A COMPARISON OF INSPIRATORY MUSCLE FATIGUE FOLLOWING
MAXIMAL EXERCISE IN MODERATELY TRAINED MALES AND FEMALES

by

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ABSTRACT

Exercise-induced inspiratory muscle fatigue (IMF) has been reported in males but there are few reports of IMF in females. The fatigability of locomotor muscle has been reported to be different between males and females, where females are more resistant to fatigue. It is not known if a gender difference exists for inspiratory muscle strength following heavy exercise. The purpose of this study was to compare inspiratory muscle strength between a group of moderately-trained males and females following maximal exercise. Specifically, the relationship between fatigue and subsequent recovery of maximal inspiratory pressure (MIP) following exercise to maximal oxygen consumption ($\dot{V}O_{2max}$) was examined in both genders. Eighteen males (23 ± 3yrs; mean$\pm$SD) and sixteen females (23 ± 2yrs) completed 10 MIP and 10 maximal handgrip strength (HG) maneuvers to establish a baseline. Subjects then performed a progressive intensity $\dot{V}O_{2max}$ test on a cycle ergometer. Post-exercise MIP and HG were assessed successively immediately following exercise and at 1, 2, 3, 4, 5, 10, and 15 min. $\dot{V}O_{2max}$ relative to fat-free mass was not statistically different between males (62 ± 7ml$\cdot$kg$^{-1}\cdot$min^{-1}) and females (60 ± 8ml$\cdot$kg$^{-1}\cdot$min^{-1}). Males had higher absolute MIP values (-cmH$_2$O) than females at all time intervals ($p<0.05$). Immediately following exercise, MIP was significantly reduced in both genders (M = 83 ± 16%; F = 78 ± 15% of baseline) but HG values were not different than resting values. MIP values remained depressed for both males and females throughout the 15 min ($p<0.05$). Differences for MIP between M and F were not statistically significant at any measurement time ($p>0.05$). IMF was observed immediately following maximal exercise in both males and females and the pattern of recovery was the same between genders.

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CHAPTER I: Introduction

1.1 Introduction

Adequate respiratory muscle function is critical for survival. The respiratory system functions to obtain oxygen (O_2) from the external environment and remove carbon dioxide (CO_2), produced by cellular metabolism, from within the body. Together with the cardiovascular system, the muscles of respiration ensure that sufficient oxygen in being delivered to the body's working muscles to produce energy. Heavy, whole-body endurance exercise has been shown to cause respiratory muscle (diaphragmatic) fatigue in healthy subjects with a variety of fitness levels (Johnson et al., 1993). The magnitude and likelihood of diaphragmatic fatigue has been determined to increase at exercise above 85% of $\dot{V}O_{2max}$ (Johnson et al, 1993). Exercise-induced inspiratory muscle fatigue (IMF) has been reported in males (Loke et al., 1982; Bye et al., 1984; Coast et al., 1990; Johnson et al., 1993; Babcock et al., 1996; Ker & Shultz, 1996; McConnell et al., 1997; Volianitis et al., 1999; Romer et al., 2002; Boussana et al., 2003, Lomax & McConnell, 2003), but there are few reports of IMF in females (Coast et al., 1999, Volianitis et al., 2001).

The fatigability of locomotor muscle has been reported to be different between males and females, where females are more resistant to fatigue (Maughan et al., 1986; Pincivero et al., 2003). Current evidence suggests that females demonstrate a greater resistance to fatigue, as measured by greater endurance in several locomotor muscles. Support for this female advantage has been shown in the back extensors (Kankaanpää et al., 1998), adductor pollicis (Fulco et al., 1999, Fulco et al., 2001), elbow flexors (Kahn et al., 1986) and knee extensors (Pincivero et al., 2003). Most studies showing greater resistance in females have used submaximal contraction intensities (e.g. 20% maximum voluntary contraction [MVC])

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to induce fatigue. The female advantage appears to decline as the contraction intensity increases (e.g. 80% or greater MVC) (Hicks *et al.*, 2001). While the differences vary, depending on the muscle group studied, only one study has concentrated specifically on the muscles of respiration (Gonzales *et al.*, 2003 [abstract]). The vast majority of studies have focused solely on a male subject pool. The few studies that have included female subjects, did not analyze their results in terms of a gender comparison (Coast *et al.*, 1999, Volianitis *et al.*, 2001). It is not known if a gender difference exists for inspiratory muscle strength following heavy exercise.

1.2   Objectives and Purpose

The purpose of this study was to characterize the changes that occur in inspiratory muscle strength between males and females following maximal exercise. Specifically, the relationship between fatigue and subsequent recovery of maximal inspiratory pressure (MIP) following exercise to $\dot{V}O_{2max}$ was examined in both genders.

General objectives

a) To better understand the consequences of fatiguing the inspiratory muscles and how they may differ in males and females.

b) To provide insight for the future development of possible gender-specific training guidelines for performance enhancement in the normal population and prevention/rehabilitation of cardiorespiratory ailments in the clinical population.

Specific objectives

a) To determine if the possible alterations in inspiratory muscle strength following a bout of exhaustive exercise are different in males and females.

b) To disseminate reliability values using a portable handheld mouth pressure meter as a reference tool for future researchers in this field.

1.3   Definitions

i. *Maximal inspiratory pressure (MIP)* – a measure of global inspiratory muscle strength, determined by the maximal inspiratory effort (inspiratory pressure) maintained for at least one second. A small leak is placed in the mouthpiece to prevent glottic closure during inspiratory measures (Müeller maneuver).

ii. *Maximal handgrip strength (HG)* – a measure of the maximum force (kg) generated by squeezing a handgrip dynamometer.

iii. *Maximal Oxygen Consumption (Uptake) ($\dot{V}O_{2max}$)* – a measure of cardiorespiratory fitness. $\dot{V}O_{2max}$ is the product of maximal cardiac output (L/min) and arterial-venous oxygen difference (ml O_2/L) (ACSM, 2000)

iv. *Inspiratory muscle fatigue (IMF)* – a decrease in volitional maximal inspiratory pressure, with demonstration of recovery with rest (NHLBI, 1990; ATS/ERS, 2002)

1.4   Delimitations

This study will be delimited by:

a)   A sample of subjects between 20 and 30 years of age from the UBC community.

b) Setting the criteria for a moderately-trained subject as having $\dot{V}O_{2max}$ between 40 and 50 $ml \cdot kg^{-1} \cdot min^{-1}$ in females and 45 and 55 $ml \cdot kg^{-1} \cdot min^{-1}$ in males, thus excluding untrained and highly trained individuals.

**Two males falling just outside these boundaries were eventually matched to two similarly trained females.

c) Comparing the genders based on $\dot{V}O_{2max}$ scores relative to fat-free mass.

d) Female participants who had a six-month history of normal menstruation (eumenorrheic). Both those who are taking oral contraceptives and those who are not will be included in the study. These females will all be tested during the early follicular phase (days 3-8) of the menstrual cycle, where estradiol concentrations ware related more closely to males.

e) The measurement of maximal handgrip strength (HG) together with MIP, to measure motivation and general body fatigue over the testing period.

f) A respiratory gas sampling rate set at 20-second intervals.

g) The methodology used to deteremine MIP, HG, and $\dot{V}O_{2max}$.

1.5 Limitations

This study will be limited by:

a) The data collection capabilities of the Sensor Medics Vmax 29 series metabolic cart and the Data Acquisition System interlaced with it.

b) The individual's metabolic response to the testing protocols.

c) The individual effort during testing procedures (e.g. the individual's ability to perform maximum inspiratory pressures, due to the volitional nature of MIP).

d) Variability in female hormone levels due to individual differences in menstrual cycles and the use of oral contraceptives (OC)

1.6 Assumptions

The following assumptions have been made:

a) The subjects' cardiorespiratory responses during the incremental cycle ergometer test were a true representation of maximal aerobic capacity once the following criteria were met: i) RER > 1.15, ii) HR within 10 beats of age predicted maximum heart rate (220-age), and iii) a plateau in $\dot{V}O_{2max}$ (either a decrease or an increase of less than 2 ml/kg/min).

b) The subjects answered all questionnaires truthfully.

c) The subjects adhered to the pre-test instructions given by the investigator.

d) Familiarization sessions to environment, equipment, and testing protocols were adequate.

e) The subjects performed to their maximal level during all testing protocols.

1.7 Rationale

Gender differences in the fatigability of locomotor muscles have been previously determined (Maughan *et al.,* 1986; Pincivero *et al.,* 2003). The assumption could be taken that respiratory muscles, as skeletal muscles, should demonstrate similar gender differences. However, there are properties unique to the respiratory muscles that may lead them to perform and fatigue in a manner different from those muscles previously studied. Unlike other skeletal muscles, respiratory muscles must contract continually, approximately 12 to 20

times per minute, for the entire duration of life (AACVPR/ACCP, 1997). They function disparate to other skeletal muscles that have the ability to relax, contract, and inevitably rest for any given duration of time (AACVPR/ACCP, 1997). Consequently, the absence of rest means that like other skeletal muscles, they are susceptible to become fatigued or injured under conditions of overload.

Some researchers believe that the greater muscle mass typically found in males is the main reason for their greater fatigability (Hicks *et al.*, 2001). Their ability to produce greater absolute force during muscle contractions, relative to females, suggests that they require a greater metabolic demand. Combined with the possibility of reduced availability of oxygen during exercise due to mechanical compression of the vascular bed, males would be expected to rely more heavily on anaerobic metabolic pathways (Russ & Kent-Braun, 2003). It had been previously suggested that males may rely on glycolytic pathways of metabolism to a greater extent than females, whereas females have a greater capacity for oxidative metabolism (Russ & Kent-Braun, 2003).

Russ and Kent-Braun (2003) also observed that ischemia eliminated the sex differences in fatigue, and the apparent female advantage of the dorsiflexors. Their results provided evidence to show that sex-based differences within the metabolic pathways utilized during muscle contraction may have been the reason for the initial sex differences in fatigue.

A number of mechanisms for the apparent gender difference in fatigue have been proposed, though it remains unclear what exactly accounts for the variation. Proposed mechanisms can be classified into three general groups; muscle mass, substrate utilization, and muscle morphology. Females typically have lower maximal voluntary contractions (MVC) for a given muscle group. Therefore, the relative contraction (as a percentage of

MVC) utilizes less absolute muscle mass for a given contraction (Hicks *et al,* 2001). Consequently, less absolute work is done by the female's muscles, causing for lower oxygen demand, delaying the onset of fatigue (Hicks *et al.,* 2001). Fat oxidation capacity has also been suggested to be greater in females compared to males, related to 4-5% lower respiratory exchange ratio (RER) during submaximal endurance exercise (Tarnapolsky, 1999). The effects of oestrogen as a glycogen-sparing tool may also be a factor. Finally, females have greater cross-sectional area and proportion of Type I fibres, enabling a greater capacity and more efficient means for oxidative metabolism (Hicks *et al.,* 2001). A fourth mechanism regarding neuromuscular activation is in the initial stages of investigation and could lead to further answers in the near future (Hicks *et al.,* 2001). This mechanism proposes differences in the pattern and level of activation of the muscle fibres, as measured by surface electromyography (EMG). It remains to be seen whether such proposed mechanisms, focused mainly on research of locomotor muscles, can be translated to the respiratory muscles as well.

Evidence has been provided to suggest that females have airways that are smaller relative to lung size than those of males (Mead, 1980). It has also been shown that smaller lung volumes and maximal flow rates for women in general may cause hyperinflation and increased prevalence of expiratory flow limitation (EFL) during strenous exercise (McClaran *et al.,* 1998). In highly fit women, a significantly higher $\dot{V}_E$, resultant of both higher VT and f_b, was observed when compared to less fit women (McClaran *et al.,* 1998). Comparing these data to men, McClaran *et al* (1998) suggested that the relatively smaller lung volumes and lower maximal expiratory flow rates in females caused them to utilize a greater percentage of ventilatory reserve during exercise. Consequently, they suggested that increasing f_b (relative

to males) would seem to be the only strategy for highly fit females to attain ventilatory requirements to meet the needs of high-intensity exercise. Therefore, it would seem that females need to work harder at maximal exercise compared to males, in order to overcome the expiratory flow limitation and subsequent increase in end-expiratory lung volume (EELV). This discrepancy in breathing mechanics, could provide a greater opportunity for inspiratory muscle fatigue to occur.

Such considerations must be accounted for when hypothesizing gender differences in respiratory muscle fatigue following maximal exercise.

1.8   Hypotheses

The following hypotheses were suggested for the possible gender difference in inspiratory muscle fatigue:

a) A significant difference will exist between the overall decline in post-test MIP in females and males, such that males will be less fatigued than females.

e.g. $MIP_{post-exercise(male)} - MIP_{baseline(male)} < MIP_{post-exercise(female)} - MIP_{baseline(female)}$, $p < 0.05$

b) Post-exercise MIPs, relative to baseline values, will portray a significant difference at each time period (Immediate, 1, 2, 3, 4, 5, 10, and 15 minutes post-exercise respectively), such that males will recover faster than females.

e.g. $MIP_{time(male)} - MIP_{baseline(male)} < MIP_{time(female)} - MIP_{baseline(female)}$, $p < 0.05$

c) Frequency of breathing (f_b) and consequently Borg dyspnea ratings (breathlessness), will be greater in females, compared to males, as measured during $\dot{V}O_{2max}$. During the same time period, tidal volume (V_T) and maximal

ventilation ($\dot{V}_E$) will be significantly higher in males than females, due to their larger mass and stature. Females will have to rely on a greater frequency of breathing at any given $\dot{V}_E$ compared to males.

Table 1. Summary of hypotheses

Hypothesis	p
a) $MIP_{\text{post-exercise(male)}} - MIP_{\text{baseline(male)}} < MIP_{\text{post-exercise(female)}} - MIP_{\text{baseline(female)}}$	$p < 0.05$
b) $MIP_{\text{immediate(male)}} - MIP_{\text{baseline(male)}} < MIP_{\text{immediate(female)}} - MIP_{\text{baseline(female)}}$	$p < 0.05$
$MIP_{1\text{(male)}} - MIP_{\text{baseline(male)}} < MIP_{1\text{(female)}} - MIP_{\text{baseline(female)}}$	$p < 0.05$
$MIP_{2\text{(male)}} - MIP_{\text{baseline(male)}} < MIP_{2\text{(female)}} - MIP_{\text{baseline(female)}}$	$p < 0.05$
$MIP_{3\text{(male)}} - MIP_{\text{baseline(male)}} < MIP_{3\text{(female)}} - MIP_{\text{baseline(female)}}$	$p < 0.05$
$MIP_{4\text{(male)}} - MIP_{\text{baseline(male)}} < MIP_{4\text{(female)}} - MIP_{\text{baseline(female)}}$	$p < 0.05$
$MIP_{5\text{(male)}} - MIP_{\text{baseline(male)}} < MIP_{5\text{(female)}} - MIP_{\text{baseline(female)}}$	$p < 0.05$
$MIP_{10\text{(male)}} - MIP_{\text{baseline(male)}} < MIP_{10\text{(female)}} - MIP_{\text{baseline(female)}}$	$p < 0.05$
$MIP_{15\text{(male)}} - MIP_{\text{baseline(male)}} < MIP_{15\text{(female)}} - MIP_{\text{baseline(female)}}$	$p < 0.05$
c) $f_b\text{(male)} < f_b\text{ (female)}$	$p < 0.05$
$Borg_{\text{dyspnea(male)}} < Borg_{\text{dyspnea(female)}}$	$p < 0.05$
$V_{T\text{(female)}} < V_{T\text{(male)}}$	$p < 0.05$
$\dot{V}_{E\text{(female)}} < \dot{V}_{E\text{(male)}}$	$p < 0.05$

1.8   Significance of the Study

Research into the training and subsequent fatigue of respiratory muscles has significant consequences to the entire spectrum of the training population, from weekend athletes to patients recovering from debilitating diseases. Investigating the degree to which inspiratory muscles fatigue after maximal exercise and developing ways to increase the

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threshold for fatigue resistance will have important repercussions for the population as a whole. Determining whether a gender difference exists within inspiratory muscle fatigue could have specific consequences for each gender.

