al.*, 1998b). Manipulating the work of breathing demonstrated how the oxygen and blood requirements of the respiratory muscles change during exercise. When the work of breathing was mechanically increased, the working muscles used approximately 71% of the total $V0_2$, and the blood flow to the exercising muscles was reduced by approximately 1.3 L/min (Harms *et al.*, 1997). When using a ventilator that reduced the work required by the inspiratory muscles, referred to as unloading, the work of breathing was reduced and total $V0_2$ was significantly reduced at the same work rate. The working muscles used approximately

89% of the total $V0_2$, and the blood flow to the exercising muscles was increased by approximately 0.8 L/min (Harms *et al.*, 1997). This study implied that the competitive relationship for blood between the working muscles and the inspiratory muscles can compromise performance.

Given the documented increase in respiratory muscle oxygen uptake and cardiac output, the question arises as to how this impacts exercise performance. By unloading the diaphragm the work of breathing was reduced and the onset of diaphragm fatigue was prevented (Babcock *et al.*, 2002). This decreased the sympathetic nervous system activity which reduces the vasoconstrictor response to exercise and maintains blood flow to the working muscles. As a result, when exercising at 90% $V0_2$ max, exercise time was extended by approximately 14% and rating of perceived exertion (RPE) of the limbs and breathing were reduced during exercise (Harms *et al.*, 2000b). In fact, reducing the work of breathing had been shown to attenuate quadriceps muscle peripheral fatigue compared to control (Romer *et al.*, 2006). This indicated that a high level of respiratory muscle work during intense endurance exercise had an effect on exercise performance and locomotor muscle fatigue.

Gender Differences in Pulmonary System Limitations

Previous investigations have provided examples of situations where the pulmonary system was not able to meet the demands placed on it by whole body endurance exercise due to: 1) EIAH; 2) Expiratory flow limitations; and 3) exercise induced diaphragm fatigue. The majority of the research in this area has been conducted exclusively using male subjects. Due to gender differences, generalization of these results to females was not possible. This has lead to the development of research

investigating the response of the female pulmonary system to whole body endurance exercise. A combination of pulmonary system structural differences and hormonal differences between males and females may possibly contribute to the differences seen in the way the female pulmonary system responds to endurance exercise.

Gender Differences in Pulmonary System Structure and EFL

The female lung has been shown to be smaller in size compared to the male lung, even after accounting for sitting height, standing height, body mass, and trunk length (McClaran *et al.*, 1998). Females have reduced airway diameter, a smaller vital capacity (Harms *et al.*, 1998a), and reduced diffusion area compared to males, which was accounted for by less alveoli as a result of smaller lungs (Thurlbeck, 1982). These structural differences are the cause of an increased occurrence of EFL in females compared to males (McClaran *et al.*, 1998). The data from males indicate that EFL typically occur in highly trained endurance males (Johnson *et al.*, 1992). Even with a similar proportion of the respiratory capacity being used, less fit women (48 ml/kg/min) have been shown to exhibit minimal EFL at maximal exercise which was contradictory to what had been shown in males. This was attributed to the smaller V_T and maximum expiratory flow rates in females creating a smaller maximum flow-volume envelope (McClaran *et al.*, 1998). To compensate for smaller tidal breathing, females increased their breathing frequency to maintain sufficient ventilation. Johnson et al. (1992) reported the maximum frequency of breathing (f_b) in males to be 58 breaths/min, while McClaran et al. (1998) reported the maximum f_b in females to be 53 breaths/min. This was not a valid comparison because males were exercising at a higher $V0_2$ max. At maximum intensity highly trained females were exercising at a $V0_2$ max of 62.9

ml/kg/min which is equivalent to approximately 83% of the male's $V0_2$ max. The maximum f_b for females was higher than the comparable f_b for males (44 breaths/min). The greater f_b, as well as an earlier onset of EFL lends evidence to an increased work of breathing in females compared to males. Aaron et al. (1992) provided evidence of an increased work of breathing in female subjects, where the only female in a group of 8 subjects had the largest percentage EFL, and the largest $V0_2$ of the respiratory muscles ($V0_{2\,RM}$) at 70 and 100% $V0_2$ max. This resulted in the highest energy requirement of the respiratory muscles despite not having the highest V_E (Aaron *et al.*, 1992).

Gender Differences in Exercise Induced Arterial Hypoxemia

As we discussed earlier, EIAH occurs in approximately 50% of highly trained males. Based on the structure of the female pulmonary system, the question was whether the prevalence of EIAH was different in females. Harms et al. (1998a) concluded that females are more susceptible to EIAH compared to males. In this study of 29 females of varying fitness levels (43 – 70 ml/kg/min), 22 of them (76%) presented a reduction in $Pa0_2 > 10$ mmHg and a decrease in $Sa0_2$ between 87 – 94% during the mid-follicular phase of the menstrual cycle (Harms *et al.*, 1998a). With just the one study, females seem to have a higher prevalence of EIAH compared to males; however, other studies have shown the prevalence to be lower. Hopkins et al. (2000) showed 4 of their 17 subjects (24%) presented EIAH, but observed the prevalence of EIAH over different phases of the menstrual cycle. Richards et al. (2004) reported 35 of their 52 subjects (67%) presented EIAH during the early follicular phase of the menstrual cycle. Richards et al. (2004) was closer to the initial estimate of Harms el al. (1998a) for the prevalence of EIAH in women, but Richards et al. (2004) used cycling instead of treadmill running

which has been shown to result in a higher $Pa0_2$ (Hopkins *et al.*, 2000). EIAH has been correlated to aerobic capacity in males, indicating that males with a $V0_2$ max greater than 150% of predicted values exhibit EIAH, whereas in females there is still a large discrepancy as to how aerobic capacity correlates to EIAH (Hopkins *et al.*, 2000;Richards *et al.*, 2004;Harms *et al.*, 1998a). The increase in EIAH prevalence in females has been partly attributed to the structure of their pulmonary system but also to a blunted hyperventilatory response to exercise. The subjects that showed the greatest amount of arterial desaturation during moderate activity (74% $V0_2$ max) did not show any significant hyperventilation (Harms *et al.*, 1998a).

There have been very few studies that have looked at the effects of EIAH on performance in women. Harms et al. (Harms *et al.*, 2000a) showed that females that are subject to EIAH, when given an hyperoxic inspirate, increased their $V0_2$ max, which was similar to what was seen in males. The difference between the two groups was that a reduction in $Sa0_2$ by at least 3% in females was required to see any significant increases in $V0_2$ max with an oxygen inspirate; whereas in males $Sa0_2$ must be reduced to 92 – 93% before the oxygen inspirate had any effect on $V0_2$ max (Powers *et al.*, 1989). This may raise some questions pertaining to the differences of pulmonary gas exchange between men and women. When this was investigated no significant differences were seen between male and female pulmonary ventilation and perfusion. In fact, females proved to have slightly better pulmonary gas exchange (Olfert *et al.*, 2004). The findings are somewhat questionable since the $V0_2$ of the matched male and female subjects ranged between 39 – 57 ml/kg/min. At this fitness level subjects did not exhibit any significant desaturation to indicate they were subject to EIAH. Despite this study, cumulative data

from Dempsey et al. (1984), Harms et al. (1998a), Hopkins et al. (2000), Olfert et al. (2004) and Rice et al. (1999) have shown that 12% of females with a $V0_2$ max less than 50 ml/kg/min had evidence of gas exchange impairment, whereas, in males less than 2% had gas exchange impairment (Harms, 2006).

Hormonal Changes in Ventilation at Rest and Exercise

The human menstrual cycle was a reoccurring cycle that repeats itself every 28 days and was associated with hormone fluctuations. For our purposes the hormones of importance are estrogen and progesterone. Estrogen concentration was highest during the follicular phase while the luteal phase was associated with peak levels of progesterone as well as with an increased concentration of estrogen. The fluctuation of estrogen and progesterone has been associated with changes in physiological functions in humans. The rate at which air leaves the lung (ventilation) at rest has been reported to be higher in females during the mid-luteal phase compared to ventilation during the mid-follicular phase in both athletic and non-athletic women (Schoene *et al.*, 1981;Slatkovska *et al.*, 2006). Increased resting ventilation during the luteal phase was thought to be caused by the increase in progesterone and estrogen. Studies in rats have shown that increasing the estrogen concentration increased the number and affinity of progesterone receptors (Brodeur *et al.*, 1986). Only when the two hormones concentrations were increased together does one see an increase in resting ventilation. When men and women are compared at rest one sees a different ventilatory response. Men seemed to have a higher resting ventilation and end tidal $C0_2$ ($PETC0_2$) compared to women during the follicular phase; however, the difference in ventilation was removed when body surface area was corrected for (Aitken *et al.*, 1986). When resting ventilation of men and women was

found to be similar, the ventilatory pattern was shown to be different; where the frequency of breathing was higher in women and the tidal volume was lower (Sebert, 1983).

Even though resting ventilation (V_E) was higher during the luteal phase, this may not have any implications on performance or $V0_2$ max. Despite the numerous studies on the effects of the menstrual cycle on exercise performance, there was still a lot of discrepancy in the results. This comes from testing during different times of the menstrual cycle and the variability of menstrual cycle hormone concentrations. As some research showed that V_E during exercise increased during the luteal phase of the menstrual cycle (Schoene *et al.*, 1981;Jurkowski *et al.*, 1981), there was also evidence that V_E during exercise did not change (Beidleman *et al.*, 1999). While there was still some confusion as to the changes in V_E, it is believed this does not have any impact on $V0_2$ max (Jurkowski *et al.*, 1981;Beidleman *et al.*, 1999).

Gender Differences in the Aerobic Capacity of the Respiratory Muscles

In the few studies that have looked at gender differences and skeletal muscle function, it has been shown that females fatigue more slowly compared to males and recover from fatigue more quickly (Fulco *et al.*, 1999;Semmler *et al.*, 1999). The results are supported by studies that indicate an enhanced oxidative phosphorylation capacity of female skeletal muscles compared to males (Nygaard, 1981). There have been some suggestions that estrogen affects muscle contractility. Sarwar et al. (1996) reported that when the circulating estrogen concentration was highest, quadriceps muscle strength and handgrip strength both increased approximately 11%, the relaxation time was slower and

the muscle was more easily fatigable compared to other phases of the menstrual cycle when estrogen concentrations were lower.