CHAPTER II: Review of Related Literature

2.1 Introduction

The interplay of the respiratory muscles, in the company of other systems within the human body, is integral to our continued existence. This critical function would lead one to assume that respiratory muscles have been studied extensively. Studies looking to determine the mechanisms forming the underlying physiology, as well as ways in which to enhance or improve the functioning of this muscle group have not been investigated thoroughly until recently. While this research area was late to catch on, it seems to be a rather hot research topic today. The basis for such research relates to the simple fact the respiratory muscles are the only skeletal muscles upon which life depends (Macklem, 1980; Farkas, 1996). The respiratory muscles work as a vital pump that can and do fail (Macklem, 1980). The importance of normal respiratory muscle function can be appreciated by considering that fatigue, injury or disease of this muscle group would result in an inability to maintain blood gas and pH levels within an acceptable range, resulting in death (Powers & Criswell, 1996). Research has quickly expanded from trying to fully understand the structure and function of the respiratory system, to trying to discover ways in which to make the system work even more effectively. Current studies are looking at pushing the respiratory system past the threshold of fatigue (Darnley *et al.,* 1999; Hart *et al.,* 2001, Kellerman *et al.,* 2000, Larson *et al.,* 1988, Romer *et al.,* 2002, Romer *et al.,* 2003, Sonetti *et al.,* 2001, Weiner *et al.,* 1992, Williams *et al.,* 2002). Consequently, respiratory muscle training programs have been developed and are being refined to help impede or delay the onset of fatigue. It must be noted that while research continues to evolve into areas of training and reducing fatigue, the mechanisms of past research have still not been confirmed. Science is seemingly moving

forward to answer questions without pausing to determine exactly why past findings resulted. Research continues to pile onto a shaky foundation that lacks specific reasoning and support for many of the findings. This lack of mechanistic interpretation of the results is concerning. This review summarizes the current literature, as well as some historical findings, and critically evaluates the findings for possible implications for future research.

2.2 The Diaphragm

While past efforts have focused directly on the heart and lungs, recent research has focused on alternative structures of the body that may indirectly influence the cardiorespiratory system. One of the key muscle groups that have been studied are the respiratory muscles. The muscles of inspiration and expiration, often considered in their individual roles, may be integral to advancing our understanding of the training and rehabilitation of the cardiorespiratory system. Specifically, much attention has been focused on the diaphragm, the key inspiratory muscle (Johnson *et al.*, 1993, Inbar *et al.*, 2000). Often overlooked in terms of its importance in sustaining life, the diaphragm has been termed, the "other vital pump" (Farkas, 1996). It could play a key role in helping to prolong and protect lives through preventative measures and rehabilitation. Thus, understanding how the diaphragm responds to exercise and the ability to resist fatigue have important health implications. Studies of the past have concentrated on large locomotor muscle groups in the attempt to better understand fatigue. Only recently have researchers looked to those muscles that indirectly service the locomotor muscles, the muscles of respiration. These muscles work together with the cardiovascular system to ensure that adequate oxygen in being

delivered to all of the working muscles. Their primary task is to displace the chest wall, moving gas in and out of the lungs to maintain arterial blood gas and pH homeostasis.

Investigating how the respiratory muscles fatigue and developing ways to increase the threshold for fatigue resistance will have important implications for the greater population. One must consider the role of the diaphragm within the physiological workings of the human body as a whole. The diaphragm, much like the heart, is continuously working over a person's lifespan. If inspiratory muscles fail, so does ventilation and tissue respiration (NHLBI, 1980).

The diaphragm is a mixed muscle comprised of a fraction of all three muscle fibre types. Histologically, half of the muscle fibres comprising the diaphragm are classified as Type I slow-oxidative, with the remainder forming approximately equal proportions of Type IIA fast oxidative-glycolytic and Type IIB fast glycolytic (NHLBI, 1980). It has been found that in all species, oxidative fatigue resistant fibres, the sum of Type I and IIA, comprise greater than 55% of the muscle (NHLBI, 1980). Relating the structure and function of the diaphragm gives this morphology an understandable evolutionary reasoning to resist fatigue.

2.3   Methodology and Technical Considerations

In order to suitably discuss exercise-induced respiratory muscle fatigue it is necessary to discuss the advancement of this field of research. The progression of the field, from the examination of fatigue induced artificially in a laboratory setting to more recent field studies looking at fatigue under various physiological conditions, will be discussed in this section.

2.3.1   Fatigue vs. Task Failure

Over the past several decades, task failure and fatigue have been used interchangeably within discussion of the respiratory muscles. Task failure is not a synonym for fatigue. Muscle fatigue is defined as a condition in which there is failure in the capacity to develop force and/or velocity, as a result of muscle activity under load, that is reversible with rest (NHLBI, 1980). Alternatively, the inability to continue a given task, based on the parameters defined by its testing protocols, characterizes task failure. The means behind this simplified definition could be attributed to muscle fatigue, however a deeper understanding of these two terms would present a more complex distinction. Task failure, during inspiratory resistive loading, has been proposed to be the outcome of inspiratory muscle fatigue. However, task failure and fatigue must be looked at as individual components acting independently and perhaps irrespective of one another. Task failure may in fact be a result of fatigue, however; it may present itself in parallel due to a number of other factors.

Three types of fatigue have been determined to occur within the respiratory muscles; central, peripheral high-frequency, and peripheral low-frequency fatigue (ATS/ERS, 2002). Central fatigue results, when contraction is limited, due to a fall in motoneural output from the central nervous system (CNS). Peripheral fatigue owes to failure at the neuromuscular junction, presented by decreased motor force output or velocity in response to direct electrical stimulation (ATS/ERS, 2002). Specifically, peripheral high and low frequency fatigue are named based on the shape of the post-fatigue muscle force frequency relationship (Aldrich, 1988). Deterioration of muscle forces in response to high (50-100Hz) and low (1-20Hz) frequency electrical stimulation defines peripheral high and low frequency fatigue respectively (Aldrich, 1988). Low frequency fatigue can occur in isolation, with high

frequency fatigue regularly coupled with some muscle force changes at lower frequencies (ATS/ERS, 2002) The loss of force at low frequencies may signify impairment of the excitation-contraction coupling mechanism within the muscles (Aldrich, 1988). At high frequencies, the decline in force generation may indicate an alteration in neuromuscular junction transmission or depreciation in action potential propagation (Bazzy & Donelly, 1993).

Task failure has been revealed in a respiratory setting on many occasions. One of the founding studies was completed by Moxham and colleagues (1980). They had subjects breathe through an inspiratory resistance until they could no longer match their intended target on 3 out of 4 breaths. They examined diaphragmatic fatigue by comparing the frequency-pressure curves (as generated by phrenic stimulation) before and after the resistive loaded task. They found that the diaphragm developed low-frequency fatigue in much the same way as other skeletal muscles.

Nickerson *et al* (1982) rendered task failure as the inability to maintain ventilation against the load, resulting in the mouthpiece coming out of the mouth of the subject. Undoubtedly, this portrays the inability to continue with the given task, though it does little in terms of determining respiratory muscle fatigue. The role of respiratory muscle fatigue in task failure has not been clearly defined. Some key psychological and physiological factors make for a strong case for factors other than muscle fatigue leading to task failure. In terms of subject compliance, possible factors other than fatigue contributing to task failure include lack of motivation and inexperience with the protocols. Initial exposures to the testing strategy have been shown to produce sensations of breathlessness and panic at higher workloads before ceasing the task (ATS/ERS, 2002). Subjects often sacrifice technique for

task success. In a physiological context, central fatigue (decreased output on the CNS), peripheral (contractile) fatigue (decreased force-generating capacity of the muscles), or the inability of the muscles to physically overcome the load due to exceeding maximum strength, have all been hypothesized as other contributing factors (Laghi *et al.*, 1998; Eastwood *et al.*, 1994; Mador & Tobin, 1992). Inspiratory resistive loading rapidly initiates a fatiguing process in the diaphragm. This results in progressive recruitment of the rib cage and expiratory muscles to handle increased ventilatory demands (Laghi *et al.*, 1998). Such findings present the difficulty in focusing loading efforts directly upon the diaphragm, without "tainting" by the accessory muscles. The breathing strategy employed by subjects is often surrendered to tolerate the resistive load more easily, with the resultant load reduced below that necessary to induce training (Enright *et al.*, 2000). It also appears that breathing strategies change from increased duration of expiration and of inspiratory muscle inactivity, to increases in their capacity to generate force (Eastwood *et al*, 1994).

McKenzie *et al* (1997) established that when breathing through a large inspiratory resistance, subjects showed a tendency to hypoventilate and proceeded to task failure without any evidence of inspiratory muscle fatigue. They showed task failure to be not fully dependent on impaired force-generating capacity of the diaphragm. In like manner, Gorman *et al* (1999) showed that task failure presented itself due to severe breathing discomfort associated with accumulation of CO_2 due to progressive hypercapnia and hypoxemia, not inspiratory muscle fatigue.

Landmark findings of Roussos and Macklem (1977) discovered that during resistive loading, subjects were able to maintain about 40% of their Pdi_{max} before the onset of task failure. Since then, several studies have found unpredictable results looking at task failure.

The given value of critical transdiaphragmatic pressure of 40% of maximum pertains to the conditions of that experiment explicitly, but it continues to be quoted. The value can change as a function of lung volume and of the duty cycle of the diaphragm (T_I/T_{tot}) (Rousso & Macklem, 1977; Macklem, 1982). More importantly, it is this difference between task failure and diaphragmatic fatigue that has eluded some researchers. It is too easy to jump to the conclusion that task failure presents itself solely due to fatigue. Continued cardiopulmonary research must strive to clearly differentiate and quantify the idea of task failure versus fatigue.

This review will now focus on its examination of research that induced and quantified respiratory muscle fatigue after exercise (Table 2). Muscle fatigue has long been deemed a limiting factor to performance. Whether it is a rehabilitation program, a casual run, or an Olympic final, the failure to continue to push the body due to muscular fatigue has led to individual plateaus in performance. Finding the capacity to overcome the inability of the muscles to continue to work at the desired rate is a decisive target.

An early study by Loke *et al.* (1982) showed that maximal inspiratory pressure (PI_{max}) measured post marathon was significantly lower than the pre-race values. This study was fundamental in suggesting the prospect of respiratory muscle fatigue after exercise. It has been said that the magnitude and likelihood of diaphragmatic fatigue increases at exercise above 85% of $\dot{V}O_{2max}$ (Johnson *et al.*, 1993). Coast *et al.* (1990) showed endurance training leads to adaptive changes in the inspiratory muscles that protect it from acute loss of strength (vs. normal subjects post-exercise). Specifically, there must be a mechanism within the body that insures that the respiratory muscles can continue to function properly even at extreme conditions. These changes could be evolutionary in nature. This would allow for

the respiratory muscles to overcome bouts of extreme exertion, not for the sake of a performance benefit, but rather for the sake of sustaining proper life function. The drive to survive may be the underlying mechanism for the general fatigue resistance of the respiratory muscles. Other studies have certainly challenged this prospect. Table 2 summarizes the remarkably inconsistent findings within the literature. The reality is that for virtually every study that was able to portray respiratory muscle fatigue, there is a counter-study that lacks significant differences. This statement is broad and generalized since the studies are not identical in methodology, but the general patterns are undeniable. Specific comparisons of studies that included similar subjects, in terms of level of training, show this variability. Studies listed below that included highly-trained individuals varied in their results. Several authors (Loke *et al.*, 1982; Chevrolet *et al.*, 1993, Boussana *et al.*, 2001; Boussana *et al.*, 2003) showed a significant decrease in respiratory muscle strength post-exercise task. However, Coast *et al.* (1990) Gonzales *et al.* (2002) and Ker & Shultz (1996) all showed no significant difference in pre and post-exercise respiratory muscle strength values.

Another difficulty in making comparisons between studies is the variability in techniques utilized to determine fatigue. While all of the studies compared inspiratory muscle (IM) strength pre and post-exercise, the measurements used varied. This lack of uniformity in terms of technique to determine fatigue is a challenge that still faces this field.

Table 2. Summary of reported changes in inspiratory muscle function after exercise

Study[a]	Participants	Exercise Task	Inspiratory Muscle (IM) Strength Measurement	Change Relative to Baseline
Loke *et al.* (1982)	n = 4 (all M)	Marathon (42.2km)	P_{Imax} pre and immediately post marathon Pdi (transdiaphragmatic pressure)	Post Marathon: 165.8 ± 11.0 cmH_2O vs. 138.5 ± 7.6 cmH_2O, $p < 0.01$
Bye *et al.* (1984)	n = 7 (all M)	Incremental test on electrically braked cycle ergometer to determine maximum working capacity (Wmax) Constant work load exercise performed at 80% of W_{max} inspiring humidified O_2 and humidified air.	Pleural (Ppl) and gastric (Pga) pressures used to determine trandisphragmatic pressure (Pdi) pre and 0.5-2 min and 2 - 5 min post-exercise	0.5-2min post-exercise: $\downarrow Pdi_{max}$ in both air ($p < 0.02$) and O_2 ($p < 0.05$) 2 - 5 min post-exercise: some recovery in Pdi_{max} in air ($p < 0.05$) and complete recovery in O_2
Coast *et al.* (1990)	Highly trained cross-country skiers; n = 6 Sedentary; n = 5	Incremental cycle to exhaustion	MIP (RV); pre & 10, 60, 120s post-ex.	Skiers: no $\downarrow$, slight $\uparrow$ (NS) Sedentary: $\downarrow$ in MIP of 10, 17, and 13%, $p < 0.05$
O'Kroy *et al* (1992)	Recreational runners; n=9 (7M, 2F)	Treadmill running: a) maximal test to exhaustion (7-14min) b) 7-min test at 90% of VO2max c) 30-min test at 60% of VO2max	MIP (RV)	MIP was not different across time or intensities
Chevrolet *et al.* (1993)	Marathoners: n = 15 Half-marathoners: n = 12	Marathon (M) and half-marathon (H)	P_{Imax} (RV); pre & 2.5h post-race Measures taken at: (mean $\pm$ SD) t_1: 11 ± 4 min. t_2: 59 ± 7 min. t_3: 139 ± 9 min.	$P_{Imax} \downarrow$ in M ($p < 0.001$) and in H ($p < 0.05$) Mean $\pm$ SD % of pre-race values (baseline): M: t_1: $75 \pm 19\%$, t_2: $81 \pm 18\%$, t_3: $86 \pm 15\%$ H: t_1: $90 \pm 9\%$, t_2: $90 \pm 10\%$, t_3: $94 \pm 14\%$