Considering the diaphragm was a skeletal muscle, it would be assumed that these traits would be carried over to diaphragm muscle function. Since there have been no studies that looked at exercise induced diaphragm fatigue exclusively in females, we can only make assumptions as to the consequences to endurance exercise.

Purpose

The purpose of this study was to determine the influence of exercise intensity and duration, on exercise induced diaphragm fatigue in normal healthy females with a broad range of fitness levels.

Hypotheses

The following hypotheses were suggested for the possible changes in transdiaphragmatic pressure (P_{di}):

a) No significant difference would exist between $P_{di\ Control}$ and $P_{di\ Post\text{-}Exercise}$ in females exercising at 75% $V0_2max$ until volitional fatigue.

b) A significant difference would exist between $P_{di\ Control}$ and $P_{di\ Post\text{-}Exercise}$ in females exercising at 95% $V0_2max$ until volitional fatigue.

An additional hypothesis was suggested for the comparison of EIDF in males and females:

a) The magnitude of EIDF post exercise will be larger in females compared to what has been previously reported in males while exercising at 95% $V0_2max$.

To determine the occurrence of EIDF at the 75 and 95% $V0_2max$ conditions, bilateral phrenic nerve stimulation was used to obtain a measurement of trans-diaphragmatic

pressure prior to and after exercise. A significant reduction in trans-diaphragmatic pressure post exercise indicated the occurrence of EIDF. A comparison of the magnitude of EIDF in females in this investigation was made against the magnitude of EIDF in males in a study conducted by Johnson et al. (1993).

Chapter III

Methods

Subjects

Ten young adult females, between the ages of 19 – 21 were used in this study. Participants that met the inclusion criteria were tested in the respiratory physiology lab at Wilfrid Laurier University. The study was approved by the Research Ethics Board of Wilfrid Laurier University. Prior to giving consent, participants were invited for a preliminary visit to the laboratory to discuss the full details and expectations of the study. During this initial visit subjects were asked to complete a Par-Q and the consent form.

Exclusion Criteria

Potential participants were excluded if they had any prior history of smoking, cardiopulmonary or respiratory illnesses. Inclusion in the study required participants to have a regular menstrual cycle. Normal resting pulmonary function was assessed on the first visit to the laboratory before the maximal aerobic capacity test. Any subjects that fell below the normal range for the ratio of forced expiratory volume in 1 second (FEV1/FVC) and forced vital capacity were excluded from the study.

Subjects that were taking triphasic birth control pills were allowed to participate in the study. Previous studies comparing the function of the respiratory system in females have also used females taking triphasic birth control, as they mimic the normal fluctuations in the hormones during the menstrual cycle (Wetter *et al.*, 2001;Guenette *et al.*, 2007). Importantly, there were no changes reported in ventilation between women taking triphasic birth control and a placebo (Lebrun *et al.*, 2003).

Bilateral Phrenic Nerve Stimulation

The phrenic nerve is the only nerve that directly innervates the diaphragm muscle. Electrically stimulating this nerve will cause the diaphragm to contract and produce trans-diaphragmatic pressure, therefore, allowing non-volitional control of the diaphragm. Electrical stimulation of the phrenic nerve was delivered superficially using two stimulating rods attached to a constant current stimulator (Digitimer DS7AH) at a frequency of 1, 10, 20, 50 and 100 Hz controlled by a computer program (Chart software V5.5 Powerlab). To confirm the phrenic nerve was stimulated supramaximally, pairs of EMG electrodes (bluesensor snap electrodes) were placed over the right and left hemidiaphragm in the seventh or eighth intercostal space midway between the anterior axillary and midaxillary line on each side of the thorax. When the phrenic nerve was stimulated the M-wave recording was passed through a bio amplifier (ADI instruments Dual Bioamplifier ML135) which was attached to the Powerlab system.

An illustration as described below is provided in Appendix 2 Figure 7. Two balloon tipped catheters (ref 47-9005, Ackrad Labs) were each attached to a separate differential pressure transducer (Validyne MP45); the pressure transducer was connected to a sine wave carrier demodulator (Validyne CD15A) and these were used to measure P_e and P_g. Lidocaine gel (2%) was used to numb the nasal passage. Approximately 3 ml of lidocaine gel was placed into a syringe tube, and the subjects were asked to squirt the gel into their nostril and sniff the gel to coat their nasal passage. Each balloon tipped catheter was individually inserted through one nostril until we could see the tip in the back of the throat. We then asked the subject to take small continuous sips of water through a straw to help swallow the balloon. One catheter was inserted to rest in the stomach and the other to rest in the lower 1/3 of the esophagus. Using a syringe, the balloon that

measured esophageal pressure was inflated with 0.8 ml of room air, and the balloon that measured gastric pressure was inflated with 1 ml of room air. To locate the phrenic nerves, the stimulating rod was activated once per second by the computer program at a sub maximal current until the phrenic nerve was found, the location was then marked for reference. The stimulation current was then increased until the M-wave amplitude reached a plateau. The stimulation current was then increased by 10% to ensure the maximal M-wave amplitude was attained. The procedure to obtain the M-wave was repeated for the stimulation control and immediately after exercise.

Bilateral phrenic nerve stimulation (BPNS) at 1 Hz (1 Hz twitch) was always performed at each subject's functional residual capacity (FRC) with subjects relaxed, nose clips on and the airway occluded. After stimulation, the occlusion was removed from the mouthpiece and the subject was asked to inhale to TLC. Single 1 Hz (twitch) stimulations were repeated 5-8 times to ensure reproducible P_{di} and M-wave measurements were obtained. Paired twitch stimulation at 10, 20, 50, and 100 hz were repeated 3-5 times. For each endurance exercise test, baseline values of diaphragm muscle stimulation and pressure generation were taken prior to exercise (pre-exercise, control period). Post exercise values for stimulation and pressure were performed immediately after exercise, 30 minutes post-exercise and then at 30 minute intervals until measurements returned to pre-exercise control values.

Transdiaphragmatic pressure was defined as the difference between esophageal pressure and abdominal pressure, it was calculated as the difference between P_e and P_g (Agostoni & Rahn, 1960). The equation to determine P_{di} is $P_g - P_e$ (P_e is a negative number). The P_{di} was automatically calculated into the integral of P_{di} ($\int P_{di}$) by the

computer. When $\int P_{di}$ is multiplied by f_b, the time integral is obtained measured in the units of cm H_2O/min. This value represents the amount of pressure generating work being done by the diaphragm. The same method will be used to calculate the time integral for P_e ($\int P_e$) which represents the pressure generating work done by all the inspiratory muscles.

We define fatigue as the reduction in electrically induced muscle force which exceeds a fall in P_{di} by at least 11%. Transdiaphragmatic pressure measure by 1, 10, 20, 50, and 100 Hz stimulation at FRC will be shown by a bar graph.

$V0_2$max Test

This test was performed on a magnetically braked cycle ergometer (Velotron CS 1.5). At the start of the test, each participant was equipped with a heart rate monitor (Polar brand) and then they mounted the cycle ergometer and made necessary adjustments for seat and handlebar height. A facemask was strapped tightly covering the mouth and nose of the subject. The mass flow meter from the metabolic cart was then securely attached to the facemask for gas analysis. Subjects sat at rest on the cycle ergometer for 3 minutes while we collected resting heart rate and respiratory data.

The test started with the subject pedaling at a work rate of 50 watts. The work rate was first increased to 100 watts after 2.5 minutes, and then each subsequent 2.5 minutes the work rate was increased by 30 watts until volitional fatigue. During the last 20 seconds of each work rate the subject's heart rate was recorded as well as a rating of perceived exertion (RPE). During exercise, ventilation (V_E), oxygen uptake ($V0_2$), carbon dioxide expiration ($VC0_2$), tidal volume (V_T) and frequency of breathing (f_b) were

collected by the metabolic cart. Maximal workload was determined by the stage with the largest average V02.

The Vmax 229 system collected the ventilatory parameters and there was a separate computer that was used to collect P_e and P_g pressure during exercise. These computer systems are not linked together. To align the exercise data, we align the end of exercise time between the two systems.

Experimental Design

This study required three visits to the laboratory. On the first visit to the lab anthropometric measurements (height and weight) were taken for each participant in the study. Subjects underwent pulmonary function tests on the metabolic cart which consisted of measuring maximal inspiratory and expiratory flow rates as well as lung volume. The metabolic cart was calibrated according to manufacturer's standards prior to each test session.

Upon completion of the pulmonary function tests, the participant was prepared for the V02 max test. After the $V0_2$max test, participants were given a cool down period to bring their heart rate below 120 beats/min.

On the second and third visit, subjects performed a whole body endurance exercise test until volitional fatigue, in a randomly assigned order, at a work rate that represented 75% or 95% $V0_2$ max. Each endurance test was conducted on day 7 ± 2 days of the menstrual cycle, during the follicular phase. Prior to exercise the BPNS procedure was performed to obtain control values for P_{di}, lung volume, and M-wave amplitude. This procedure was repeated again immediately post exercise, 30 minutes post exercise and 60 minutes post exercise. Figure 8 in Appendix 2 shows an illustration of the time course of

measurements. The follicular phase for testing was chosen for 2 reasons. First of all, most studies that compare the response of the pulmonary system across genders conduct their studies during the follicular phase of the menstrual cycle, so the data we collect will allow us to relate our results to other studies. The follicular phase was chosen because the concentration of progesterone was still low which limits its effects on ventilation.

Data Analysis:

One-way ANOVA with repeated measures was used to determine differences between pre and post exercise BPNS data. Transdiaphragmatic pressure post exercise, 30, and 60 minutes after exercise was compared against control values to determine the occurrence and recovery from EIDF. When the F value was significant Fisher's LSD post hoc test was used to determine significant differences in pairwise comparisons.

Maintaining M-wave amplitude and consistent lung volume are 2 important assumptions in this study. Repeated measures ANOVA was used to identify any significant differences in the group mean of the M-wave amplitude and lung volume during the various stimulation data collection periods.