				Average value for all three BPNS frequencies (mean ± SEM) 85%: 27.5 ± 2.0 vs. 22.6 ± 2.8, $p < 0.05$ 95%: 24.9 ± 1.8 vs. 21.5 ± 2.0, $p < 0.05$ Mueller (Pdi): 85%: 108.6 ± 10.2 cmH_2O vs. 97.1 ± 8.4 cmH_2O, NS 95%: 108.9 ± 8.1 cmH_2O vs. 108.2 ± 7.2 cmH_2O, NS Expulsive + Mueller (Pdi): 85%: 175.1 ± 4.2 cmH_2O vs. 155.9 ± 3.6 cmH_2O, $p < 0.05$ 95%: 167.4 ± 6.1 cmH_2O vs. 147.0 ± 7.3 cmH_2O, $p < 0.05$
Johnson *et al.* (1993)	n = 12 (all M) (VO_{2max} 61 ± 4 ml•kg^{-1}•min^{-1})	Whole body exercise to exhaustion at 85% (31 ± 8 min) and 95% VO2max (14 ± 3 min) n = 10 treadmill, n = 2 cycle ergometer	Supramaximal BPNS at 1*, 10, and 20 Hz (FRC) Mueller maneuver (Pdi_{max}) (FRC) Mueller maneuver (Pdi_{max}) plus expulsive manuever	
Babcock *et al.* 1995)	n = 11 (8 M, 3 F) (VO_{2max} 52.4 ± 0.7 ml•kg^{-1}•min^{-1})	One normoxic (arterial O2 saturation 96-94%) and one hypoxic (inspiratory O2 fraction = 0.15 and arterial O2 saturation 83-77%) n = 7 treadmill, n = 5 cycle ergometer	Supramaximal BPNS at 1*, 10, and 20 Hz (FRC) *completed at one-half inspiratory capacity and FRC Mueller maneuver (Pdi_{max}) (FRC)	1Hz: norm. Pdi ↓ -25.8 ± 4.1%, $p < 0.003$; hyp. Pdi ↓ -22.0 ± 2.2%, $p < 0.001$ 10Hz: norm. Pdi ↓ -25.7 ± 6.6%, $p < 0.04$; hyp. Pdi ↓ -19.6 ± 4.6%, $p < 0.02$ 20Hz: norm. Pdi ↓ -10.4 ± 4.1%, $p < 0.4$; hyp. Pdi ↓ -13.9 ± 4.2%, $p < 0.04$ Mueller (Pdimax): norm. 11% decrease: 89.1 ± 8.4 cmH_2O vs. 79.3 ± 8.0 cmH_2O, $p < 0.05$ hyp. 9.6% decrease: 102.7 ± 6.7 cmH_2O vs. 92.8 ± 7.7 cmH_2O, $p < 0.06$
Babcock *et al.* (1995)	n = 9 (7 M, 2 F) (VO_{2max} 63.2 ± 0.9 (SE) ml•kg^{-1}•min^{-1})	Compared diaphragm fatigue in whole body exercise to exhaustion (86 - 93% VO2max for 13.2 ± 2.0 min) with voluntary hyperpnea mimicing exercise. n = 8 treadmill, n = 1 cycle ergometer Subgroup (n = 7): voluntary hyperpnea Pdi ~ 50% greater than exercise Pdi levels	Supramaximal BPNS at 1*, 10, and 20 Hz (FRC) Mueller manuever (Pdi_{max}) (FRC)	Average value for all three BPNS frequencies: Whole body exercise (WB): -26.0 ± 2.9%, $p < 0.01$ Supramimic (SM): -22.6 ± 5.6%, $p < 0.05$ Mimic (M): -9.5 ± 4.8%, $p > 0.07$ Mueller (Pdimax): WB: 100.8 ± 14.4 cmH_2O vs. 98.4 ± 14.0 cmH_2O, NS SM: 99.5 ± 9.8 cmH_2O vs. 96.7 ± 9.4 cmH_2O, NS M: 104.9 ± 9.4 cmH_2O vs. 92.4 ± 11.1 cmH_2O, NS

Babcock *et al.* 1996)	20 M, 4 F 11 High Fit (H): (VO$_{2max}$ 69.0 ± 1.8 ml•kg^{-1}•min^{-1}) 13 Fit (F): (VO$_{2max}$ 50.4 ± 1.7 ml•kg^{-1}•min^{-1}) p < 0.05	Endurance exercise (88-92% VO2max); H: 15.2 ± 1.7 min., F: 17.9 ± 2.6 min. n = 19 treadmill, n = 5 cycle ergometer	BPNS at 1, 10, and 20 Hz (FRC)	H: Pdi ↓ -23.1 ± 3.1%, p < 0.01 F: Pdi ↓ -23.1 ± 3.8%, p > 0.05
Ker & Shultz (1996)	Ultra-marathon runners; n = 10 (8M, 2F)	Ultra-marathon (87km)	P$_{Imax}$ pre and 3 days post race (RV)	109 ± 21 cmH$_2$O vs. 115 ± 13 cmH$_2$O, NS, p > 0.37
McConnell *et al.* (1997)	Moderately-trained; n= 24 (all M) (VO$_{2max}$ 53.8 ± 5.2 ml•kg^{-1}•min^{-1})	Multi-stage incremental shuttle run to volitional fatigue (distance = 20m)	P$_{Ipeak}$ and P$_{Iave}$ (RV); pre & within 3 min. post-exercise	P$_{Ipeak}$ and Piave both ↓, p < 0.001
Coast *et al.* (1999)	n = 11 5 M: 50 ± 3.7 ml•kg^{-1}•min^{-1} 6 F: 40.1 ± 2.5 ml•kg^{-1}•min^{-1}	Exhaustive cycle at 60 RPM, with load ↑ 1 kg/ 3 min. Hyperpnea (HP): matched V$_E$ and f$_b$ from last min. of each stage in cycle test Control (C): sit for same duration as exercise	MIP (RV)	MIP ↓ 12 mmHg (15%), p , 0.005 No ↓ MIP with HP or C trials
Volianitis *et al.* (1999)	Rowers: n = 11 Non-rowers: n = 12	Rowers: incremental test to volitional exhaustion - wind-resistance braked rowing erg. Non-rowers: incremental test to volitional exhaustion - electromagnetically braked cycle erg.	MIP (RV)	Rowing: MIP ↓ 7.0 ± 2.0%, p < 0.01 Cycling: MIP ↓ 2.2 ± 3.0%, NS
Boussana *et al.* (2001)	Triathletes (all M) : n = 12	1. incremental cycel test (VO$_2$max) 2. 20 min. cycle followed by 20 min. run. [C-R] 3, 20 min. run followed by 20 min. cycle [R-C]	P$_{Imax}$ (FRC); pre & post-task P$_{Imax}$1' (measured after R-C T$_{lim}$ task)	Post C-R: 130.0 ± 3.8 cmH$_2$O vs. 126.7 ± 4.3 cmH$_2$O, p < 0.05 Post R-C: 129.6 ± 4.3 cmH$_2$O vs. 123.7 ± 4.9 cmH$_2$O, p < 0.05 Post R-C P$_{Imax}$1': 121.2 ± 3.9 cmH$_2$O vs. 111.2 ± 5.5 cmH$_2$O, p < 0.001

Volianitis et al. (2001)	Competitive rowers: n = 14 (7 M, 7 F)	6 min. all out rowing simulation preceded by: 1. submaximal rowing warm-up (SWU) 2.specific rowing warm-up (RWU) 3. specific rowing warm-up plus respiratory warm-up (RWU plus)	MIP (RV)	Deficits in IM strength: SWU: 10.2 ± 1.4%, RWU 11.1 ± 1.3%, RWUplus 4.2 ± 0.3%, p< 0.05 relative to other warm-up conditions
Gonzales et al. (2002) [abstract]	Competitive cyclists; n = 7 (all F) (VO_{2max} 49.4 ± 7.3 ml·kg^{-1}·min^{-1})	Progressive intensity cycle to volitional fat. using personal cycle (Computrainer)	P_{Imax} pre and immediately post task	NS ↓ in Pimax, p = 0.39
Boussana et al. (2003)	Triathletes (all M) : n = 10	1. incremental cycle test (VO_2max) 2. 20 min. cycle [C] 3. 30 min. run [R] 4. 20 min. cycle followed by 20 min. run. [C-R]	P_{Imax} (FRC); pre & post-task	Post C: 132.4 ± 4.9 cmH_2O vs. 125.7 ± 5.6 cmH_2O, p < 0.05 Post R: 131.8 6.1 cmH_2O vs. 129.6 6.4 cmH_2O, p > 0.05 Post C-R: 133.7 ± 4.0 cmH_2O vs. 126.9 ± 5.2 cmH_2O, p < 0.05

[a] Studies listed in chronological order

2.3.2 Current Assessments of Respiratory Muscle Fatigue

Over the course of history, respiratory muscle testing has endured several different procedures to confirm and measure the degree of respiratory muscle fatigue. Numerous protocols have been operationalized to induce muscle fatigue and will be discussed within the context of their findings. Indicators are often based on feasibility and availability of equipment, combined with subjective preference of the given laboratories. While all of these protocols vary in their degree of invasiveness, ease of use, and measures produced, they have been shown to produce valid and reliable results. Some key methods for determining respiratory muscle fatigue will be critiqued and analyzed in terms of technical considerations that need to be addressed. It must be noted that a suggestion has been made that it may be possible only to infer fatigue retrospectively by its reversibility after a period of rest (NHLBI, 1980).

2.3.2.1 Supramaximal Bilateral Phrenic Nerve Stimulation

The phrenic nerve solely innervates the diaphragm. Thus, phrenic nerve stimulation (PNS) allows for the investigation of the diaphragm without interference from other respiratory muscles. Equally, the artificial stimulation of PNS eliminates the authority of the central nervous system. Supramaximal bilateral phrenic nerve stimulation provides perhaps the most effective means of measuring diaphragmatic fatigue in humans. The involuntary nature of the muscle contraction, due to the stimulation, counteracts many of the limitations of inducing fatigue through voluntary contraction. The strength of the protocol becomes apparent when it is compared in relation to some of the other techniques utilized over the course of the advancement of respiratory muscle fatigue research. However, the invasive nature of the procedure means that it is not a viable option for all laboratories. Two techniques, electrical and magnetic stimulation, will be discussed.

The procedure most often used for bilateral electrical phrenic nerve stimulation follows (Johnson *et al.*, 1993; Babcock *et al.* 1995a; Babcock *et al.*, 1995b; Babcock *et al.*, 1996). Intranasally, two balloon-tipped catheters are positioned, one in the stomach to measure gastric pressure (Pga), and one in the lower third of the esophagus to measure esophageal pressure (Pes). Transdiaphragmatic pressure (Pdi) is the difference between pleural pressure (Ppl) and abdominal pressure (Pab), and is equated to the difference between Pga and Pes (Agostini *et al.*, 1960). The summation of these two variables gives transdiaphragmatic pressure (Pdi), since Pes is usually negative; Pdi = Pga – Pes. To record compound muscle action potential (M wave) resulting from phrenic nerve stimulation, surface electromyogram (EMG) electrodes are placed over each hemidiaphragm in the sixth or seventh intercostals space near the costal margin. The surface stimulation site is marked

approximately 2cm above the clavicle on the subject's neck. By increasing the stimulation current until no change in M-wave amplitude is found, the maximal M-wave can be determined. Further increasing the current by 50% above the maximum level insures the supramaximal nature of the protocol. Continuous monitoring of the subject's lung volumes throughout the stimulation tests is established by a wedge spirometer, connected to the subject. The subject is able to gain feedback visually with an oscilloscope display of the lung volume. Stimulation is given 9-12 times at 1Hz at functional residual capacity (FRC), at 3-5 times at 10Hz and 20Hz respectively. Tetanic stimulations last 400ms in duration and are performed at four lung volumes. The resultant Pes, Pga, Pdi, and M-waves are collected for analysis. The total force generated by the diaphragm is reflected by the Pdi value. The Pdi's are analyzed for peak tension (maximum increase in tension above the Pdi baseline), contraction time (time interval required from the initiation of the stimulation until the Pdi reached its peak), and one-half relaxation time (time period required for the Pdi to decline to one-half the peak pressure). Babcock *et al* (1995a, 1995b, 1996) calculated the fatigue index by using the mean percent change in the BPNS Pdi at each stimulation frequency.

There are also some disadvantages to using this technique. In terms of subject comfort, the intensities needed to produce supramaximal stimulation can be uncomfortable. It is difficult to maintain optimal contact between the stimulating electrode and the nerve. Locating the nerve itself can be complicated, therefore necessitating multiple stimulations. This can alter the results through potentiation. At high intensities, it is possible to confuse PNS with brachial plexus stimulation. Sources of error for BPNS include the following; 1) maintenance of nerve stimulation, 2) ensuring that muscle length remains constant for each stimulation and 3) occurrence of twitch potentiation (Babcock *et al.*, 1995a; Babcock *et al.*,

1995b; Babcock *et al*, 1996). Traveline *et al.* (1997) utilized a technique where a plaster mold was fitted around the anterior abdomen to prevent diaphragm shortening. This minimized outward displacement of the abdomen during stimulation. However, a standardized technique to bind the abdomen has not been conferred. Similarly, time constraints may lead to a possible underestimation of diaphragmatic fatigue. An evident time lapse of approximately five to eight minutes during preparation of the subject, from the end of exercise to the first post-exercise measurement and stimulation, accounts for this discrepancy (Babcock *et al.*, 1996). The delicate nature of the technique means that immense technical expertise is required to conduct the testing.

Bilateral phrenic nerve stimulation can also be performed using a magnetic stimulation (Laghi *et al.*, 1995; Similowski *et al.*, 1998). Two protocols currently used include cervical magnetic stimulation (CMS) and unilateral anterior-lateral magnetic stimulation (ATS/ERS, 2002). The former induces bilateral diaphragmatic contraction, while the latter focuses on the more specific hemidiaphragmatic function. Subjects are instructed to flex their neck to approximately 60 degrees (Laghi *et al.*, 1995; Similowski *et al.*, 1998) and the magnetic coil is placed over the cervical spine. The coil is moved between the C_5 and C_7 vertebrae, with the subject instructed to relax at FRC, to determine optimal stimulation (Laghi *et al.*, 1995; Similowski *et al.*, 1998). The stimulus is then increased until no further increases in transdiaphragmatic twitch pressure (Pdi_{tw}) are observed. This position is marked for all succeeding stimulations to be performed. Anterior lateral magnetic stimulation utilizes a similar technique, however, the magnetic coil is placed over the upper part of the sternum.

Paired stimulation is a technique that is often used to determine high frequency fatigue. Two magnetic stimulators, separated by a short time interval (e.g. 10-200ms [5-100Hz]), produce a response (pTw) that is the sum of the two twitches (T1 and T2). Subtraction of T1 from pTw, reflects the contractile properties of the muscle. The resultant diaphragm T2 force–frequency curves are then analyzed (Hughes *et al.*, 1999).

Magnetic stimulation, as opposed to electrical, has been shown to be more comfortable for the subjects. This provides a less invasive means of garnering equally acceptable results. However, magnetic stimulation is still believed to be less specific than electrical stimulation. Some problems, with coactivating muscles innervated by the brachial plexus, have been reported (ATS/ERS, 2002). Since these techniques all measure the diaphragm specifically, none actually assess global respiratory muscle fatigue.

2.3.2.2 Maximal Respiratory Pressures

Maximal respiratory pressures provide a meaningful measurement of global respiratory muscle function, while assessing both the combined peripheral and central components of fatigue. Regarding the respiratory muscles specifically, Black and Hyatt (1969) conducted the most influential study pertaining to muscle strength measurements. They looked at maximal inspiratory pressure (PI$_{max}$) as an indicator of respiratory muscle strength. Their protocols form the basis for the standards still in use today. Maximal expiratory pressure (PE$_{max}$) was measured near total lung capacity (TLC) after a maximal inspiration. Maximal inspiratory pressure was measured near residual volume (RV) after a maximal expiratory (Müeller) maneuver (Black & Hyatt, 1969). The pressures were maintained for at least one second and were repeated until two satisfactory measurements

were recorded. A small leak was placed in the mouthpiece to prevent glottic closure during inspiratory measures.

Measurements of MIP have been shown to be greatest at residual volume (RV), with a decrease at FRC and a decline to zero at total lung capacity (TLC) (Farkas *et al*, 1996). Similarly, MEP has been shown to be greatest at TLC with a progressive decline at FRC and RV, due to the decrease in lung volume. Maximal pressures generated at the mouth are misleading. They must be corrected for, as they reflect pressures generated from active muscle contraction as well passive recoil components of the respiratory systems (Farkas *et al.*, 1996). In order to assess pressures, generated by the respiratory muscles as a single unit (P_{MUS}), without the effects of recoil (P_{RS}), one must keep in mind that P_{RS} is approximately – 40 cm H_2O at TLC, zero at FRC, and +40 cm H_2O at RV (Farkas *et al.*, 1996).

Unfortunately, tremendous inconsistency has been shown in maximal respiratory pressures. These pressures are thought to be linked to the following two behaviours: the procedures used for measurement, and the extreme variability in features of the study population (Bruschi *et al.*, 1992). Insight is given to the immense variability in the exact testing protocols. No less than 8 different procedures used to measure the same task over the past several years have been employed (Table 3). It is possible that the variability is simply a result of the lack of specificity in the protocols.

The volitional nature of maximal pressures tasks are said to be inconclusive because the test conditions are difficult to control and are not sufficiently objective or independent of total body fatigue (Johnson *et al.*, 1993). There is incredible lack of specificity. Similarly, while serial measurement of MIPs after exercise can provide a relatively simple means of tracking changes in inspiratory muscle strength after exercise, the serial measures may be

fatiguing in themselves. Thus, serial measurement presents its own set of problems. This involves setting the frequency of measurements far apart such that the testing procedures do not become fatiguing. Conversely, the measures must be close enough together to track changes in MIP without giving too much time for recovery.