Chapter IV

Results

Subjects:

Ten female subjects, average age of 20 years (range 18 – 22 years), gave written consent to participate in this study. Descriptive characteristics of the subjects are listed in Table 1.

Table 1. Subject characteristics

Subject Characteristics and Resting Pulmonary Function		
	Mean ± S.D.	Range
Age (years)	19.7	18 – 21
Height (cm)	161.54	154.94 – 165.10
Weight (kg)	63.45	51.82 – 79.09
$V0_2max$ (ml kg^{-1} min^{-1})	53.5	39.4 – 64.4
$V0_2max$ FFM (ml kg^{-1}[FFM] min^{-1})	74.33	60.61 – 78.40
TLC (L)	5.35	4.84 – 6.04
FRC (L)	2.84	2.25 – 3.56
Menstrual Cycle Length (Days)	29	27 – 32

Exercise Response

The average workload for participants exercising at 75% $V0_2max$ was 142 watts and for 95% $V0_2max$ was 166 watts, with an average exercise time of 22:40 (range 13:54 to 30:00) and 8:49 (range 6:52 to 13:39) respectively. The ventilation rate increased an average of 44% and 52% after approximately 20% of exercise at 75 and 95% $V0_2max$ respectively. The V_E response at both workloads was a result of a constant V_T and a progressively rising f_B throughout exercise (Figure 1).

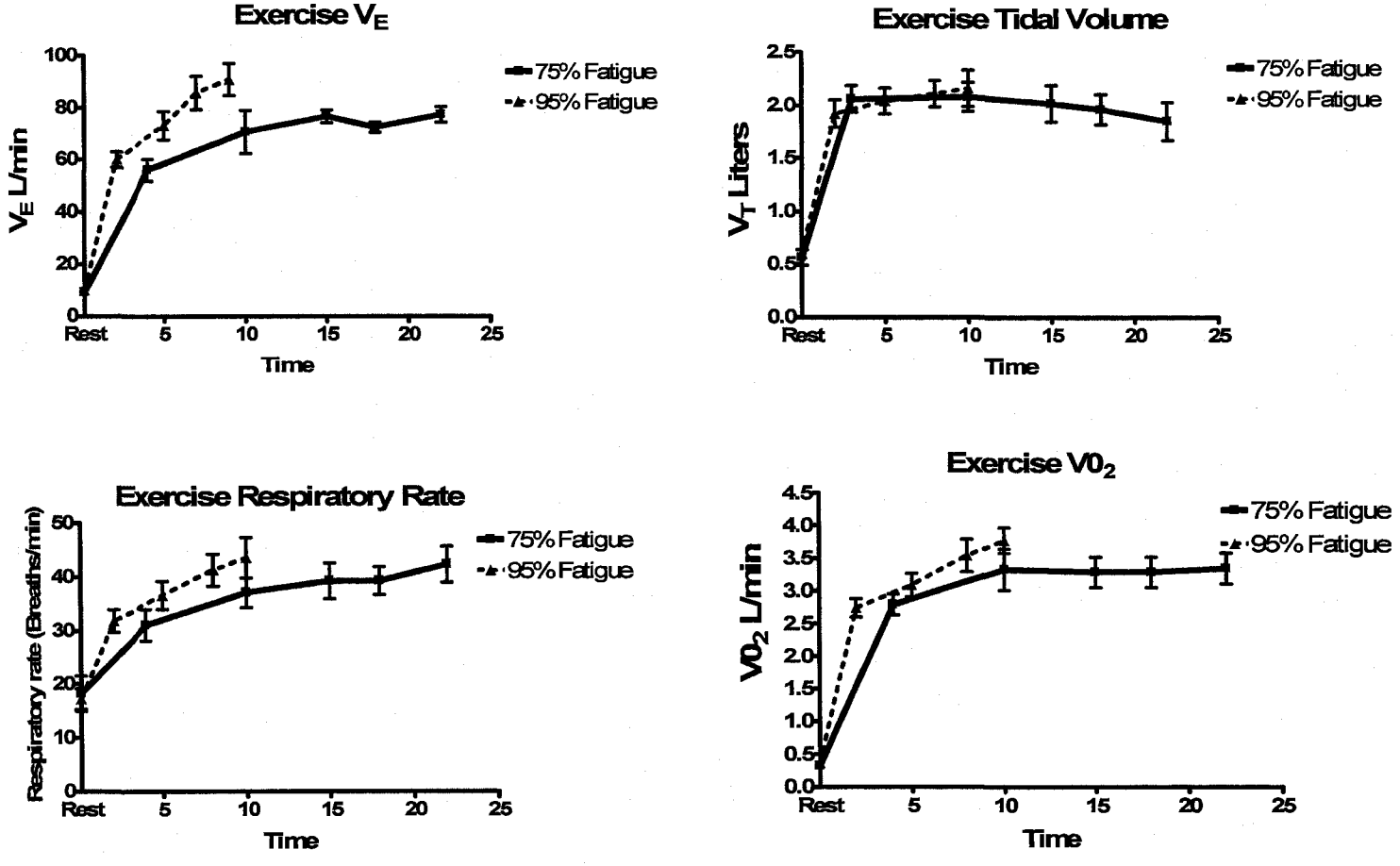
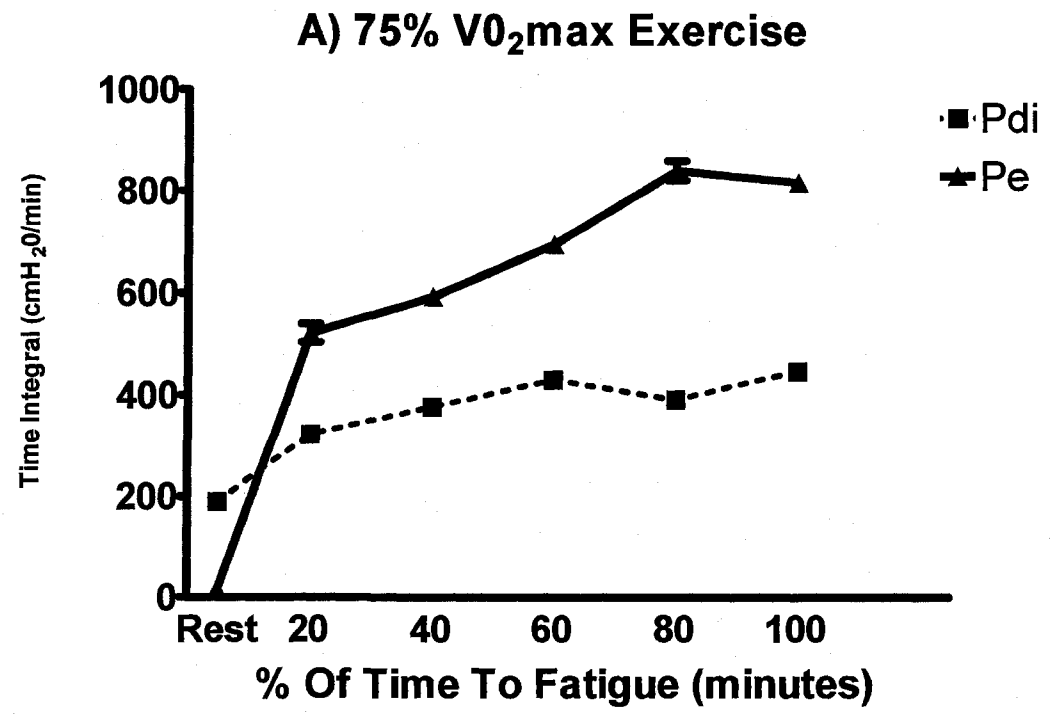
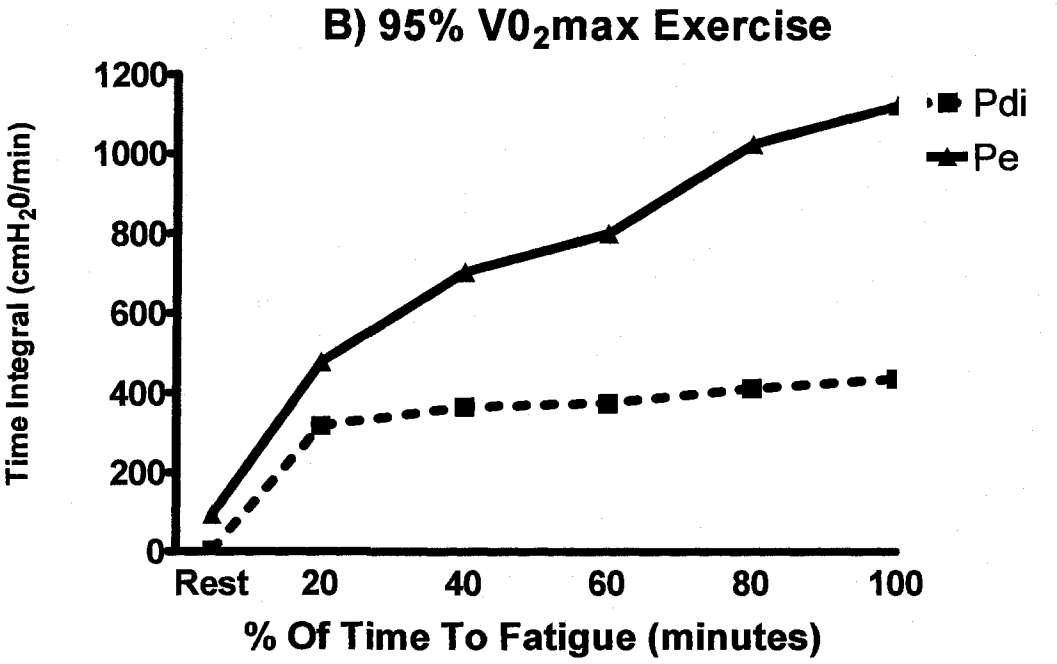


Figure 1 Ventilatory parameters measured during exercise at 75 and 95% $V0_2$max
A) Ventilation measured while exercising at 75 and 95% $V0_2$max. B) Tidal Volume measured while exercising at 75 and 95% $V0_2$max. C) Respiratory rate measured while exercising at 75 and 95% $V0_2$max. D) $V0_2$ measured while exercising at 75 and 95% $V0_2$max. Each data point represents the group mean ± S.E.