Table 3. Summary of maximal inspiratory pressure (MIP) protocols

Study[a]	Participants	Lung Volume	Inspiratory Muscle (IM) Strength Measurement
Black & Hyatt (1969)	120 normal adults (60M, 60F); 10M and 10F in each decade from 20 to 70 years and a group over 70 years	near RV	Highest value of two technically satisfactory measurements (~9% variation)
Coast et al. (1990)	Highly trained cross-country skiers; n = 6 Sedentary; n = 5	RV	Mean of highest three trials
Mador & Tobin (1992)	n = 7 (6M, 1F)	FRC	Highest of 3 reproducible measurements
O'Kroy et al (1992)	Recreational runners; n=9 (7M, 2F)	RV	Three measures within 5mm Hg - average of two highest
Eastwood et al. (1994)	Healthy highly-motivated volunteers; n=7 (5M, 2F)	FRC RV	Highest of 3 reproducible measurements (within 5% of each other) Highest of 3 reproducible measurements (within 5% of each other)
Wen et al (1997)	Pediatric and adult subjects with suspected IM weakness; n = 178	RV	"Short MIP": average of first 3 highest values with 5% variability "Long MIP": average of 3 highest values with 5% variability
McConnell et al (1997)	Moderately trained males; n = 24	N/A	PIpeak: highest of two measures within 5% PIave: average of two measures within 5%
McConnell & Copestake (1999)	Healthy elderly; n = 41 (18M, 23 F)	RV	3 technically acceptable readings with the highest of the plateau of measures used
Volianitis et al. (1999)	Rowers: n = 11 Non-rowers: n = 12	RV	Highest of 3 measures with 5% variability or within 5 cm H2O difference
Boussana et al. (2001)[32]	Triathletes (all M) : n = 12	FRC	Highest value of 3 reproducible measurements; variability <10%
Sonetti et al. (2001)	Competitive cyclists; n = 9 (all M)	RV	Highest of 4 maximal efforts at 20s intervals

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Romer et al. (2002)	Repetitive sprint sport athletes; n = 24 (all M)	RV	Highest value of 10 maximal efforts at 30s intervals
Williams *et al.* (2002)	Collegiate distance runners; n=7 (5M, 2F)	RV	Average of highest 2 values from multiple trials
Boussana *et al.* (2003)	Triathletes; n = 10 (all M)	FRC	Highest value of 3 reproducible measurements; variability <10%

[a] Studies listed in chronological order

Increasing skepticism towards these procedures may have been the driving force for finding alternative methods utilizing the guiding principals of maximal respiratory pressures. Maillard and colleagues (1998) investigated the use of sniff nasal inspiratory pressure (SNIP) as a substitute to previous methods of testing inspiratory muscle strength and failure. This technique mimics that of the maximal respiratory pressures generated orally, with measurements taken nasally, through one nostril. They showed the technique to provide reliable and valid measurements and clearly presented SNIP as a non-invasive volitional test of inspiratory muscle strength.

While there certainly remains room for improvement, maximal pressures provide a simple and straightforward means of measuring global respiratory muscle strength and fatigue.

2.3.2.3 Electromyography (EMG)

The failure of muscle to produce force during high levels of sustained contraction, or as produced by dynamic efforts against loads, is associated with a shift of the EMG power spectrum from higher to lower frequencies (Badier *et al.*, 1994). This principle forms the foundation of utilizing EMG to identify respiratory muscle fatigue. Unfortunately, surface EMG provides the opportunity for significant contamination of results (cross-talk) due to the nature of the protocol. Placing EMG cutaneously means that there is increased risk of

recording accessory muscles in combination with the diaphragm that is being pinpointed. The introduction of needle EMG as a measurement tool may decrease the chances of such errors. Chen et al. (1996) provided some of the initial discoveries in utilizing power spectral analysis of diaphragmatic needle EMG, to detect diaphragmatic fatigue. They found the procedure to be highly feasible while providing reliable results. A monopolar needle was inserted at a right angle to the chest wall, with the subject lying supine, superior to the costal margin, between the anterior axillary and medial clavicular lines, through the external oblique or rectus abdominus muscles, external and internal intercostals, and finally the diaphragm. Recognition of diaphragmatic activity was easily established by the regular firing pattern with each inspiration. Thus, needle EMG of the diaphragm allows recording of the higher frequencies with little to no contamination from the chest wall and abdominal muscles (Chen et al., 1996). Sources of error relate to sampling errors and noise, with the main problem being the induction of selective sampling, since unlike surface EMG, needle EMG only records from muscle fibres in the area immediate to the needle. Introduction of the needle into the chest wall to measure single motor unit firing frequencies presents a risk of pneumothorax (ATS/ERS, 2002).

2.4   Exercise-Induced Respiratory Muscle Fatigue

Babcock et al. (1995a) found that significant diaphragmatic fatigue occurred after intense whole-body exercise (85% $\dot{V}O_{2max}$) in normoxic and hypoxic conditions. They established that exercise time in hypoxia was one-third shorter than normoxia and recovery time for Pdi to baseline (normal) was significantly longer. A further study by Babcock et al. (1996) showed that heavy endurance exercise to exhaustion (aerobic exercise) demonstrated significant decrease in force production of the diaphragm in response to low frequency

phrenic nerve stimulation. In opposition to the studies of the Johnson *et al.* (1993) and

Babcock *et al.* (1995a, 1996) groups, time to exhaustion during constant-load resistive

breathing was found to be significantly reduced after exhaustive cycling at 65, 75, 85, or 95%

$\dot{V}O_{2peak}$ and that the reduction was independent of intensity (Perret *et al.*, 2000). However,

the former study was specific to the diaphragm, while the latter aimed to investigate the role

of extra-diaphragmatic muscle. Therefore, the divergent results are a result of the

comparison of diaphragmatic fatigue vs. global respiratory performance. Gandevia and

McKenzie (1985) showed that during prolonged inspiratory efforts or following fatigue of

the diaphragm, subjects were able to activate the diaphragm maximally.

Time to fatigue has been shown to depend on age and height in healthy adults, and is

correlated with maximal inspiratory pressure (Fiz *et al.*, 1998). Maximum static respiratory

pressures [MSRP] (e.g. MIP/MEP) show statistically significant negative correlations with

age and statistically positive correlations with physical activity (McConnell & Copestake,

1999). Bruschi *et al.* (1992) showed that age generally did not have much influence even in

subjects over 55 years of age, as previously noted by Black and Hyatt (1969). Conversely,

they demonstrated that body surface area (BSA: $\sqrt{height[cm] x weight[kg] / 3,600}$)

significantly related to respiratory pressures in both males and females younger than 55 years

of age. Similar contradictions were discovered by McKenzie *et al.* (1997) who showed no

support for the concept of diaphragmatic central fatigue when tested with maximal static

inspiratory efforts.

Gandevia and McKenzie (1985) and McKenzie *et al.* (1992) found that deterioration

in diaphragmatic performance occurred when intra-abdominal pressure was elevated. They

pointed to the fact that this mechanism within the body may help explain why this pressure is

rarely elevated for a prolonged period of time during the course of daily activity. There are mechanisms within the body that assure that the needs of the respiratory muscles may be favoured to the locomotor muscles. Coast *et al.* (1990) sought to determine if a differential effect existed between trained and untrained subjects in terms of pre and post-exercise maximal inspiratory pressures. Their results showed that endurance training leads to adaptive changes in the inspiratory muscles. Training the muscles protect them from acute loss of strength (vs. normal subjects post-exercise). A clear differential in post-exercise MIP was presented. The trained group showed no evidence of respiratory muscle dysfunction, while the sedentary subjects presented a 10-17% decrease in post-exercise MIP. McConnell's group (1997) found that there was no correlation between height, mass, body mass index (BMI), or $\dot{V}O_{2max}$ at either pre-exercise P_{Ipeak} or P_{Iave}. Subjects with higher inspiratory pressures were not necessarily the largest, or most aerobically fit. However, while both these indicators declined following the exercise task, it was noted that subjects with the strongest inspiratory muscles displayed the least fatigue. They suggest that strong inspiratory muscles afford some protection against inspiratory muscle fatigue. Similarly, greater absolute strength may mean that a smaller relative demand for force generation is required during exercise. Specifically, there must be a mechanism within the body that insures that the respiratory muscles can continue to function properly even at extreme conditions. These changes could be evolutionary in nature. This allowed for the respiratory muscles to overcome bouts of extreme exertion, not for the sake of a performance benefit, but rather for the sake of sustaining proper life function. The drive to survive may be the underlying mechanism for the general fatigue resistance of the respiratory muscles.

2.5   Inspiratory Muscle Training – Fact or Fiction?

The study of exercise-induced respiratory muscle fatigue has led to related field of study, inspiratory muscle training (IMT). Since groups have been able to present respiratory muscle fatigue post-exercise (Table 2), new studies have been looking at ways in which to help guard these muscles from fatigue. Robinson and Kjeldgaard (1982) concluded that running could improve ventilatory muscle strength and endurance in healthy, previously sedentary individuals. They demonstrated significant improvements in maximum voluntary ventilation (MVV) after a 20-week running endurance program. This initial discovery explored overall whole body muscular endurance and its effects on the respiratory muscles. More specifically relating to the training of the inspiratory muscles, Inbar *et al.* (2000) concluded that 10 weeks of specific inspiratory muscle training (SIMT) increased the respiratory muscles' capacity in highly trained athletes. This was measured by a significant increase in MIP. Interestingly, there was no associated increase in aerobic capacity, as determined by $\dot{V}O_{2max}$ or in arterial O_2 desaturation during the maximal graded exercise challenge.

More recently, Williams and colleagues (2002), using highly-fit competitive athletes showed that respiratory muscle strength and endurance could be improved with IMT. In like manner, this improvement was not transferable to whole-body endurance exercise at 85% $\dot{V}O_{2max}$.

Kellerman *et al.* (2000) showed that high intensity inspiratory muscle training (IMT) led to increase in respiratory muscle strength as measured by MIP. Subjects used a spring-loaded threshold inspiratory trainer, which allows for adjustable threshold loads. Extrapolation of such findings, into the training population, led Volianitis *et al.* (2000) to

show that incorporating a respiratory muscle warm-up, along with a sport-specific warm-up, is more effective than a specific rowing warm-up or submaximal whole-body warm-up as a preparation for performance. This was demonstrated when they incorporated the threshold respiratory trainer along with a specific rowing warm-up (Volianitis *et al.*, 2000). In an earlier study (Volianitis *et al.*, 1999) this same group found that inspiratory muscle strength could be enhanced with preliminary activity, a warm-up phenomenon similar to that found in other skeletal muscles.

Weiner *et al.* (1992) taking examples from the clinical population, showed that in patients with mild asthma, specific inspiratory muscle training (SIMT) was associated with a decrease in the perception of dyspnea. They also demonstrated a decrease in $beta_2$ -agonist consumption, after use of a threshold inspiratory muscle trainer. Sánchez Riera *et al.* (2001) also concluded that inspiratory muscle training relieved dyspnea, increased the capacity to walk, and improved quality of life in COPD patients. Unfortunately, results were once again met with past insignificance. A metaanalyis conducted by the American College of Chest Physicians (ACCP) and the American Association of Cardiovascular and Pulmonary Rehabilitation (AACVPR) Pulmonary Rehabilitation Guidelines Panel showed that change in PI_{max} after respiratory muscle training was not significant (1997). They cited inadequate frequency, intensity and/or duration and small loads (less than 30%) to be the most probable causes of failure. Further development of the principles, learned through the study of specific diseased populations, should lead to considerations that can have important consequences to the healthy and training populations (Levine *et al.*, 2001).

Conversely, Sonetti *et al.* (2001) found that the effect of respiratory muscle training on exercise performance in highly trained cyclists did not exceed that of the placebo group.

Significant increases were found in MIP among both groups, though the differences between them were not significant.

Like many issues that begin with scientific research, the corporate world has jumped on the idea of training the respiratory muscles with the development of several brands of respiratory muscle trainers. Unfortunately, recent studies (Hart *et al.*, 2001) critiquing the effectiveness of such products, have been met with harsh criticism. Speculation, with published results refuting the positive claims of these products, has arisen due in large part to sample sizes that are too small to present generalizations to the greater population. This has led to an increasing feud between researchers and those with a financial interest in the products.

Readers are pointed at a very good review by Sheel (2002) that looked at respiratory muscle training in a healthy population.

2.6 Post-Fatigue Recovery

The past ten years have presented a spin-off of respiratory muscle fatigue research. While some laboratories continue to stress the diaphragm to determine levels of fatigue, others are now looking at post-fatigue recovery to help find further answers to the underlying mechanism of respiratory muscle fatigue. Laghi *et al.* (1995) found that diaphragmatic fatigue, as indicated by a decrease in Pdi twitch (Pdi_{tw}) induced by magnetic stimulation of the phrenic nerves, showed some recovery after 8 hours. However, the diaphragm was not fully recovered by 24 hours. Travaline *et al.* (1997) investigated the recovery of Pdi following induction of diaphragmatic fatigue. They were able to show a 50% reduction in Pdi_{tw} at FRC, with a return to 72% of baseline after 3 hours and complete recovery by 20-25

hours. Post-fatigue Pdi_{max} was reduced to 75%, but recovered to 87% by 3 hours and 100% by 25 hours. Such findings pose interesting consequences for athletes competing on consecutive days. Ker and Schultz (1996) found that while inspiratory muscle strength was normal 3 days after an ultra-marathon, inspiratory muscle endurance, as measured by time to fatigue (T_{lim}) was still impaired. This was in contrast to initial results by Loke *et al.* (1982) who showed that MIP falling 16% immediately at the end of a marathon. The lack of recovery up to 24 hours post-exercise induced diaphragmatic fatigue means that athletes competing on consecutive days may be at a physiological disadvantage.

2.7   Implications of Research

2.7.1   Lessons from the COPD population

Levine *et al.* (2000) presented some very interesting ideas to help extrapolate findings in the COPD clinical population to that of sports science. Knowing that ATP consumption exceeded ATP generation during fatiguing exercise, they sought to present molecular and chemical evidence that the human diaphragm may adapt to severe COPD through a decrease in ATP consumption and an increase in ATP generation. They theorized that a decrease in diaphragmatic ATP utilization could be a possible method for increasing fatigue resistance. Specifically, they hypothesized severe COPD characterized a transformation from fast myosin heavy chains (MHC) to slow. Slow MHCs have been indicated to have a lower ATPase than fast MHCs, decreasing ATP consumption. Using SDS-Page Electrophoresis and immunocytochemistry, they were able to portray results of this transformation from fast-to-slow diaphragmatic MHCs that was consistent with their hypothesis. Similarly, they used succinic dehydrogenase (SDH), as a marker of oxidative phosphorylation occurring during to citric acid (Kreb's) cycle to provide evidence of greater ATP generation in subjects with

severe COPD. They suggested that COPD diaphragms appear to have a greater capacity for oxidation phosphorylation, relative to controls. They believed that it could be possible that endurance training in the healthy population could lead to similar diaphragmatic adaptations, though no conclusive studies have been confirmed. A similar method could be used in between-gender studies to examine possible variances in the metabolic processes within males and females.

2.7.2 Animal Models

The delicate and vital nature of the human respiratory system makes it a difficult area to study intricately, due to the invasiveness of the procedures that would be required to answer those questions that have eluded science. Blood flow to the respiratory muscles, in exercising humans, is not yet measurable (Aaron et al., 1992). The use of animal subjects has done a great deal to provide further discoveries.

Based on previous findings using rats, it had been suggested that an increase in mitochondrial volume would increase fat utilization, slow the utilization of muscle glycogen and blood glucose, and produce less lactate during exercise of a given intensity, possibly helping to delay fatigue (Holloszy & Coyle, 1984; Powers & Criswell, 1996).

Citing the work of Lewis and colleagues (1992), who showed that glucocorticoid administration promoted atrophy of the fast fatiguing type IIb and IId/x fibres, with no effect on slow fatiguing type I and type IIa fibres, Fletcher et al., (2000) tested the hypothesis that in vitro diaphragmatic fatigue resistance could be enhanced in animals (rats) treated with glucocorticoids. Their rationale related to the fact that due to the above atrophy, the glucocorticoid-treated rats would present a higher percentage of fatigue resistant fibres.

Indeed, they showed that costal strips of glucocorticoid-treated rats possessed an enhanced resistance to fatigue. The greater percentage of cross-sectional area consisting of slow fatiguing fibres, thus enhancing fatigue resistance, was indicated as the physiological mechanism for their findings.