Figure 2 displays the average time integrals of P_e and P_{di}, ($\int P_e$/min, $\int P_{di}$/min) throughout exercise at 75 and 95% $V0_2$max. The $\int P_e$ represents the pressure developed by all of the inspiratory muscles (diaphragm and accessory muscles) and the $\int P_{di}$ represents the pressure developed by the diaphragm during exercise. During exercise at 75% $V0_2$max the $\int P_e$/min and $\int P_{di}$/min increased 58 and 38% respectively from 20% of time to fatigue, and $\int P_e$/min and $\int P_{di}$/min increased 135 and 37% from 25% of time to fatigue during exercise at 95% $V0_2$max.


A) 75% V0$_2$max Exercise
1000
800
600
400
200
0
Time Integral (cmH$_2$0/min)
Pdi
Pe
Rest
20
40
60
80
100
% Of Time To Fatigue (minutes)


B) 95% V0$_2$max Exercise
1200
1000
800
600
400
200
0
Time Integral (cmH$_2$0/min)
Pdi
Pe
Rest
20
40
60
80
100
% Of Time To Fatigue (minutes)

Test-Retest Reliability $V0_2max$ and Transdiaphragmatic Pressure

The coefficient of variation (CV) was determined to indicate the reproducibility of the participants exercise workload ($V0_2max$) and the test-retest reliably of the measure of the P_{di}.

Table 2. Coefficient of variation data for $V0_2max$ and P_{di}

	$V0_2max$	P_{di}
C.V.	7.7%	11.2%

The test-retest reliability for the measurement of $V0_2max$ was performed within a period of at least 1 week between tests. The C.V for the $V0_2max$ was on average 7.7% with a range from 0.48 to 16.3%. The C.V. for P_{di} was determined by taking initial measurements and repeating the measurements 1 hour later. The C.V for the P_{di} was on average 11.2% with a range of 8.6 – 15.3%. Based on the C.V. we can only say EIDF is present if the P_{di} response to supramaximal stimulation has been reduced by more than 11% post exercise otherwise changes in P_{di} could be due to measurement error.

Power Calculations

A power calculation was performed using the PS Power and Sample Size Calculation program. Utilizing the group means of P_{di} from 95% $V0_2max$ exercise at control to post exercise the following power was calculated.

Table 3. Calculated power across all frequencies of stimulation

Stimulation	Power
1 Hz	0.91
10 Hz	0.12*
20 Hz	0.19
50 Hz	0.79
100 Hz	0.06*

* indicates a difference of less than 10% between control and post exercise

Based on the calculated power data in Table 3, we can confidently state that our sample size of 8 participants was sufficient to be able to display a significant reduction in P_{di} post exercise at 95%$V0_2$max.

Reproducibility of M-Wave Amplitude And Lung Volume

The reproducibility of the M-wave amplitudes across all frequencies of stimulation at 75 and 95 % $V0_2$max are shown in Appendix I (Table 5 and 6). According to Table 5, the M-wave amplitude on the right side indicates a large variability across all time points compared to the control value at 75% $V0_2$max, especially at 1, 10, and 20 Hz stimulation; however these differences did not reach statistical significance ($P > 0.05$). The M-wave amplitude for the left side at 75% $V0_2$max is very consistent, staying well within 20% of control values.

Table 6 shows the mean peak M-wave amplitudes across all levels of stimulation and all time points for exercise at 95% $V0_2$max. The M-wave amplitudes are very consistent for all frequencies of stimulation. The highest level of variability was a 34.77% increase compared to control values observed on the right side at 50 Hz stimulation at 60 minutes post exercise; however this did not reach statistical significance ($P > 0.05$).

Lung volume measurements were taken during stimulation to ensure consistency of diaphragm muscle length and the results are displayed in Appendix I (Table 7). For the 75% $V0_2$max exercise condition, there does appear to be some changes in lung volume across time; however the difference from control did not reach statistical

significance ($P > 0.05$). For the 95% $V0_2max$ condition there were two points where the lung volume was statistically significantly reduced from control values.

Consistent lung volume and supramaximal stimulation of the diaphragm are the two most important variables to control in the BPNS technique. These two variables help to ensure any changes observed in P_{di} are due specifically to changes that occur in the muscle; therefore, it was crucial that there was consistency in these measurements.

Menstrual Cycle Length

The endurance exercise protocols for the second and third visits were performed within ± 2 days of the seventh day of the menstrual cycle (follicular phase). The menstrual cycle for all participants ranged from 25 -33 days in length. Participants tracked the start date of their menstrual cycle over a four month period. The length for up to 3 menstrual cycles is reported in Table 4. This was done to insure subjects had a regular menstrual cycle.

Table 4. Menstrual cycle length for each participant

Subject Number	Number Of Days In Menstrual Cycle		
1	35	31	30
2	31	27	30
3	33	33	32
4	28	28	27
5	29	31	
6	30	28	
7	29	30	31
8	24	27	29
9	28	29	28
10			

Diaphragm Response to Electrical Stimulation

Measurements of P_{di} taken prior to exercise were used as baseline control values. Any reduction in the post exercise P_{di} by more than 11% of control was considered to be an indication of the presence of EIDF based on the C.V. otherwise the difference could be

due to measurement error. The immediate post exercise stimulation actually occurred on average 10 minutes post exercise due to the requirement to set up the instrumentation after exercise. Figures 3 represent the mean peak P_{di} of the diaphragm in response to BPNS to 1 Hz stimulation at all time points for the 75% $V0_2$max condition. There was no significant reduction in P_{di} post exercise with 1 Hz stimulation in the 75% $V0_2$max condition. Similarly, there was also no reduction in P_{di} shown post exercise at 10, 20, 50, and 100 Hz stimulation in the 75% $V0_2$max condition as shown by Figure 4.

There was a significant reduction in P_{di} post exercise with 1 Hz stimulation in the 95% $V0_2$max condition ($P < 0.05$). The fall in P_{di} averaged $30 \pm 7\%$ post exercise with a trend that suggests recovery in P_{di} to control values within 30 minutes post exercise (Figure 5). Figure 6 illustrates the response of the diaphragm to BPNS at 10, 20, 50, and 100 Hz of stimulation for the 95% $V0_2$max conditions. In addition to 1 Hz, P_{di} was also significantly reduced post exercise at 50 Hz stimulation by approximately 24%.

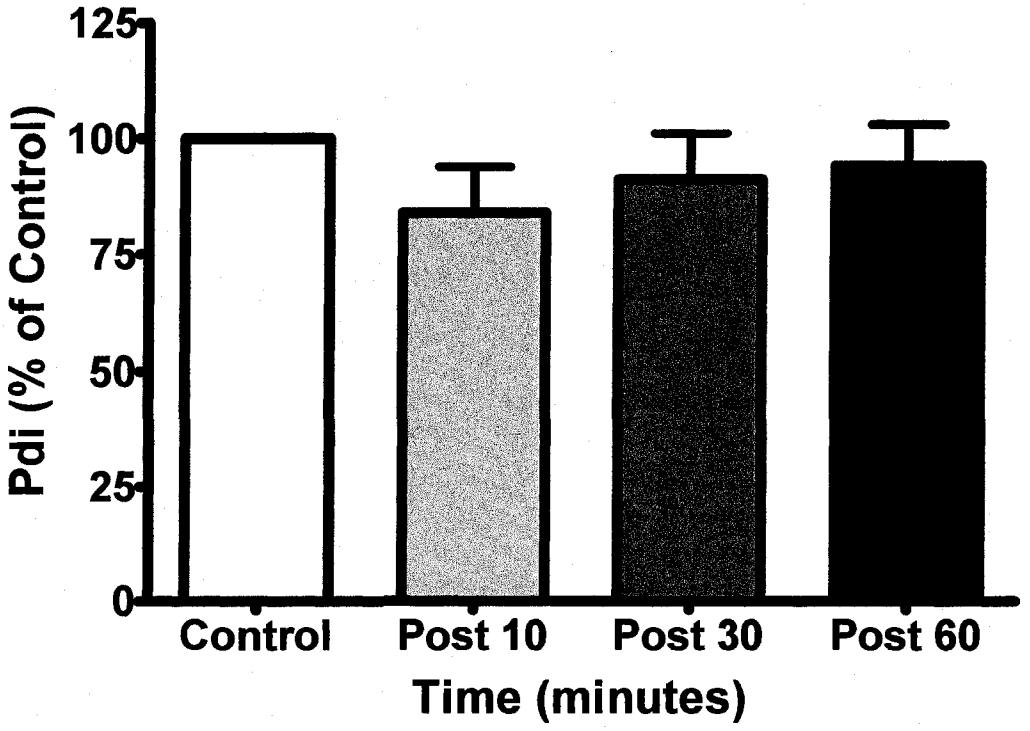


Figure 2. Response to BPNS at 1 Hz at 75% $V0_2$max (means ± S.E.).
Values are expressed as a percentage of control. Control, values obtained prior to exercise; Post, values obtained immediately after exercise; 30 mins, values obtained 30 minutes after exercise; 60 mins, values obtained 60 minutes after exercise.

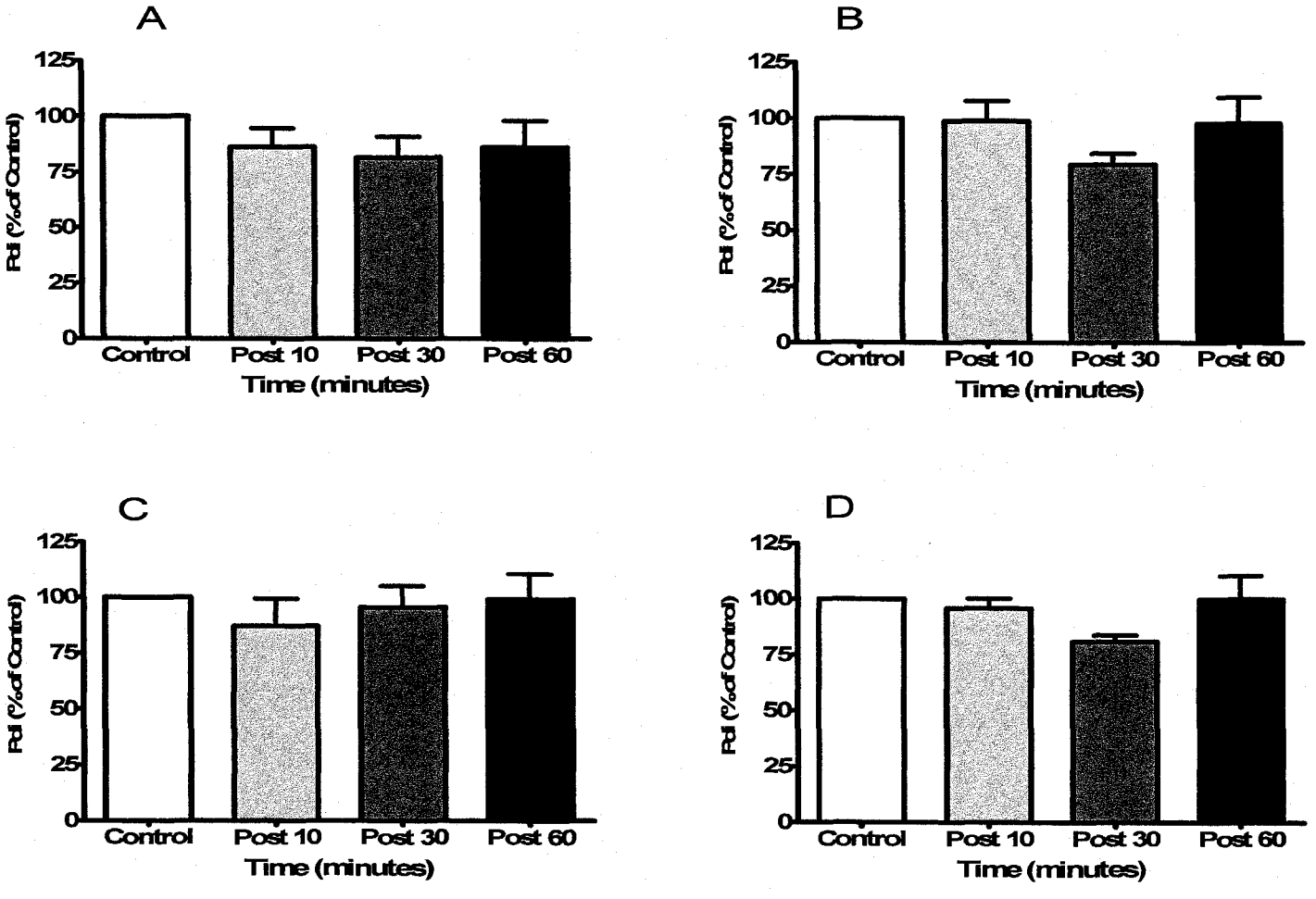


Figure 1. Response to BPNS at 10 Hz at 75% $V0_2max$ (means ± S.E.).
BPNS at A) 10, B) 20, C) 50, and D) 100 Hz. Values are expressed as a percentage of control. Control, values obtained prior to exercise; Post, values obtained immediately after exercise; 30 mins, values obtained 30 minutes after exercise; 60 mins, values obtained 60 minutes after exercise. (*, value is significantly different from Control $P < 0.05$)

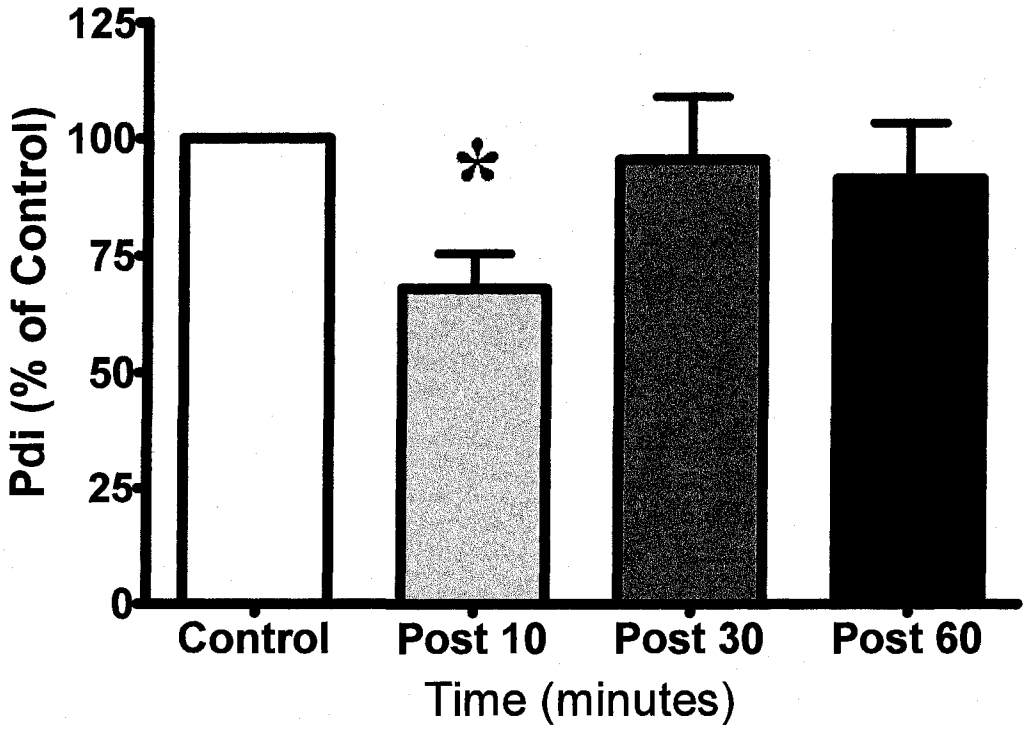


Figure 4. Response to BPNS at 1 Hz at 95% V02max (means ± S.E.).
Values are expressed as a percentage of control. Control, values obtained prior to exercise; Post, values obtained immediately after exercise; 30 mins, values obtained 30 minutes after exercise; 60 mins, values obtained 60 minutes after exercise. (*, value is significantly different from Control $P < 0.05$)

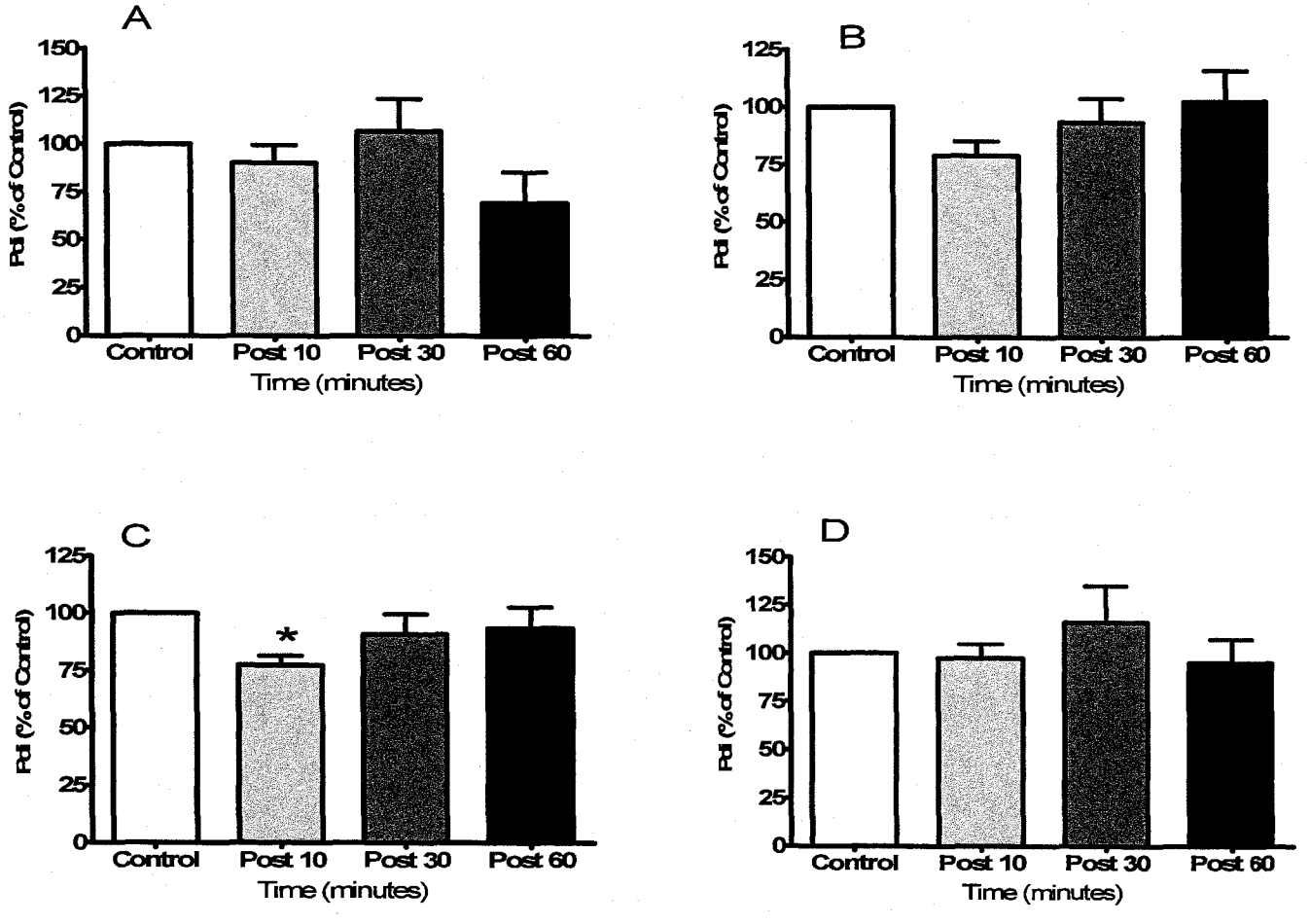


Figure 1 Response to BPNS at 95% V02max (means ± S.E.).
BPNS at A) 10, B) 20, C) 50, and D) 100 Hz. Values are expressed as a percentage of control. Control, values obtained prior to exercise; Post, values obtained immediately after exercise; 30 mins, values obtained 30 minutes after exercise; 60 mins, values obtained 60 minutes after exercise. (*, value is significantly different from Control $P < 0.05$)

Chapter V

Discussion

The purpose of this study was to determine if EIDF occurs in females with a broad range of fitness levels. Fatigue was defined as "a loss in the capacity of the muscle for developing force and/or velocity of a muscle shortening, resulting from muscle activity under load, which is reversible by rest" (NHLBI Workshop, 1990). This was indicated by a significant reduction in P_{di} post exercise versus pre exercise values. Significant declines in P_{di} were observed following whole body endurance exercise at 95% $V0_2$max with BPNS at 1 and 50 Hz stimulation post exercise which implies EIDF did occur. Recovery from fatigue was shown to occur after approximately 30 minutes after exercise.