Fregosi and Dempsey (1986) looked to compare diaphragm muscle glycogen utilization with that in locomotor muscles following exercise at various intensities in rats. They showed that respiratory muscle glycogen stores were spared during exhaustive exercise, while substantial glycogen utilization was shown in plantaris. They also showed that the [total creatine] and [ATP] did not change systematically in any of the muscles. During exhaustive exercise, their observation of elevated [lactic acid] without glycogen utilization, suggested increased lactate uptake not production, within the respiratory muscles. This led them to conclude that locomotor muscles most likely fatigue and limit exercise performance well in advance of respiratory muscle fatigue.

Translation of these conclusions within the animal model to the results of our study could lead one to assume that the Borg RPE (locomotor muscle discomfort) probably should have been significantly greater than those for dyspnea. That certainly was not the case for most subjects. If locomotor muscles fatigue and limit exercise performance well in advance of respiratory muscles, wider differences between ratings for dyspnea and leg discomfort should have been seen. Similarly, HG ratings could have been at risk due this postulated whole-body fatigue.

Research looking at the changes endured by the respiratory muscles of animals during exercise has given some very valuable information. In 1986, Manohar examined the changes in blood flow and oxygen supply to the respiratory, limb muscles, and internal organs during

maximal exertion in ponies. He demonstrated that the circulatory response of the exercise pony consisted of substantial vasoconstriction in the renal and splanchnic tissues. This redistribution of blood flow was greater to the diaphragm (on a per-weight basis) than in limb locomotor muscles. It was also accompanied by an overall increase in CO, ~16%, to the inspiratory and expiratory muscles. Data implied that diaphragmatic work and its O_2 requirements may not be less than other forcefully contracting muscles (e.g. gluteus medius and biceps femoris) showing similar increases in metabolic need.

Two years later, Manohar (1988) looked to determine if differences existed between blood flow to the costal and crural regions of the equine diaphragm, as well as the fraction of cardiac output (CO) needed by the diaphragm at near-maximal exercise. It was shown that similar costal and crural diaphragmatic perfusion observed in resting ponies was not preserved during exercise, with the costal diaphragmatic blood flow being significantly larger. Differences in metabolic O_2 requirements of these tissues during exercise were given as a possible reason. CO perfusing the diaphragm was also increased substantially during near-maximal exercise, with the foremost allocated to the costal diaphragm. However, this increase still only related to 2.2% of the CO during near-maximal exercise. Manohar (1988) recommended that previous estimates of respiratory muscle $\dot{V}O_2$ comprising $\geq 20\%$ of total-body $\dot{V}O_2$ during maximal exercise appear unlikely in light of the findings of his study.

There are still many controversial issues within this field of research seemingly relating back to the fact that there has been no concise explanation for the mechanisms involved in respiratory muscle fatigue and recovery. Research findings within the animal world may help to uncover further mechanisms in the future.

An intriguing factor of respiratory muscle research has to do with perhaps the longest running dilemma in exercise physiology, the factors limiting $\dot{V}O_{2max}$. Bassett and Howley (1997) stated that, in exercising humans, $\dot{V}O_{2max}$ is limited by the ability of the cardiorespiratory system to deliver oxygen to the exercising muscles. While this idea has been controversial, it has been demonstrated that an increase in oxygen delivery can increase $\dot{V}O_{2max}$, suggesting oxygen supply limitation (Richardson et al., 1999). Harms et al., (2000) showed that respiratory muscle unloading, reduced $\dot{V}O_2$ and caused hyperventilation. This demonstrated that the work of breathing normally incurred during sustained, heavy breathing exercise (90% $\dot{V}O_2$) has a significant influence on performance. It may be possible to shed further light on this topic, through intensive investigation into the role of respiratory muscles and their fatigability in an attempt to produce an improvement in aerobic capacity. Before such steps can be made, the foundation must be set through standardized methodologies.

There remains to be a "gold standard" approach to measurement of respiratory muscle fatigue. The risk of error seems to be high, with several options to measure the same variables. Currently, it has been said that serial measurement of respiratory muscle pressure generation in response to electrical and magnetic stimulation is arguably the best technique to directly assess the development of respiratory muscles fatigue (ATS/ERS, 2002). It is also believed that such techniques offer the utmost potential for future innovation into an objective test of respiratory muscle fatigue (ATS/ERS, 2002).

While extensive research has been conducted over the past quarter century, this area of research is still in its infancy. Twenty years ago Macklem (1980) declared the inspiratory muscles as the, "last remaining essentially uninvestigated vital organ." While laboratories

around the world are publishing rather vast amounts of research concerning this topic, the results, as presented, remain controversial.

CHAPTER III: Methodology

3.1   Facilities and Instrumentation

Participants who met the inclusion criteria (see 3.2 Subjects) were evaluated at the John M. Buchanan Exercise Science Laboratory within the Aquatic Centre of the University of British Columbia. Anthropometrical measurements (height and weight) were taken for each participant prior to the study. Fat-free mass was estimated for each subject through densitometry (Lacy & Hastad, 2003). Body density (Db) equals body mass divided by body volume. To calculate body volume, a hydrostatic weighing technique, based on Archimedes' principle was used (Appendix I). The homemade apparatus consisted of a chair mechanism suspended to an overhanging scale, submerged within a large tank filled with water. The resultant body density value was then plugged into Siri's Equation (1961) to determine percent body fat (Appendix I).

Maximal inspiratory pressures, as produced during a brief, quasi-static contraction (Müeller maneuver), reflect the capacity of the global inspiratory muscles to generate force (Larson *et al,* 1993). MIPs were taken with a portable handheld mouth pressure meter (Micro Mouth Pressure Meter (MP01), Micro Medical, UK). The device could withstand an operating pressure of $\pm$ 350cm H_2O ($\pm$ 5PSID) and a burst pressure of + 1400cm H_2O ($\pm$ 20 PSID). The mouth pressure meter utilized piezo resistive pressure sensing technology to achieve accuracy and long-term stability in a hand held unit. The maximum effort sustained for at least one second was calculated with the largest negative pressure displayed. Values were reported as positive numbers (cm H_2O). The maximum measurement error of the mouth pressure meter was $\pm$ 3%, as published by the manufacturer (Micro Mouth Pressure Meter (MP01), Micro Medical, UK).

Similar handheld devices have been widely used of late, due to their ease of use, portability and accuracy (McConnell *et al.,* 1997, 1999; Volianitis *et al.,* 1999, 2000; Romer *et al.,* 2002; Lomax & McConnell, 2003). Portable measurements of maximum mouth pressures have been deemed reliable and accurate in the normal and patient population, when compared to measures utilizing a pressure transducer (Hamnegård *et al.,* 1994). The mouth pressure meter was calibrated against a water manometer once per year based on the manufacturers recommended guidelines.

Using a standardized protocol, in which the highest value of five trials within 10% was used, maximized reliability of inspiratory muscle strength measurements. Specifically, the highest of 3 technically satisfactory & reproducible measurements (variation < 10%) were determined to be the pre-exercise MIP (Black & Hyatt, 1969, Hayot *et al.,* 2000, Boussana *et al.,* 2001). All MIPs were recorded from near residual volume (RV) to provide maximal values of inspiratory mouth pressure. Test-retest reliability was determined as maximal inspiratory pressure recordings were obtained on two separate visits. A coefficient of variation of 10-15% was the general guideline for acceptability (Black and Hyatt, 1969). A more recent study presented greater precision in MIP measurements, closer to 9%, thus portraying better reliability (Aldrich & Spiro, 1995).

Maximal aerobic fitness was evaluated by an incremental stage $\dot{V}O_{2max}$ bicycle ergometer test, using the Sensor Medics Ergometrics 800 bicycle ergometer (Sensor Medics, Yorba Linda CA USA) and the Sensor Medics Vmax 29 series metabolic measurement cart (Sensor Medics, Yorba Linda, CA USA). Breath-by-breath values were averaged over 20 second intervals and displayed on the monitor. Similar to other respiratory muscle fatigue studies that have utilized a bicycle ergometer, all participants began stage one pedaling at a

50W with subsequent increases of 30W per minute, until task failure (exhaustion) (Boussana *et al.*, 2003). This protocol allowed for maximum oxygen consumption ($\dot{V}O_{2max}$) to be achieved within 5 to 15 minutes, thus providing ample time to fatigue the respiratory muscles (Bye *et al.*, 1984).

3.2   Subjects

The subjects were composed of 18 male and 16 female (34 total) healthy university-aged (18-30 years) volunteers who were non-smokers and had no chronic or acute respiratory conditions (e.g. asthma, cold, flu, etc.), as self-reported. Female participants were limited to those who were eumenorrheic over the past six months. The females completed a short survey indicating oral contraceptive use and history.   The study utilized both those consuming oral contraceptives ($n = 10$) and those who were not ($n = 6$).   All female participants were tested during the early follicular phase of the menstrual cycle, (days 3 - 8) where the estradiol concentration started at levels comparable to males (Lebrun *et al* 1995, Frankovich & Lebrun 2000, Tarnapolsky *et al* 2001). Progesterone levels were also close to zero during this phase. This was important because it had been suggested that progesterone could enhance ventilatory drives and stimulate ventilation (Chen & Tang, 1989). Avoiding testing during the luteal phase, where progesterone levels peak, guarded against this possible confounding variable. Chen and Tang (1989) also indicated that respiratory muscle strength and pulmonary function (e.g. 12-sec maximal voluntary ventilation (MVV), forced vital capacity (FVC), forced expiratory volume (1 sec) (FEV_1), FEV_1/FVC, V_T, f_b, and duty cycle) were unchanged when comparing the results from the midfollicular and midluteal phases. However, the results of Janse de Jonge *et al.* (2001) suggested that fluctuations in female

reproductive hormone concentrations, over the course of the menstrual cycle, do not affect muscle contractile characteristics. All testing corresponded with the female participants' selection of the appropriate testing date to coincide with their early follicular phase. Therefore, the familiarization task was always completed prior to the exercise task.

Prior to testing, informed consent was given and all subjects completed the *Par-Q & You Questionnaire* (CSEP, 2002) to confirm their positive health status and ability to complete the required exercise tasks. As per the guidelines of the *Par-Q*, if the subject answered "yes" to one or more of the questions, the subject would have been required to present signed medical documentation from their physician stating their ability to be a subject prior to being accepted in the research study. Such a situation did not arise during the course of this study. Signed letters of consent presenting information, time commitment and possible risks involved in the investigations were obtained from all participants prior to the study. Ethical approval was obtained from the UBC Office of Research Services Clinical Research Ethics Board with the use of human subjects in accordance with the Declaration of Helsinki.

3.2.1 Exclusion Factors

Exclusion factors included a history of smoking or respiratory illness, chronic or acute respiratory conditions (e.g. asthma, cold, flu, etc.), leg or knee injury (e.g. patellar injury, knee ligament damage, etc.), and failure to provide medical clearance for those indicating "yes" to one or more questions on the *PAR-Q & YOU* (CSEP, 2002) questionnaire. Since the study focused on moderately trained individuals, the criterion was initially defined as those individuals having $\dot{V}O_{2max}$ scores between 40 and 50 ml·kg^{-1}·min^{-1} for females and 45 and 55 ml·kg^{-1}·min^{-1} for males (Aaron *et al.,* 1992; O'Kroy *et al.,* 1992; Coast *et al.,* 1999;

Volianitis *et al.,* 2001; Williams *et al.,* 2002). However, there were a few subjects that fell just outside the set ranges. These data were included in the results because the findings were matched between the genders. That is, for the two males that were just outside of the range, were matched to two females that were also outside of the given range. These criteria excluded the recruitment of untrained and highly trained individuals, helping to assure a greater likelihood of inducing respiratory fatigue, since it had been suggested that the respiratory musculature may not fatigue in aerobically trained athletes, as shown in a study testing female cyclists during short-term maximal exercise (Gonzales *et al.,* 2002 [abstract]).

3.2.2 A Priori Power, Effect Size, and Sample Size Calculation

A priori power and sample size calculations were completed with the *G Power* software package (Erdfelder *et al.,* 1996). The results of a pilot study (see below) provided the data that for the calculation table (Table 4). Utilizing group means (change in MIP compared from baseline to immediately following the $\dot{V}O_{2max}$) and standard deviations, the required sample size was calculated as follows by *G-Power*:

Table 4. Effect-size calculation table using pilot study data

Mean Group 1	17
Mean Group 2	11
Sigma (within each group)	6
Effect size d	1.00
Alpha	0.05

Using the resultant effect size (d = 1.00) and setting the alpha level (α) at 0.05, the following total sample size values were calculated (Table 5):

Table 5. Power and sample size determination

Power	Actual Power	Delta	Critical	Total Sample Size
0.95	0.9548	3.3912	t(44) = 1.6802	46
0.90	0.9023	3.0000	t(34) = 1.6909	36
0.85	0.8684	2.8284	t(30) = 1.6973	32
0.80	0.8241	2.6458	t(26) = 1.7056	28

With the above calculations, it was found that with an alpha level set at 0.05 and power set at 0.80, a sample size of 28 (14 male and 14 female) would be required for the purposes of the research study.

3.3 Testing Procedures

The study consisted of a cross-sectional design that used a physiological approach to determine gender differences in inspiratory muscle fatigue following maximal aerobic whole body exercise. Day 1 incorporated anthropometrical measurements, hydrostatic weighing and a familiarization task that was used in preparation for the data collection involved with the maximal aerobic test on Day 2. For some subjects, the familiarization task was a repeat of what they had previously completed as part of the initial pilot and MIP reliability studies (Part II of the pilot study).

3.3.1 Session One: Intro, Hydrostatic Weighing and Familiarization Task

Prior to any measurements, the consent form was discussed and reviewed by the participant and the co-investigator. The participants had the opportunity to give consent immediately, or bring the form back prior to testing on Day 2. The *Par-Q and You*

questionnaire was then administered. Anthropometrical measurements (height and weight) followed the questionnaire.

Since the average female has a higher percentage of body fat (5-10%) and lower muscle mass compared to the average male, it had been suggested that maximal aerobic capacity be indicated relative to fat-free mass (Cureton & Sparling, 1980; Tarnapolsky et al., 2001). To determine fat-free mass, hydrostatic weighing was utilized. This procedure helped negate the possibility of selecting females that are heavier than males, which could occur with a between-gender comparison based upon absolute $\dot{V}O_{2max}$ (Tarnapolsky et al., 2001).

3.3.1.1 Hydrostatic Weighing:

Before entering the hydrostatic weighing tank specific instructions (as follows) were discussed to all participants. These instructions were discussed and any questions were answered by the investigator prior to the subjects entering the tank. Subjects descended into the tank slowly and were seated in the suspended chair with their hands holding the vertical support bars attached to the chair, below the level of the water. The participants were advised to pat out any air bubbles within their swimsuits. Particular attention was paid to the male subjects, since they tended to wear swimsuits (shorts) that trapped air within the legs and pockets. Subjects were also advised to keep their feet suspended within the water, or placed on the horizontal bar connected parallel to the seat below them. They were told to avoid touching their feet on the base of the water tank at any time during the testing procedure. Once seated comfortably with the air removed from their swimsuits, the subjects were instructed to submerge their heads completely under the water and breathe out as much air from their lungs as possible by blowing bubbles. Simplified, the subjects bent their heads

forward, completely under the water and blew out bubbles and held with no air in their lungs until the investigator tapped the side of the tank to confirm a valid reading (approximately 2-4 sec). The subjects were told to complete the task under slow control to minimize the oscillations of the attached scale. At least four measurements were taken to confirm a reliable measurement. All subjects were advised to come out of the water to regain their breath if they felt any discomfort during the process, or if they felt that they could no longer hold their breath underwater.

All males had their fat-free mass measured during their Day 1 testing appointment. While this procedure was similar to most females, there were a few cases where the fat-free mass was determined after completion of the Day 2 testing. This occurred because the time interval between Day 1 and 2 testing was deemed too long, (max = 32 days) having to wait for the early follicular phase, providing a greater opportunity for changes in overall and fat-free mass.