Descriptive Measures

The mean age of all participants in our study was 20 years old with an average $V0_2$ peak of 53.5 ml/kg/min. This was lower than the average age and $V0_2$peak of the males in the Johnson et al. (1993) study to which gender comparisons are being made. The mean age of participants was younger than other studies examining the female pulmonary system, but the average $V0_2$ peak was similar (McClaran *et al.*, 1998;Harms *et al.*, 1998a;Richards *et al.*, 2004). Females were able to maintain a 95% $V0_2$max workload for an average of 9 minutes and 26 seconds, while the males (Johnson *et al.*, 1993) were able to maintain this workload for an average of 14 minutes. The study started off with the intension to study females with a wide range of fitness levels; however values for $V0_2$, f_b, V_E, and V_T were similar to other studies investigating females exercising at maximal intensity.

No reduction in P_{di} was observed in the 75% $V0_2$ peak condition at all levels of stimulation, which implies diaphragm fatigue did not occur. The occurrence and magnitude of EIDF was dependent on the amount of work performed by the diaphragm during whole body endurance exercise. The results suggest the V_E required while exercising at 75% $V0_2$max in females was not sufficient enough to result in EIDF. It should be noted that some but not all of the subjects exhibited a fall in the Pdi response to BPNS post exercise. This will require further study.

A significant reduction in P_{di} was observed in the 95% $V0_2$ peak condition which does suggest EIDF did occur. The data indicated transdiaphragmatic pressure was reduced by 30% post exercise and that recovery from fatigue occurred within approximately 30 minutes post exercise. The higher ventilatory workload required while exercising at 95% $V0_2$max was sufficient enough to cause EIDF. During exercise the f_b at 75% and 95% $V0_2$max was similar (42.3 and 43.6 breaths/min respectively) and it was the larger V_T while exercising at 95% $V0_2$max compared to 75% $V0_2$max (2.16 and 1.85 L/min respectively Figure 1) that accounted for the increase in ventilation.

Limitations

There are two major assumption involved with the BPNS technique 1) bilateral supramaximal stimulation of the phrenic nerve, and 2) consistent length of the diaphragm reflected by lung volume.

Supramaximal stimulation of the phrenic nerve was monitored by measuring M-wave amplitude. Supramaximally stimulating the phrenic nerve will produce the largest M-wave possible and implies all motor units associated with the diaphragm have been recruited; hence the muscle was being fully activated. Our study protocol allowed us to

monitor and ensure maximal M-wave amplitude was initially being achieved during stimulation; in addition, the reproducibility of the M-wave amplitudes at each stimulation frequency prior to and after exercise confirmed maximal force production was achieved.

The second assumption, muscle length, was an important factor in terms of producing repeatable magnitudes of force. Muscle stimulation studies require that supramaximal stimulation occurs at a similar muscle length. The force-length relationship of muscle illustrates how constant stimulation of muscle at different length exhibited different force responses. Studies on phrenic nerve stimulation of human diaphragm muscle have displayed increases in P_{di} with increasing lung volume (Braun *et al.*, 1982;Hamnegard *et al.*, 1995). The length of the diaphragm cannot be directly measured so lung volume was used as a surrogate measure for muscle length. Supramaximal stimulation and muscle length are the 2 most important factors to control. By keeping these factors consistent we can be certain that any changes in P_{di} are specifically a result of changes in the muscle. The consistency of lung volume measurements implies diaphragm muscle length was kept constant during BPNS.

A brief period of voluntary muscle contraction results in enhanced contractile force, a situation that is referred to as potentiation (Rassier & Macintosh, 2000). The study protocol requires repetitive involuntary supramaximal stimulation of the phrenic nerve followed by a submaximal inspiratory manoeuvre to TLC. The subsequent inspiratory manoeuvre was what makes potentiation a potential cause for concern. Mador et al. (1994) have shown that one maximal inspiratory manoeuvre can potentiate the diaphragm, with the number of inspirations having no effect on the magnitude and duration of potentiation. This was an important factor to consider especially after

exercise. Mador et al. (1994) monitored the decay in potentiation after performing a diaphragmatic maximal voluntary contraction, showing the effects of potentiation were eliminated after 8 minutes; therefore, the 10 minute delay in our first measurements post exercise was beneficial for minimizing any effects of potentiation caused by exercise. Our study protocol also allowed us to control for the effects of potentiation caused by supramaximal stimulation. Vandervoort et al. (1983) found in the Tibialis Anterior muscle, a contraction which exhibited 25 or 50% of maximum force produced little or no potentiation. It had been shown that a single supramaximal stimulation of the diaphragm produces approximately 20% of maximal P_{di} (Wragg *et al.*, 1994), and potentiation of the diaphragm produced by a submaximal contraction at 33% maximal P_{di} will last just less than 60 seconds (Mador *et al.*, 1994). Therefore by allowing 60 seconds between stimulations we did reduce the effect of potentiation on P_{di}.

The BPNS technique was the preferred method in terms of measuring P_{di}; however the benefits of the accuracy of this technique are also accompanied by some limitations. Before BPNS can occur the location of the phrenic nerve must be marked, and the pressures and the placement of the balloons must be checked. Prior to exercise, this does not pose a problem; however, this does delay post exercise measurements. On average post exercise measurement started approximately 10 minutes after exercise. Hence we may be underestimating the true reductions in P_{di} especially at high frequencies of stimulation.

Muscular fatigue occurs due to decrements in central and peripheral factors. The BPNS technique measures diaphragm fatigue caused by peripheral factors in the muscle but fails to determine the effect of inadequate motor drive. Previous studies have

indicated that central motor drive does play a role in diaphragm fatigue (Bellemare & Bigland-Ritchie, 1987); however, this was beyond the scope of this investigation.

The structural and hormonal differences that exist between men and women are thought to influence the response of the pulmonary system to endurance exercise. Fluctuating concentrations of estrogen and progesterone have been shown to affect ventilation during exercise. Our study was designed to measure diaphragm fatigue during the follicular phase of the menstrual cycle, specifically on day 7 of the menstrual cycle, to limit the effects of progesterone on ventilation. Despite the fact participants were asked to track their menstrual cycle over a four month period to ensure regularity, we were unable to specifically measure progesterone levels during testing without taking blood samples. If specific implications are proposed as to the effects of progesterone on the effects of pulmonary system during exercise, blood samples must be taken.

Implications for The Response To Exercise

Exercise induced diaphragm fatigue has been shown to occur in males after whole body endurance exercise by using bilateral phrenic nerve stimulation (Johnson *et al.*, 1993). A truly objective measure of EIDF in females has never been shown before. The purpose of our study was to determine, 1) does EIDF occur in females with whole body endurance exercise, and 2) are females susceptible to EIDF while exercising at a workload lower than 85% $V0_2max$? To our knowledge, our study was the first to show that EIDF does occur in females performing whole body endurance exercise for an average of 10 minutes. This was indicated by a significant reduction in P_{di} at 1 and 50 Hz stimulation post exercise. Return of P_{di} to control values at approximately 30 minutes post exercise suggests recovery from EIDF occurred at this time. The second objective

was to determine if EIDF occurred while exercising at a workload lower than 85% $V0_2max$ (specifically 75% $V0_2max$). We decided to choose this workload based on the previous research that shows females are susceptible to EFL and EIAH at reduced workloads and have lower fitness levels compared to males (McClaran *et al.*, 1998;Harms *et al.*, 1998a). According to our data, females did not present any statistically significant reductions in P_{di} in the 75% $V0_2max$ condition. There were three subjects that did present a reduction in P_{di} at 1 Hz stimulation ($P_{di\ Twitch}$) post exercise; however, there was a decreased likelihood that EIDF would occur in females exercising at 75% $V0_2max$.

In whole body endurance exercise, an increase in oxygen requirement of the respiratory muscles can potentially have an impact on locomotor muscle fatigue. The theory suggests a competitive relationship between the diaphragm and the locomotor muscles exists during exercise. As exercise intensity increased to near maximal levels, an increased oxygen requirement and metabolic cost of the respiratory muscles presents a limitation in blood flow to locomotor muscles causing a limitation to work output (Babcock *et al.*, 2002;Harms *et al.*, 1997;Romer *et al.*, 2006;Harms *et al.*, 1997). Exercise at 95% $V0_2max$ resulted in a 135% increase in $\int P_e$ throughout exercise until fatigue, the $\int P_{di}$ increased by approximately 37% throughout exercise. The much larger increase in the $\int P_e$ indicates a much larger increase in the contribution of the accessory muscle to inspiration during exercise. The considerable increase in the accessory muscle activation suggests a reduced ability of the diaphragm to generate the required pressure to support ventilation at this work load. This was supported by the large reduction in the twitch P_{di} observed post exercise. There has been speculation that the strategy of

recruitment of accessory inspiratory muscles throughout exercise may be different between genders (Bellemare *et al.*, 2003). A difference in breathing strategy could be an indication that the diaphragm may have a reduced contribution to inspiration during exercise (this will be discussed in more detail below).

Gender Differences in Exercise Induced Diaphragm Fatigue

The purpose of this study was to determine if females were more susceptible to exercise induced diaphragm fatigue than males. The data suggests females are not more susceptible to EIDF than males as our data have shown no significant reduction in P_{di} post exercise in the 75% $V0_2max$ condition. This theory was generated due to the anatomical composition of the female pulmonary system. The smaller lungs, airways, and reduced diffusion area are thought to be the cause of the increased occurrence of EFL (McClaran *et al.*, 1998) and EIAH (Harms *et al.*, 1998a) in females. The increased susceptibility specifically to EFL in females would suggest the work required to ventilate the lung by the diaphragm would potentially be higher at similar exercise work rates compared to males. Evidence of this has recently been shown in a study conducted by Guenette et al. (2007) that highlighted an important point about the comparison of exercise intensities between genders. Comparing the work of breathing between genders at a percentage of their $V0_2max$ does not provide a fair representation because females have a substantially smaller maximal V_E compared to males. Comparing the work of breathing against the ventilation rate allows us to see the true differences that occur. The work of breathing was shown to be almost twice as high in females compared to males when ventilation was over 90 l/min (Guenette *et al.*, 2007). These findings help to explain why this investigation did not find EIDF in females exercising at 75% $V0_2max$.

The females in our study reached a peak ventilation of 82.3 l/min while exercising at 75% $V0_2max$. Even though females generally have a higher work of breathing than males the peak ventilation was not high enough to substantially increase the work of breathing to induce fatigue of the diaphragm.

Females exercising at 95% $V0_2max$ with a peak V_E of 95.7 l/min, exhibited a 30% reduction in P_{di} induced by 1 Hz stimulation, while Johnson et al. (1993) indicated an approximate 14% reduction in P_{di} in males with a peak V_E of approximately 150 l/min. Based on the data from Guenette et al. males exercising with a ventilation of 150 l/min still have a slightly higher work of breathing compared to females exercising with a ventilation of 95 l/min. If females have a smaller work of breathing, why do they exhibit a substantially larger magnitude of EIDF post exercise, and why is the rate of recovery from EIDF faster in females than males? Since this is the first study to date to investigate EIDF in females there are no theories to explain this phenomenon.

The results from previous studies investigating gender related differences in muscle fatigue can help to explain our results. The data from the Johnson et al. (1993) study reported that while the males were exercising, the diaphragm was producing approximately 62% max force during whole body endurance exercise.

One may assume that while females are engaging in intense whole body endurance exercise their diaphragm was also operating at a submaximal level. Females producing submaximal skeletal muscular contractions have been shown to present an advantage in muscular fatigue resistance compared to males. A study by Fulco et al. (1999) investigated gender related differences in the fatigability of skeletal muscle. Participants performed a submaximal endurance exercise protocol at 50% MVC with a

50% duty cycle of 5 seconds to task failure. The relevant findings indicated women displayed a faster recovery from skeletal muscle fatigue compared to males (Fulco *et al.*, 1999). Further studies have confirmed this finding but also suggested females present a slower rate of fatigue with submaximal exercise compared to males (Russ & Kent-Braun, 2003;Ditor & Hicks, 2000;Hunter *et al.*, 2004). Since the diaphragm is a skeletal muscle, the same enhanced pattern of recovery from fatigue might be expected to occur. The cellular mechanisms behind the quick recovery from fatigue are beyond the scope of this study, but based on previous studies of muscular fatigue, the mechanism may be related to gender differences in muscle metabolism and/or the magnitude of the work of breathing.

The interpretations of the results from this study are not in agreement with the few studies that have investigated gender related differences of respiratory muscle fatigue. A study by Gonzales et al. (2006) reported an earlier onset of respiratory muscle fatigue in females, and a similar magnitude of reduction in respiratory muscle strength between genders after exercise. Another study by Ozkaplan et al. (2005) also found a similar reduction in respiratory muscle strength and reported no difference in the rate of recovery of the respiratory muscles between genders. Several important differences between our study and that of Gonzales et al. and Ozkaplan et al. must be noted. These previous studies are similar in terms of the factors they measured; our study specifically focused on fatigue of the diaphragm, while the other two non-specifically measure fatigue of the overall inspiratory muscles.

The most noteworthy difference between these studies was the method used to determine fatigue. Gonzales et al. and Ozkaplan et al. used a measurement of maximal

inspiratory pressure (MIP) to determine inspiratory muscle fatigue. This was not the preferable method to measure inspiratory muscle fatigue because it is a volitional manoeuvre which leaves too much control on the end of the patient; therefore, you have to rely on the patient's motivation to produce maximal contractions. This manoeuvre also required the contribution of the diaphragm and the accessory inspiratory muscles. This limits the interpretation of their results due to the inability to determine the magnitude of fatigue specifically in the accessory muscles or in the diaphragm. The method we employed, BPNS, is an objective way of measuring EIDF because it allowed us to bypass the central nervous system to maximally contract the diaphragm; hence, changes in the response to supramaximal stimulation can be specifically measured. The second important difference to draw attention to is the method employed to cause diaphragm fatigue. Gonzales et al. (2006) used a resistive breathing exercise to cause fatigue. While this method may result in respiratory muscle fatigue the subjects don't illicit the same physiological responses that occur with intense whole body endurance exercise. Ozkaplan et al. (2005) used an incremental resistive cycling test to induce respiratory muscle fatigue. The protocol employed by Ozkaplan et al. was more physiologically relevant; however, others have shown using BPNS that EIDF does not occur using this exercise protocol.

In addition to determining the occurrence of EIDF in females, this investigation also monitored the recruitment patterns of the inspiratory muscles throughout exercise. To date, no other study has reported data on accessory inspiratory muscle recruitment in females during whole body exercise. The extremely large increase in $\int P_e$ throughout exercise suggests the diaphragm may be incapable of generating the large pressure

required during exercise so the accessory muscles must increase their contribution. This may be due to differences in the morphology of the diaphragm muscle between genders. Bellemere et al. (2003) have reported that females have a shorter diaphragm than males having the same height. The consequence is an inability for females to create a comparable volume displacement and flow rate to that of their male counterparts. This alone provides further evidence that women require a greater contribution of the accessory inspiratory muscles during exercise.

Any differences observed in EIDF between males and females could be related to gender differences in cardiac output, substrate utilization, or fatigue resistance to submaximal exercise and possibly many other factors. Only recently has there been large devotion to investigating gender related differences to exercise. In addition to providing intriguing results, this study should help to stimulate the further development of gender related physiological studies.

Future Directions and Applications

The first suggestion I would make is to replicate this study with a larger number of participants. Our data only indicates significant reductions in P_{di} post exercise. Our sample size was more than sufficient to identify the occurrence of EIDF in females. Further studies would benefit by using a larger number of participants would allow us to determine how different factors influence the occurrence of EIDF in females. In addition to the variables collected in our study, we need to determine the percentage of the P_{di} capacity produced during exercise.

The fact estrogen and progesterone have been shown to have effects on muscle contractility and ventilation would indicate that investigating EIDF through different

phases of the menstrual cycle may produce interesting results. This data may lead into further speculation as to whether females may possess a competitive edge while competing during specific phases of the menstrual cycle.

Patients with COPD and CHF have been shown to have a reduced tolerance to aerobic exercise partially caused by an earlier onset of dyspnea. Clinicians have been using inspiratory muscle training devices to reduce the effects of dyspnea during exercise in people with pulmonary diseases. The study data has suggested that females have a larger magnitude of EIDF than males with whole body endurance exercise. Since the only data to date have been collected on males, the results have been generalized and applied to females. Further research into the use of inspiratory muscle training devices is required to determine if the current training protocols are most effective.

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Appendix 1

Table 5. Reproducibility of M-Wave peak amplitudes at 75% $V0_2max$

75% $V0_2max$								
	RMW (a.u.) Mean Amplitude				LMW (a.u.) Mean Amplitude			
	Control	Post	30 mins	60 mins	Control	Post	30 mins	60 mins
1 Hz	100	191.79	192.58	185.58	100	100.76	104.61	96.86
S.E.	0	60.72	62.88	58.19	0	1.41	3.06	
10 Hz	100	203.97	194.47	198.08	100	99.25	104.44	98.99
S.E.	0	70.03	60.86	65.40	0	99.25	2.51	2.71
20 Hz	100	189.74	184.61	182.12	100	100.28	103.63	100.29
S.E.	0	61.48	56.52	57.47	0	0.85	1.50	1.79
50	100	119.83	123.97	120.93	100	99.51	104.50	96.89
S.E.	0	16.32	16.14	16.31	0	2.22	2.99	1.24
100 Hz	100	124.46	125.10	124.15	100	99.76	98.50	98.47
S.E.	0	16.98	16.98	17.37	0	0.47	0.92	1.83

M-wave values are expressed as a percentage of control values across all frequencies of stimulation. Peak values are measured prior to exercise, immediately after exercise and 30 and 60 minutes after exercise. RMV, right M-wave; LMW, left M-wave; Control, values obtained prior to exercise; Post, values obtained immediately after exercise; 30 mins, values obtained 30 minutes after exercise; 60 mins, values obtained 60 minutes after exercise.

Table 6. Reproducibility of M-Wave peak amplitudes at 95% $V0_2$max

95% $V0_2$ max								
	RMW (a.u.) Mean Amplitude				LMW (a.u.) Mean Amplitude			
	Control	Post	30 mins	60 mins	Control	Post	30 mins	60 mins
1 Hz	100	114.56	114.69	113.44	100	96.73	99.56	94.35
S.E.	0	17.66	20.75	19.64	0	2.26	0.70	1.33
10 Hz	100	110.46	113.19	108.10	100	102.62	119.04	114.11
S.E.	0	17.69	17.75	20.53	0	3.38	13.29	12.39
20 Hz	100	116.62	112.27	113.04	100	101.77	102.54	100.60
S.E.	0	18.13	18.70	18.94	0	3.93	3.15	1.11
50	100	115.94	118.68	134.77	100	100.13	100.91	98.93
S.E.	0	14.27	15.52	16.48	0	2.89	2.80	1.63
100 Hz	100	111.62	119.15	128.68	100	99.78	101.54	101.02
S.E.	0	14.61	20.21	21.15	0	2.25	1.36	2.50

M-wave values are expressed as a percentage of control values across all frequencies of stimulation. Peak values are measured prior to exercise, immediately after exercise and 30 and 60 minutes after exercise. RMV, right M-wave; LMW, left M-wave; Control, values obtained prior to exercise; Post, values obtained immediately after exercise; 30 mins, values obtained 30 minutes after exercise; 60 mins, values obtained 60 minutes after exercise.