3.3.1.2 Familiarization Task:

The familiarization task was comprised of five measurements of maximal inspiratory pressure (MIP) and maximal handgrip (HG) maneuvers. MIP was measured using a handheld mouth pressure meter (Micro Mouth Pressure Meter (MP01), Micro Medical, UK). The participants were instructed to expire to residual volume (RV) and then inspire maximally to generate the greatest inspiratory pressure. Producing each maximal effort from RV has been suggested to control for the initial length of the inspiratory muscles (Volianitis *et al*, 2001). More importantly, measuring MIP from RV ensured that the highest pressures would be recorded. Subjects were instructed to place a nose clip over their nostrils upon expiration to ensure maximal recordings from the mouth. They were advised not to put on

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the nose clip before expiration, to avoid undue comfort (popping) within the inner ear, during the initial expiratory maneuver.

The participants were verbally encouraged by the investigator throughout all trials to assist in performing maximal efforts. This task was repeated ten times over two days of testing, with one minute of rest between trials, since a learning effect has been previously reported with MIP (Wen *et al.*, 1997).

Maximal handgrip strength measurements were recorded in alternating fashion after each MIP measure. Subjects were advised to hold the handgrip dynamometer (T.K.K. Grip A, Takei, Tokyo, Japan) in their dominant hand with their arm abducted, and then exhale and squeeze as hard as they could while adducting the arm back to their midline. They were told to avoid making contact with the dynamometer with any part of their bodies. Handgrip strength was measured in order to look at motivation and central effort, in an attempt to help differentiate IMF from generalized whole-body fatigue (Fuller *et al.*, 1996, Coast *et al.*, 1999). Inhibitions of voluntary efforts, due to reduced motivation and attention, as well as reduced motor drive resultant of afferent feedback from the contracting muscle (e.g. pain) are factors that exemplify central fatigue (Asmussen & Mazin, 1978). Failure of action potential propagation and disruption of processes within the muscle itself (e.g. calcium release, excitation-contraction coupling) results in peripheral fatigue (Bigland-Ritchie *et al.*, 1978). The measurements allowed for a better view of the physical consequences and physiological responses of the entire body to maximal exercise.

3.3.2   Session Two: $\dot{V}O_{2max}$, HG, and MIP Fatigue Measurements

On the test day, participants were instructed not to eat, as well as avoid drinking alcohol or caffeine 2 hours before testing, to avoid the introduction of confounding factors to human physiological functions.   Prior to the arrival of the participants, the O_2 and CO_2 analyzers were calibrated with the use of known certified gas concentrations (Praxair Canada Inc., BC).   The mass flow sensor for the metabolic cart (Sensor Medics, Yorba Linda, CA USA) was calibrated using a 3.0 L calibration syringe (Sensor Medics, Yorba Linda, CA USA), at various flow rates (slow, medium and fast paced strokes) according to the manufacturer's instructions.   The flow sensor was recalibrated prior to each subject, as per the manufacturer's guidelines.   Subjects were fitted with a heart rate monitor (Polar Sport Tester, Polar Electro Oy, Kempere, Finland) as soon they arrived at the lab so that resting data could be evaluated, as well as in preparation for measurements during the exercise task. Each participant was then re-weighed, with the resultant mass verified and compared to that measured on the initial visit.   Each participant then alternated at least 5 MIPs and 5 HGs as a pre-test baseline.   Subjects performed a maximum of 9 MIPs in order to get their top three values within the acceptable range of variability.   The highest value within 10% of the other trials was considered the maximum effort (Black & Hyatt, 1969, Hayot *et al.,* 2000, Boussana *et al.,* 2001).

Upon completion of the pre-trial tasks, the subjects mounted the electrically braked Sensor Medics Ergometrics 800 bicycle ergometer (Sensor Medics, Yorba Linda, CA USA). The seat height and handlebars were adjusted to suit the individual participants.   The subjects were clipped into their pedals once proper adjustments were made.   The face mask connecting the collecting tubes to the Sensor Medics Vmax 29 series metabolic measurement

cart (Sensor Medics, Yorba Linda, CA USA) was then secured tightly around the mouth and nose of the participant. Each subject was then asked to exhale maximally to ensure that no air was leaking around the perimeter of the mask. Once the mask was secured the subject began their warm-up, consisting of 5 minutes of cycling against a load of 50W. This was the same load used during the first minute of the test. The participant then completed a 5-13 minute test of maximal aerobic capacity on an electrically-braked bicycle ergometer (Sensor Medics, Yorba Linda CA USA). The workload started at 50W and increased by 30W per minute. Participants were advised to keep their RPM at 75rpm minimum and were told to continue cycling until exhaustion. Values were recorded on a breath-by-breath basis, averaged over 20-second intervals.

The task was terminated at volitional fatigue, when the pedal cadence fell below 45 rpm. A maximal test was confirmed based on pre-determined indicators; respiratory exchange ratio (RER = $\dot{V}CO_2 / \dot{V}O_2$) above 1.1, HR within 10 beats of age predicted maximum heart rate (220-age), and a plateau in $\dot{V}O_{2max}$ (either a decrease or an increase of less than 2 ml/kg/min (ACSM, 2000). $\dot{V}O_{2max}$ was determined by averaging the highest $\dot{V}O_2$ values over two consecutive 20-second intervals.

Each subject was asked to give ratings of perceived exertion using a modified Borg scale (Borg & Noble, 1974; Borg, 1982). At one-minute intervals during the exercise task, each subject was asked to rate dyspnea and locomotor muscle fatigue (Harms *et al.*, 2000). A visual representation of the scale was placed in front of the subject while they continued to pedal. They were simply asked to point to the value corresponding to the breathing and leg discomfort respectively (Appendix II). When the subjects were asked to rate breathlessness, they selected a number between 0 and 10 (including fractions) whose corresponding words

most appropriately described the sensation of breathlessness at that particular time. Subjects were instructed to score only their sensation of breathlessness and ignore other sensory stimuli such as throat irritation (Burdon *et al.*, 1982).

HR measures were taken simultaneously with the Borg ratings, providing a view of the changes incurred within HR over time period of exercise. Immediately following the termination of the task, the face mask was removed from the face and the subject was led from the cycle ergometer to the chair (placed 1m away) for the first post-exercise MIP and HG measures. Further MIP and HG recordings were taken at one-minute intervals after the immediate MIP. Measures taken at one-minute intervals lasted up to 5 minutes, where the subjects had subsequent 5 minute breaks between the final two measurements take at 10 and 15 minutes post-exercise. HR measures were continually recorded, corresponding to each MIP/HG measurement interval.

3.3.2.1 Reliability

In order to assess reproducibility of the research method, a sub sample of 6 subjects (3 females and 3 males) completed testing (day 2) on two separate occasions. These subjects completed the entire protocol on two separate occasions, separated by at least one day; (M: 6.5±6 days, range: 1-20 days; F: 14.6±10 days, range: 1-32 days; mean ± SD), to investigate the reliability of the measurement outcomes. The time interval between measures was greatest in one female subject due to having to wait for the next appropriate phase of the menstrual cycle.

Reliability of MIPs was also predetermined in a pilot study using 8 subjects (4 M, 4F; 22±1.3yrs) with coefficients of variation values ranging from 8-9% (7.94%-8.77%) over all 9 trials, and 2-3% (2.15% - 2.61%) for the top 3 trials, in males and females respectively.

3.3.2.2 Intensity

Intensity of work was measured through heart rate using a heart rate monitor (Polar Sport Tester, Polar Electro Oy, Kempere, Finland). The modified Borg Scale was also used to evaluate intensity through measurement of ratings of dyspnea (breathlessness) and ratings of perceived exertion (locomotor muscle discomfort). Much like the known linear relationship between HR and $\dot{V}O_2$, RPE is also linearly related to $\dot{V}O_2$ (Noble & Borg, 1972). Relations of the pre-fatigue and post-fatigue scores were used to determine if the participants worked at similar intensities. Wilson and Jones (1991) showed that there was a significant correlation ($p = 0.0001$) between breathlessness and minute ventilation. The Borg scale continues to be used extensively as a reliable technique for studying the sensation of breathlessness over time in both clinical (Iandelli *et al.,* 2002, Lewis *et al.,* 2003, Fierro-Carrion *et al.,* 2004) and healthy populations (Harms *et al.,* 2000; Volianitis *et al.,* 2001). A comparison of these values also gave insight to the motivation levels of the participants, allowing one to see if the subjects worked at the same intensity during all tasks. Similarly, Borg scores for the locomotor muscles were compared to those for dyspnea to provide a general model of fatigue for each individual.

3.3.2.3 Motivation

A further, more stringent method of determining motivation during the required tasks occurred in the form of a maximal handgrip strength test, modified from that used by Fuller *et al.* (1996) and Coast *et al.* (1999). The average of the two highest values was used as the maximal value. This additional test was used to confirm that the anticipated decreases in post- exercise MIP values were not the result of reduced motivation (Fuller *et al.,* 1996) or

general whole-body fatigue (Coast *et al.*, 1999), but rather due to fatiguing of the respiratory muscles.

3.4   Data Analysis

A comparison between male and female subjects was made to determine the difference in the rate of decline in MIP for the respiratory task, and maximal handgrip strength (HG) for the motivation task. These comparisons were made using a group (2) by time (9) repeated measures analysis of variance (ANOVA). A similar group (2) by time (5) repeated measures ANOVA was used for analysis of pooled time. Such analyses allowed for evidence of fatigue to be established within each specific sex. Values of $p < 0.05$ were considered to be statistically significant and were set *a priori*. All other variables tested (height, weight, age, etc.) were analyzed using a one-way ANOVA. Pearson-product moment correlation co-efficients and significance probabilities were calculated between MIP_{rest}, MIP_{1-2} (average MIP over approximately the first 2 minutes post-task, e.g. immediate to 2 minutes), anthropometric variables, and maximal exercise variables. A significance level of 5% was used for all correlations.

All values are reported as mean $\pm$ SD, unless otherwise noted (some tables mean $\pm$ SEM). All statistical analyses were performed using the 11.0 version release of SPSS for Windows (SPSS Inc., Chicago IL).

CHAPTER IV: Results

4.1 Descriptive Measures

The subjects were divided into two groups on the basis of their gender (Table 6). Testing occurred over two days (M: 6.5 ± 6.0; F: 14.6 ± 4.7 days; mean ± SD). All subjects were approximately the same age, with the males being significantly taller and heavier compared to the females. The fat-free mass of the groups varied in absolute terms and were converted to percentages of fat-free mass of 84.1% (16% body fat) in males and 74.6% (25% body fat) in females respectively.

Table 6. Descriptive statistics for male and female subjects

	Males	Females
n	18	16
Age	23.2 ± 0.6	22.6 ± 0.5
Height (m)	1.80 ± 0.0	1.68 ± 0.0†
Mass (kg)	77.5 ± 2.2	61.8 ± 2.1†
Fat-Free Mass [FFM] (kg)	63.1 ± 1.6	46.1 ± 1.3†
BMI	24.0 ± 0.5	21.7 ± 0.6†

Values are means ± SEM; *n* , no of subjects; BMI, basal metabolic index
† Significantly different from male group (p<0.05)

Resting HR values (Table 7) were similar between the genders (p>0.05). However, males had significantly higher (statistically) maximal handgrip strength (HG) values, as well as maximal inspiratory pressure (MIP) (p<0.001).

Table 7. Resting values for male and female subjects

	Males	**Females**
n	18	16
Hrrest, bpm	68.9 ± 2.1	68.3 ± 1.9
HG$_{rest}$, kg	51.6 ± 1.6	30.5 ± 1.2†
MIP$_{rest}$, -cmH$_2$O	153.4 ± 6.7	106.4 ± 4.7†

Values are means ± SEM; *n*, no of subjects; HR$_{rest}$, heart rate @ rest

HG$_{time}$, maximal handgrip strength @ rest

MIP$_{time}$, maximal inspiratory pressure @ rest

† Significantly different from male group (p<0.05)

4.2 Comparisons at $\dot{V}O_{2\ max}$

Maximal exercise testing (Table 8) showed that males had significantly higher maximal oxygen consumption ($\dot{V}O_{2\ max}$) values statistically (p<0.05) when compared to the females, both in absolute terms (L•min^{-1}), and relative to mass (ml•kg^{-1}•min^{-1}). However, when these values were converted relative to fat-free mass, the $\dot{V}O_{2\ max}$ (ml•kg[FFM]$^{-1}$•min^{-1}) values were not statistically different between the genders (p>0.05). No statistically significant relationship existed between height, mass, fat-free mass, or $\dot{V}O_{2\ max}$ with pre-exercise (MIP$_{rest}$) or post-exercise MIP (MIP$_{1-2}$) values.

The respiratory parameters during maximal exercise showed some varying results. The male subjects had significantly greater minute ventilation ($\dot{V}_E$) and tidal volume (VT) compared to the females (p<0.05). The males also had higher respiratory exchange ratio values (RER) (p<0.05).

Table 8. Comparison of selected parameters between male and female groups at $\dot{V}O_{2\,max}$

	Males	Females
n	18	16
$\dot{V}_{O2max}$ (L•min^{-1})	3.87 ± 0.1	2.76 ± 0.1†
$\dot{V}_{O2max}$ (ml•kg^{-1}•min^{-1})	50.3 ± 1.6	44.9 ± 2.1†
$\dot{V}_{O2max}$ FFM (ml•kg[FFM]$^{-1}$•min^{-1})	61.6 ± 1.6	59.8 ± 1.9
$\dot{V}_E$ (L•min^{-1})	163.4 ± 6.2	105.4 ± 5.2†
RER	1.24 ± 0.0	1.19 ± 0.0†
VT (L)	3.22 ± 0.1	2.12 ± 0.1†
fb	48.6 ± 2.1	49.1 ± 1.5
Hrpeak, bpm	191.1 ± 2.2	182.6 ± 1.8†
Maximal External Power (W)	274.9 ± 16.7	218.7 ± 11.2
Dyspnea (Borg)	7.89 ± 0.4	7.50 ± 0.4
Leg (Borg)	8.33 ± 0.4	8.00 ± 0.5

Values are means ± SEM; *n*, no of subjects; $\dot{V}O_{2max}$, maximal oxygen consumption; $\dot{V}_E$, minute ventilation; RQ, respiratory quotient; VT, tidal volume; fb, frequency of breathing; HR, heart rate; Borg, ratings of perceived exertion (RPE) Maximal External Power; W
† Significantly different from male group ($p<0.05$)

There was no statistically significant difference between the frequency of breathing, ratings of perceived exertion (leg) and dyspnea of the females when compared to the males ($p>0.05$). Peak HR values were significantly higher in males compared to females. The males also exerted statistically significantly higher maximal power compared to the females ($p<0.05$).

4.3   Comparisons Post-exercise (recovery)

Maximal handgrip measures showed significant differences between the genders at all time intervals (Table 9) ($p<0.05$). The males presented handgrip strength measures that were approximately 20kg greater than the corresponding measure in females. There was no statistically significant difference compared to baseline values in either gender.