Table 7. Lung volume measurements at 75 and 95% $V0_2max$

Lung Volume Measurements								
	75% $V0_2max$				95% $V0_2max$			
	Control	Post	30 mins	60 mins	Control	Post	30 mins	60 mins
1 Hz	100	84.83	87.65	102.72	100	97.53	79.85	77.46
S.E.	0	7.01	3.41	7.75	0	10.79	7.70	10.81
10 Hz	100	0.57	82.68	66.01	100	86.18	10.23	13.86
S.E.	0	86.11	8.50	24.90	0	8.64	65.75	64.40
20 Hz	100	87.17	80.98	133.81	100	107.27	123.47	77.87
S.E.	0	4.01	8.31	27.43	0	16.03	33.39	7.35
50	100	82.21	85.39	99.12	100	82.32	80.16*	77.54*
S.E.	0	6.89	5.03	8.26	0	7.83	6.20	6.76
100 Hz	100	98.79	86.20	100.81	100	105.3	98	96.97
S.E.	0	2.18	7.10	4.08	0	5.07	9.22	8.66

Lung volume values are expressed as a percentage of control FRC measurements across all frequencies of stimulation. Values are measured prior to exercise, immediately after exercise and 30 and 60 minutes after exercise. Control, values obtained prior to exercise; Post, values obtained immediately after exercise; 30 mins, values obtained 30 minutes after exercise; 60 mins, values obtained 60 minutes after exercise.

(*, value is significantly different from Control $P < 0.05$)

Appendix 2

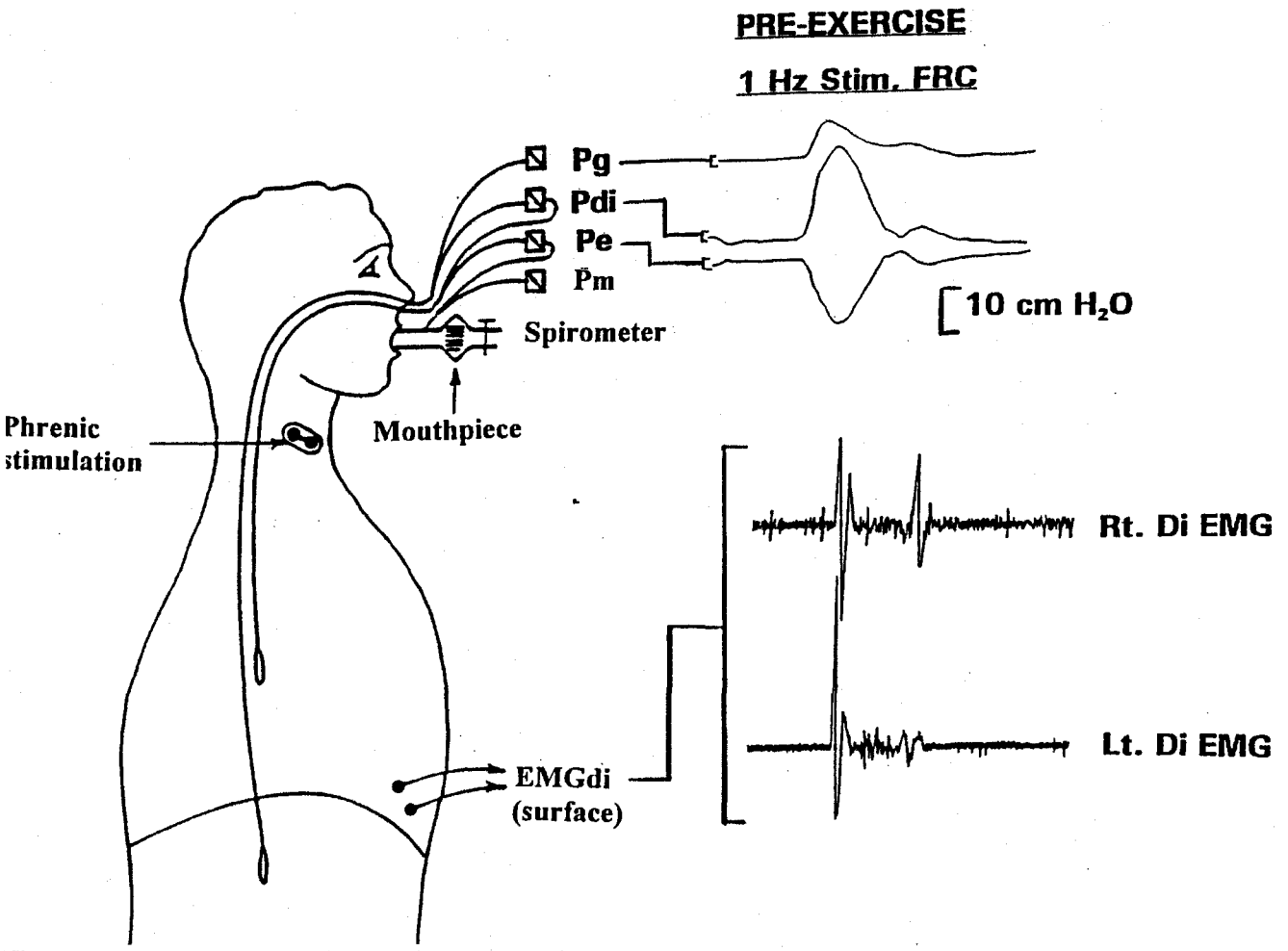

Figure 6 Illustration of experimental instrumentation

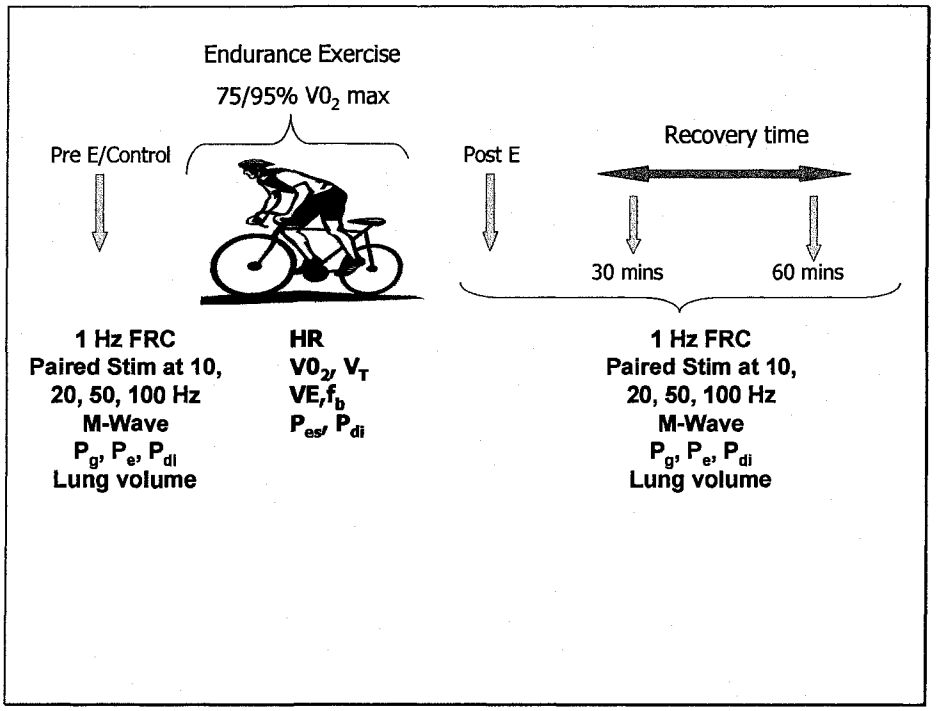

Figure 7 Illustration of timeline of measurements for the experimental protocol

Appendix 3

Glossary

Alveolar Arterial 0_2 Difference (A-a$D0_2$) – The difference in the partial pressure of oxygen between the alveolar and arterial blood

Bilateral Phrenic Nerve Stimulation (BPNS) – Application of electrical stimulation to the phrenic nerves bilaterally

Chronic Heart Failure (CHF) – A condition in which the heart's ability to pump oxygenated blood to the body is inadequate to meet the body's needs

Chronic Obstructive Pulmonary Disease (COPD) – A disease in which the lungs are damaged making it difficult to breathe

Dyspnea – Difficult or laboured breathing

End Expiratory Lung Volume (EELV) – The volume of air remaining in the lungs after an expiration

End inspiratory Lung Volume (EILV) – The volume of air in the lungs after an inspiration

End Tidal $C0_2$ ($PETC0_2$) – The concentration of carbon dioxide at the end of expiration

Esophageal pressure P_e – Surrogate measure of pleural pressure

Exercise Induced Arterial Hypoxemia EIAH – A condition where blood transport is reduced to a point that it no longer meets the needs of the working muscle

Exercise Induced Diaphragm Fatigue (EIDF) – Defined in this investigation by a reduction in P_{di} by more than 11%.

Expiratory Flow Limitation EFL – A mechanical constraint that limit the rate of expiration

Fatigue – A loss in the capacity of the muscle for developing force and/or velocity of muscle shortening, resulting from muscle activity under load which is reversible by rest

Functional Residual Capacity (FRC) – The volume of air remaining in the lungs after a normal expiration

Gastric pressure P_g – Surrogate measure of abdominal pressure

Maximal Voluntary Contraction (MVC) – The amount of force produced with one voluntary contraction

$Pa0_2$ – Measure of the partial pressure of oxygen in the blood

Residual Volume (RV) – The volume of air left in the lung after a maximal expiration

$Sa0_2$ – Arterial oxygen saturation. Represents the amount of oxygen attached to haemoglobin in the blood

Tidal Volume (V_T) – The amount of air that can be voluntarily moved out of the respiratory system with one breathe

Time integral of P_e ($\int P_e$) – A calculation of the integral of the P_e for one breath multiplied by the frequency of breathing. Represents the pressure produced by the accessory inspiratory muscles during exercise

Time integral of P_g ($\int P_g$) – A calculation of the integral of the P_g for one breath multiplied by the frequency of breathing. Represents the pressure produced by the diaphragm muscle during exercise

Total Lung Capacity (TLC) – The complete capacity of the lungs

Trans-diaphragmatic pressure (P_{di}) – A measure of the pressure created across the diaphragm during contraction. Calculated by $P_e - P_g$.

$V0_2$peak – The peak rate of oxygen uptake by the muscle during exercise

Ventilation (V_E) – The rate of air movement between the lungs and the atmosphere