Table 9. Pre- and post-exercise values for raw maximal handgrip strength (HG) values

	Males	**Females**
n	18	16
HG_{rest}, kg	51.6 ± 1.6	30.5 ± 1.2†
HG_I, kg	51.8 ± 1.8	31.3 ± 1.2†
HG_1, kg	50.2 ± 1.7	30.9 ± 1.2†
HG_2, kg	50.2 ± 1.9	29.8 ± 1.3†
HG_3, kg	49.4 ± 1.9	30.3 ± 1.1†
HG_4, kg	48.6 ± 1.6	29.2 ± 1.2†
HG_5, kg	48.7 ± 1.5	28.3 ± 1.3†
HG_{10}, kg	51.1 ± 1.9	30.1 ± 1.4†
HG_{15}, kg	50.5 ± 1.3	29.7 ± 1.4†

Values are means ± SEM; n, no of subjects;
HG_{time}, maximal handgrip strength @ time
† Significantly different from male group (p<0.05)

The conversion of the raw values (kg) into percentages of baseline maximal handgrip strength showed no significant differences between the genders (Figure 1). Specifically, the trends between the genders were very similar. The average values over 15 minutes also showed no change relative to baseline, with the HG values at 98% and 97% of baseline for males and females respectively. However, the results showed that the serial measurements may have been fatiguing in themselves, since the average HG values for each gender, as well as for the group as a whole, were significantly different than baseline at the 3-5 minute post-exercise interval (p<0.05). Consequently, the HG measures in both genders returned to approximate baseline levels at the 10 to 15 minute time interval.
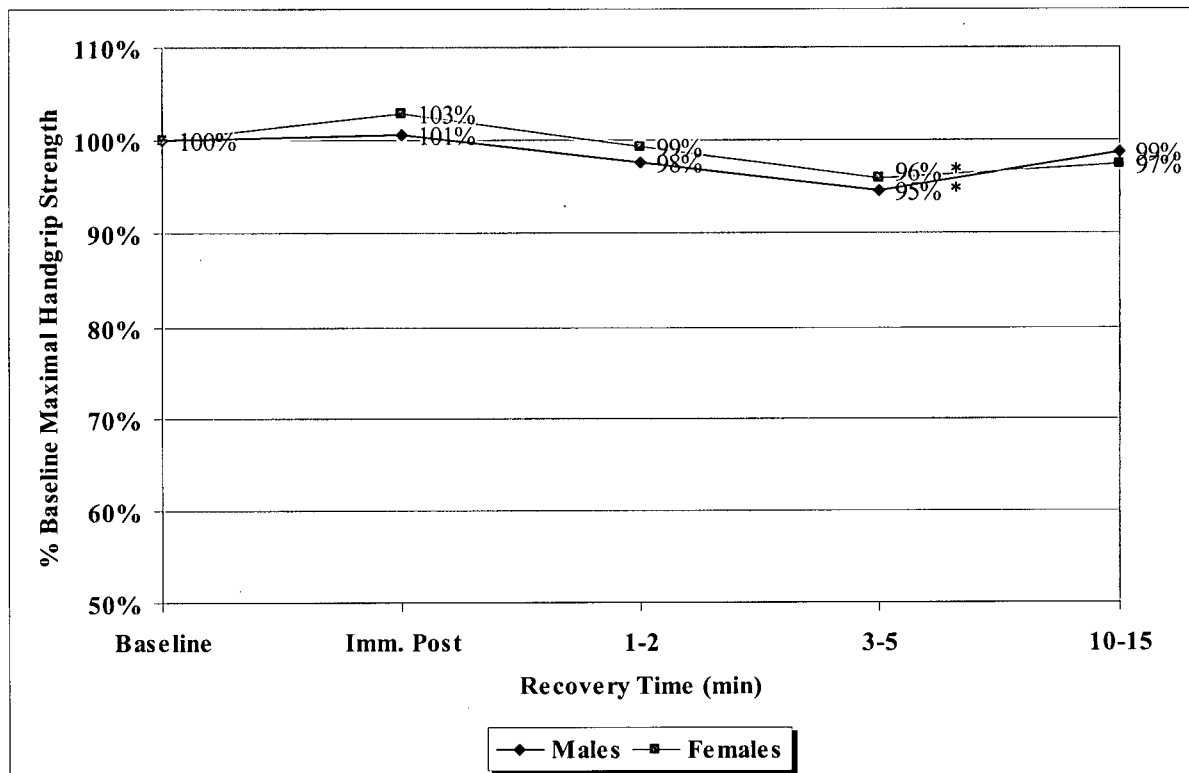
Figure 1. Baseline and recovery of maximal handgrip strength (kg) post- maximal exercise (F_{GENDER} = 0.286; p>0.05; F_{TIME} = 11.638; p<0.001; $F_{GENDER*TIME}$ = 0.742; p>0.05)
(*Significantly different than baseline, p<0.05)

The use of the portable mouth pressure meter allowed for measurements to be taken very rapidly post-exercise. MIP and HG were recorded in that same order for each time interval. The average time for the first MIP measurement was within 20 seconds of volitional fatigue (20.6±7.1s, mean ± SD). Subsequent MIPs were recorded at one-minute intervals to this initial (immediately post-exercise) measurement.

MIP measures, as absolute values, showed significant differences between the males and females at all time intervals (Table 10). This trend was similar to that portrayed within HG.

Table 10. Pre- and post-exercise values for maximal inspiratory pressure (MIP) values

	Males	Females
n	18	16
MIP_{rest}, -cmH_2O	153.4 ± 6.7	106.4 ± 4.7†
MIP_I, -cmH_2O	126.7 ± 7.8	83.8 ± 5.6†
MIP_1, -cmH_2O	131.3 ± 7.1	95.1 ± 5.8†
MIP_2, -cmH_2O	134.2 ± 6.6	94.2 ± 5.4†
MIP_3, -cmH_2O	135.9 ± 7.0	95.4 ± 5.4†
MIP_4, -cmH_2O	133.8 ± 6.6	90.9 ± 4.9†
MIP_5, -cmH_2O	136.4 ± 5.6	93.8 ± 4.5†
MIP_{10}, -cmH_2O	141.7 ± 7.1	95.0 ± 4.6†
MIP_{15}, -cmH_2O	144.4 ± 7.4	98.1 ± 4.8†

Values are means ± SEM; *n*, no of subjects;
MIP_{time}, maximal inspiratory pressure @ time
† Significantly different from male group ($p < 0.05$)

These same values as a percentage of baseline MIP, showed a similar pattern of recovery following maximal exercise in both males and females (Figure 3). Both males and females showed a significant drop in MIP values immediately following exercise, that remained in tact for 15 minutes post-exercise. MIP recovered slightly when the average of the 1-2 minute time interval was calculated. Consequently, average MIP values for the females dropped again at the 3-5 minute time interval, only to increase once again by the 10-15 minute interval. After the initial drop in males, their values consistently rose to 15 minutes. Thus, it can be seen that save the 1-2 minute interval, % of baseline MIP values for the females were consistently lower than the males. However, these differences between the genders at each time interval were not statistically significant.
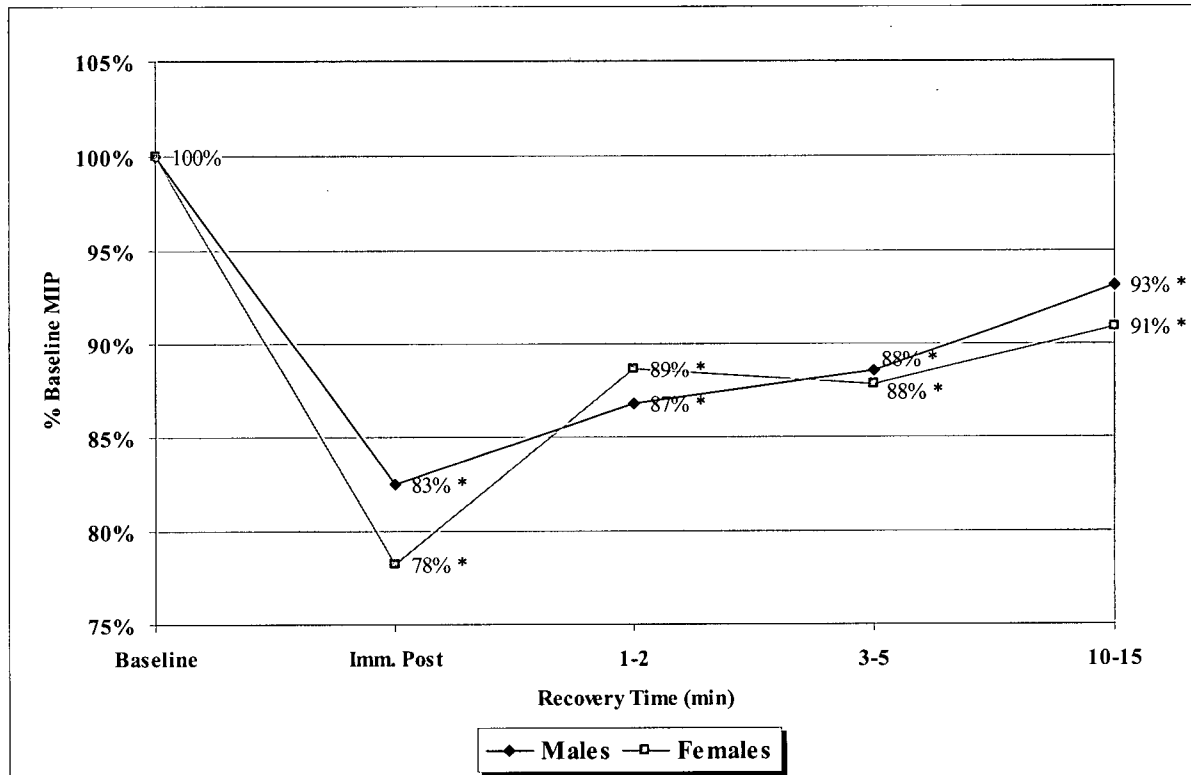
Figure 2. Baseline and pooled percent recovery of maximal inspiratory pressure post-maximal exercise
($F_{GENDER} = 0.456$; $p>0.05$; $F_{TIME} = 22.385$; $p<0.001$; $F_{GENDER*TIME} = 0.992$; $p>0.05$)
(*Significantly different from baseline, $p<0.05$)

Baseline MIP values portrayed no significant correlation with aerobic fitness, as

predicted using the cycle ergometer ($\dot{V}O_{2\,max}$) (Table 11). Pearson-product moment

correlation co-efficients did not demonstrate any significant correlations between MIP_{rest} with

any anthropometric variables, or maximal exercise variables.

Table 11. Pearson product moment correlations between resting maximal inspiratory
pressure (MIP) values and maximal exercise variables

		Age (yrs)	Height (cm)	Mass (kg)	Fat-free Mass (kg)	$\dot{V}O_{2max}$ (L•min⁻¹)	$\dot{V}O_{2max}$ (ml•kg⁻¹•min⁻¹)	$\dot{V}O_{2max}$ FFM (ml•kg[FFM]⁻¹•min⁻¹)
MIPrest	*Correlation*	0.108	-0.121	-0.186	-0.068	0.178	0.016	0.127
	Sig. (1-tailed)	0.271	0.248	0.146	0.352	0.157	0.464	0.237

Pearson Product Moment Correlations
† Significant correlation (p<0.05)

Overall, the results showed that maximal exercise led to a decrease in MIP with no associated decrease in HG up to 15 minutes post-exercise. The general trends were similar between the male and female subjects, with a sudden drop in MIP followed by a gradual increase, with a trend towards resting values by 15 minutes. HG values remained steady at approximately baseline values over 15 minutes, but fatigue was indeed presented at the 3 to 5 minute time interval, most likely due to the nature of the serial measurements.

4.4 Test-retest Reliability for MIP, HG, and $\dot{V}O_{2max}$

Co-efficient of variation (CV) values for $\dot{V}O_{2\,max}$ (Table 12) was slightly lower in males than females. Overall, CV values for both males and females showed that the subjects' performance for the re-test varied within 4.2% of their $\dot{V}O_{2\,max}$ test.

Table 12. Test-retest values for 6 subjects (3M; 3F) who completed $\dot{V}O_{2\,max}$ tests on two separate occasions (mean ± SD)

Subject	Sex	$\dot{V}O_{2max}$ (L•min^{-1})	$\dot{V}O_{2max}$ (ml•kg^{-1}•min^{-1})
MEAN	M	3.527 ± 0.1	48.1 ± 1.6
CV		2.5	3.3
MEAN	F	2.885 ± 0.2	46.8 ± 2.5
CV		5.9	5.6
MEAN	TOTAL	3.206 ± 0.1	47.4 ± 2.1
CV		4.2	4.5

Values are means ± SD
CV, coefficient of variation (%)

Maximal handgrip strength testing showed CV values ranging from 1-8% over each of the time intervals for the individual genders (Table 13). When looked at as a group, (pooled data for males and females) CVs ranged from approximately 2-6%, presenting reliable measures.

Table 13. Test-retest values for 6 subjects (3M; 3F) who completed the HG test protocols on two separate occasions (mean ± SD)

Subject	Sex	Pre	Imm.	1	2	3	4	5	10	15
						Time Interval				
MEAN	M	50.5 ± 2.4	50.5 ± 1.4	48.9 ± 1.8	48.1 ± 0.7	45.8 ± 0.9	45.2 ± 0.7	44.8 ± 3.2	46.1 ± 2.7	45.8 ± 2.5
CV		4.9	3.0	3.6	1.5	2.1	1.6	7.4	5.6	5.6
MEAN	F	33.2 ± 1.2	31.9 ± 2.5	32.9 ± 0.4	31.0 ± 1.9	32.5 ± 1.9	32.6 ± 1.1	31.3 ± 1.7	31.3 ± 0.7	31.3 ± 1.1
CV		3.5	7.8	1.1	6.1	6.0	3.2	5.3	2.1	3.2
MEAN	TOTAL	41.9 ± 1.8	41.2 ± 1.9	40.9 ± 1.1	39.5 ± 1.3	39.2 ± 1.4	38.9 ± 0.9	38.0 ± 6.7	38.7 ± 1.7	38.5 ± 1.8
CV		4.2	5.4	2.3	3.8	4.0	2.4	6.3	3.9	4.4

CV, coefficient of variation (%)

A comparison of the MIP reliability measures (Table 14), showed greater variation in females (3-12%) relative to males (3-9%). When viewed as a group, the CVs presented values in the 3-9% range, once again showing slightly greater variability than the handgrip measures. However, these values were still considered quite consistent for the subjects, with CVs below 10% generally considered quite positive, and these values presenting as much lower (Black & Hyatt, 1969; Aldrich & Spiro, 1995).

Table 14. Test-retest values for 6 subjects (3M; 3F) who completed the MIP test protocols on two separate occasions (mean ± SD)

Subject	Sex	Pre	Imm.	1	2	3	4	5	10	15
						Time Interval				
MEAN	M	154.3 ± 10.8	132.2 ± 8.2	137.8 ± 4.5	139.6 ± 7.8	137.8 ± 4.5	131.0 ± 7.1	133.3 ± 4.7	137.5 ± 12.5	138.0 ± 10.8
CV		7.3	6.4	3.1	5.4	3.7	5.5	3.7	9.2	8.1
MEAN	F	122.7 ± 5.2	111.3 ± 11.8	117.5 ± 4.0	113.7 ± 13.2	118.2 ± 5.9	112.8 ± 8.7	119.2 ± 8.7	121.0 ± 9.9	119.0 ± 8.0
CV		4.2	10.9	3.4	11.6	4.9	7.5	7.2	8.0	6.6
MEAN	TOTAL	138.5 ± 8.0	121.8 ± 10.0	127.7 ± 4.2	126.6 ± 10.5	128.0 ± 5.2	121.9 ± 7.9	126.3 ± 6.7	129.3 ± 11.2	128.5 ± 9.4
CV		5.8	8.6	3.3	8.5	4.3	6.5	5.4	8.6	7.4

CV, coefficient of variation (%)

4.5   Hypotheses Verification

Hypothesis	p
a) $MIP_{\text{post-exercise(male)}} - MIP_{\text{baseline(male)}} < MIP_{\text{post-exercise(female)}} - MIP_{\text{baseline(female)}}$	$p < 0.05$
b) $MIP_{\text{immediate(male)}} - MIP_{\text{baseline(male)}} < MIP_{\text{immediate(female)}} - MIP_{\text{baseline(female)}}$	$p < 0.05$
$MIP_{1\text{(male)}} - MIP_{\text{baseline(male)}} < MIP_{1\text{(female)}} - MIP_{\text{baseline(female)}}$	$p < 0.05$
$MIP_{2\text{(male)}} - MIP_{\text{baseline(male)}} < MIP_{2\text{(female)}} - MIP_{\text{baseline(female)}}$	$p < 0.05$
$MIP_{3\text{(male)}} - MIP_{\text{baseline(male)}} < MIP_{3\text{(female)}} - MIP_{\text{baseline(female)}}$	$p < 0.05$
$MIP_{4\text{(male)}} - MIP_{\text{baseline(male)}} < MIP_{4\text{(female)}} - MIP_{\text{baseline(female)}}$	$p < 0.05$
$MIP_{5\text{(male)}} - MIP_{\text{baseline(male)}} < MIP_{5\text{(female)}} - MIP_{\text{baseline(female)}}$	$p < 0.05$
$MIP_{10\text{(male)}} - MIP_{\text{baseline(male)}} < MIP_{10\text{(female)}} - MIP_{\text{baseline(female)}}$	$p < 0.05$
$MIP_{15\text{(male)}} - MIP_{\text{baseline(male)}} < MIP_{15\text{(female)}} - MIP_{\text{baseline(female)}}$	$p < 0.05$
c) $f_b(\text{male}) < f_b(\text{female})$	$p < 0.05$
$Borg_{\text{dyspnea(male)}} < Borg_{\text{dyspnea(female)}}$	$p < 0.05$
$V_{T\text{(female)}} < V_{T\text{(male)}}$	$p < 0.05$
$\dot{V}_{E\text{(female)}} < \dot{V}_{E\text{(male)}}$	$p < 0.05$

CHAPTER V: Discussion

5.1   Descriptive Measures

The mean age of 23 years for all subjects in the present study falls within the range of many other studies examining inspiratory muscle fatigue (McConnell *et al.*, 1997, Coast *et al.*, 1999; Volianitis *et al.*, 1999; Boussana *et al.*, 2001; Volianitis *et al.*, 2001). The mean age was also shown to be slightly younger than the subject populations used in most studies prior to 1995. Predicted MIP values were calculated based on the regression equations used by Black and Hyatt (1969); Males: MIP = 143 – 0.55A; Females: MIP = 104 – 0.51A. The average values were found to be -131.1 cm H_2O in males, and -92.5 cm H_2O in females. Our results showed that the genders performed equally well, with males achieving 117.6% of predicted MIP, while females achieving 115.1%. Statistically significant group characteristic differences in height, mass, and fat-free mass were typical and anticipated for the males and females.

5.2   Comparisons at $\dot{V}O_{2max}$

The level of training of the subjects, as interpreted through $\dot{V}O_{2max}$ scores, was comparable to several studies that used moderately trained, as opposed to highly trained subjects (Aaron *et al.*, 1992a; Aaron *et al.*, 1992b; O'Kroy *et al.*, 1992; Babcock *et al.*, 1996; Coast *et al.*, 1999; Volianitis *et al.*, 2001; Williams *et al.*, 2002). The statistically significant difference in relative $\dot{V}O_{2max}$ (ml·kg^{-1}·min^{-1}) between genders was consistent with other studies that included both male and female subjects (Coast *et al.*, 1999, Volianitis *et al.*, 2001). As projected based on the suggestions of Cureton & Sparling (1980) and Tarnapolsky

et al. (2001), the significant differences in $\dot{V}O_{2max}$, both absolute ($L \cdot min^{-1}$) and relative ($ml \cdot kg[FFM]^{-1} \cdot min^{-1}$) were negated once presented relative to fat-free mass ($ml \cdot kg^{-1} \cdot min^{-1}$).

Values for $\dot{V}_E$, VT and fb at $\dot{V}O_{2max}$ were consistent with other studies using moderately trained males and females within a similar age range (Aaron *et al.*, 1992a, Aaron *et al.*, 1992b, Coast *et al.*, 1999). More stringent comparisons of $\dot{V}_E$, VT, and fb at $\dot{V}O_{2max}$ relative to previous studies were not possible in some cases. Some studies used so few (one to five) females in their subject pool, that their analyses were grouped, not always providing indications of the differences between males and females (Babcock *et al.*, 1996; Volianitis *et al.*, 2001, Williams *et al.*, 2002).

The vast majority of literature in the area of exercise-induced inspiratory muscle fatigue focused solely on male subjects. Focusing on studies that utilized males with similar levels of training showed similar values for $\dot{V}O_{2max}$, $\dot{V}_E$, VT, and fb (Harms *et al*, 1997, Harms *et al*, 1998, McConnell *et al*, 1997, Volianitis *et al*, 1999).

5.3   Post-exercise (recovery) Comparisons

5.3.1   Maximal Handgrip Strength (HG)

It was hypothesized that handgrip measures would not be different after exercise, serving to indicate that the resultant changes in MIP were not due to generalized fatigue or decreased central effort (Coast *et al.*, 1999). Handgrip measures in absolute terms were significantly higher ($p < 0.001$) in males compared to females. This is similar to the results of Coast *et al.*, (1999). Miller and colleagues (1993) suggest that greater strength in males could primarily be due to larger muscle fibres. The conversion of handgrip measures, to percentages of baseline maximum strength, presented slightly unusual results. Significant differences ($p < 0.05$) were seen when measures at the 3-5 minute time interval were

compared to baseline. These results could signify that the serial measures for handgrip strength were in themselves fatiguing. In both males and female subjects, the handgrip measures presented similar trends, with handgrip strength falling from the 1-2 minute to 3-5 minute intervals, followed by an increase by the 10-15 minute mark. There was also no significant difference between baseline HG and HG measures averaged over 15 minutes, in the males and females. It was likely that these results were due to the frequency of the measurement tasks, not due to overall body fatigue caused by maximal exercise. This idea was supported by the fact that handgrip measures were not different than baseline in the first 2 minutes after exercise, as well as 10 to 15 minutes post-fatigue. This showed that the exercise task did not present an obstacle for overall whole-body fatigue, as demonstrated by a lack of difference immediately post-exercise. Similarly, these measures returned to baseline at the 10 and 15 minute time intervals, when more adequate time was given for rest between measurements. Consequently, this apparent lack of overall fatigue in handgrip strength, with a corresponding decrease in MIP, suggested that the exercise task did indeed fatigue the respiratory muscles specifically.

5.3.2 Maximal Inspiratory Pressure (MIP)

These moderately-trained university-aged males and females portrayed a significant decline in their ability to reproduce baseline maximal inspiratory mouth pressures following an incremental cycle to volitional fatigue. The failure to develop force and/or velocity, as a result of muscle activity under load, that is reversible with rest, defines fatigue (NHLBI, 1980). These data therefore contribute further support to the growing literature suggesting that maximal exercise can induce fatigue in the muscles of inspiration (Boussana *et al.*, 2001;

Volianitis *et al.*, 2001; Gonzales *et al.*, 2002 [abstract]; Romer *et al.,* 2002; Boussana *et al.*, 2003, Gonzales *et al.*, 2003 [abstract]; Lomax & McConnell, 2003).

These results are of particular·importance because they show that serial measurement of MIPs using a portable mouth pressure meter was a robust method for quantifying fatigue. Specifically, it showed that the one-minute intervals were a short enough time frame to demonstrate valid measures of fatigue. The noninvasive protocol of this tool could make it all the more attractive for future research.

The differences between the genders, when comparing the decrease in maximal inspiratory muscle strength at each of the time intervals, were not significant ($p > 0.05$). The subject groups presented similar patterns of fatigue and consequent recovery. Such results could suggest that the underlying mechanisms concerning fatigue and recovery may actually be similar for males and females.

5.3.3 Borg Ratings of Perceived Exertion and Dyspnea

Killian and colleagues (1992) showed that at maximal exercise, dyspnea could possibly be more limiting than leg fatigue in subjects with chronic airflow limitation (CAL). Their study compared a CAL group against a large control group. A closer look at the control group, forming a sample group that was similar to the subjects used in our study, showed that their results for ratings of dyspnea exceeded leg effort in 22% of the subjects, the rating of leg effort exceeded dyspnea in 36% of control subjects and both were rated equally in 42% control subjects, respectively. In comparison, our study showed that leg effort exceeded dyspnea in the majority of subjects, 59% (M: 61%; F: 56%), with dyspnea ratings exceeding leg effort in only 15% (M; 6%; F: 25%). Both were rated equally in 26%

of the subjects (M: 33%; F: 19%). However, much like the results of Killian *et al.* (1992), when analyzed in groups (in this case by gender) the overall means showed no statistically significant difference. Harms *et al.* (2000) also showed no significant difference between leg RPE and dyspnea Borg ratings in their subjects.

5.4 Exercise-induced Inspiratory Muscle Fatigue (IMF) and the Implications of the Timeline for Recovery

The decline in post-exercise MIP presented in our study is consistent with several studies that have looked at exercise-induced inspiratory muscle fatigue (Coast *et al.*, 1999; Volianitis *et al.*, 1999; Boussana *et al.*, 2001; Volianitis *et al.*, 2001; Gonzales *et al.*, 2002 [abstract]; Boussana *et al.*, 2003; Gonzales *et al.*, 2002 [abstract]; Romer *et al.*, 2002; Lomax & McConnell, 2003). While these findings are still variable, it seems that a greater number of studies were able to present data to confirm that exercise induced fatigue as measured by MIP (see above), versus those that are not (Coast *et al.*, 1990; Johnson *et al.*, 1993; Perret *et al.*, 1999). It is important to keep in mind that whole body endurance exercise in healthy subjects has been discovered to cause diaphragmatic fatigue in a wide range of fitness levels (Johnson *et al.*, 1993).

MIP values in our study remained depressed in both groups for the entire 15 minute post-exercise timeline. These findings are virtually identical to Coast *et al.* (1999), who showed a 15% drop in MIP post-maximal exercise that remained depressed for 15 minutes. These findings are also consistent with other studies that have shown inspiratory muscle fatigue to occur after exhaustive exercise and remain present for up to 24 hours post-task (Laghi *et al.*, 1995; Chevrolet *et al.*, 1993; Hill *et al.*, 1991). Laghi *et al.* (1995) showed that recovery of diaphragmatic contractility was not complete 24 hours after induction of

diaphragmatic fatigue. This statement related to the precision of the techniques used to determine transdiaphragmatic pressure (Pdi), as opposed to MIP, a volitional measure.

Chevrolet *et al.* (1993) demonstrated a significant decrease in MIP following both a half-marathon and a full-marathon that lasted for more than 2.5 hours. A greater degree of fatigue was seen in the marathon runners, compared to the half-marathoners. In comparison to our study, one would have to only consider their first measurement post-task ($t_1 = 11 \pm 4$ min), since their next two intervals were well after the final time interval used in our study ($t_2 = 59 \pm 7$ and $t_3 = 139 \pm 9$ min). The marathoners presented MIP values that were approximately 73% of baseline. The half-marathoners presented greater MIP values, at approximately 90% of baseline, though all values were still significantly different compared to baseline. Our results were consistent with these values. It was interesting to see that the shorter duration maximal exercise task used in our study provided results in between those of the much longer duration half- and full- marathons. However, with the short duration exercise task used in our study, it was evident that both males and females, while still presenting IMF, were almost fully recovered by 15 minutes post-task.

In like manner, Loke *et al.* (1982) suggested the development of fatigue owing to a decrease in MIP in four healthy male runners after a marathon (42.2km). They compared pre-test (2 hours prior to marathon) with post-test (21-60 minutes post-marathon) MIPs and found them to be significantly different. Once again, a true comparison was difficult since the time interval used for post-task measures far exceeded the final serial measurement used in our study.

Hill and colleagues (1991) portrayed a significant decrease in MIP after the bicycle, and run events of a triathlon, but not so after swimming. Their subjects had recovered fully

by 24 hours. They concluded that reductions in respiratory muscle strength could be dependent on the type of exercise performed. Specifically, they implied that the paced, relatively low-frequency, large tidal volume breathing pattern utilized in swimming could have been a contributing factor. Lomax & McConnell (2003) recently provided contradictory evidence, showing IMF following a maximal 200m swim (~6 min).

Volianitis *et al.* (2000) presented MIP values that were lower after 6 minutes of maximal rowing. The use of competitive rowers reiterated Johnson and colleagues' (1996) suggestion that even those with high levels of aerobic fitness were not impervious to inspiratory muscle fatigue.

Boussana and colleagues (2001) also looked at the components of a triathlon and duathlon, investigating the effects of the cycle-run and run-cycle successions. They showed significant differences between pre- and post-values, but no differences between the successions. Romer *et al.* (2002) also provided evidence of a decrease in MIP within 2 minutes of completing a 20km and 40km bicycle time trial.

Boussana and colleagues (2003) also showed that respiratory muscle fatigue induced by prior exercise was neither reversed nor heightened by further exercise. However, Mador and Acevedo (1991) presented data that IMF could impair subsequent high-intensity exercise performance, possibly through an alteration in breathing pattern during exercise.

The timeline for recovery could have important implications for the training population, especially those participating in exhaustive activity on successive days. Athletes competing in multiple day tournaments, or events lasting several days to weeks in duration (e.g. Tour de France, Race Across America, etc.) could have to deal with the consequences of

fatigued respiratory muscles, as well as locomotor. Obviously, the risk increases for those competing several times within the same 24 hour period.

5.5 Gender Differences in Inspiratory Muscle Fatigue

Our study suggests that recovery from inspiratory muscle fatigue post-exercise was similar between males and females. The lack of significant differences for percent baseline MIP between the groups at any time interval, combined with a visual trend in recovery when graphed, made it difficult for us to conclude otherwise. The initial interpretation of the results of this study is consistent with the findings of Gonzales and colleagues (2003). They determined no gender difference in respiratory muscle fatigue (RMF) following exhaustive exercise. Their results showed that both genders demonstrated similar reductions in MIP immediately following maximal exercise, with no difference observed between genders. They concluded that RMF was similar between genders following exhaustive exercise; however they went on to suggest that females demonstrated a slower rate of recovery.

Several important differences must be noted between our study, and that of Gonzales *et al* (2003). Their methodology differed in the fact that the endurance exercise test (EET) consisted of pedaling at ~80% of maximal work rate (WR_{max}), as opposed to an incremental cycle to fatigue ($\dot{V}O_{2max}$). Also, they measured MIP immediately following exercise, and not again until 15, 25, and 35 minutes post-task. A constant load breathing test was also performed immediately following EET. They showed that females produced lower MIP values at 15, 25, and 35 minutes post exercise, compared to baseline and the male subjects. The inclusion of the constant load breathing test following EET may have confounded their results. It is highly likely that the overall gender differences in fatigue viewed at these later time intervals may have been due in part to their additional breathing tests. The lack of

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significant difference between genders could suggest that the mechanisms for fatigue and subsequent recovery were similar.

5.5.1 Possible Mechanisms

Rochester and Arora (1983) presented six major determinants of respiratory muscle strength in normal subjects; i) age, ii) sex, iii) general muscular development iv) muscle force-length relationship, v) muscle force-velocity relationship, and vi) force-frequency relationship. These factors were further grouped into those that reflect the state of the contractile machinery under optimal conditions (i-iii), and those that reflect either the state of the actin-myosin interaction (iv-v) or the state of the muscle activation (vi). Further, they presented the following 6 causes of respiratory muscle fatigue: 1) inhibition of neural drive; 2) failure of transmission across the neuromuscular junction; 3) excessive force and duration of contraction; 4) impaired muscle blood supply; 5) impaired excitation-contraction coupling; and 6) depletion of muscle energy stores. It can be seen that the causes that they chose to focus upon relate more to the general factors influencing fatigue of virtually any skeletal muscle, as opposed to mechanisms related directly to the function and structure of the diaphragm and respiratory system. However, several other research groups have extrapolated and built upon the general causes cited by Rochester and Arora (1983).

The use of oxygen as a fuel source for muscles led Macklem (1980) to equate muscle efficiency to the gas mileage of an automobile. Low muscle efficiency equated to low mileage, meaning the muscle consumes more oxygen to perform a given level of work. Babcock and colleagues (1995a) looked at the hypoxic effects on exercise-induced diaphragmatic fatigue in normal healthy subjects. They suggested that experiencing the same

amount of fatigue in a shorter time frame during hypoxic exercise may have been due to increased EFL; decreased O_2 transport to the diaphragm and/or increased circulatory metabolites.

Like all skeletal muscles, the ability of the diaphragm to generate maximal force for a given neural activation is dependent on the initial length of the muscle filaments actin and myosin. For the same level of activation, the tension produced is much less for muscles working at a shorter length (Smith & Bellemare, 1987). Specific to the muscles of inspiration, muscle fibre length is a function of the lung volume. Larger lung volumes are representative of shorter inspiratory muscles, which in turn generate weaker force. Therefore hyperinflation and the related increase in end-expiratory lung volume (EELV) perpetuate inspiratory muscle fatigue. The resultant MIPs seen in our study could suggest similar degrees of shortening between the genders, resulting in similar fatigue. Conversely, similar fatigue may have been representative of increased shortening within one sex, to equate the fatigue values, if a difference within shortening does indeed exist.

The diaphragm can be divided into two regions conceptually, the diaphragmatic dome, and the zone of apposition (Levine et al., 2000). The diaphragmatic zone is the upper region of the diaphragm adjacent to the lung, while the zone of apposition is directly apposed to the rib cage. The reduced length of the diaphragm has particular consequences for this zone of apposition. Fitting (2000) described the work of the diaphragm to that of a piston. A shorter zone of apposition results in a shorter range of motion, despite of maximal tension generated. Cassart and colleagues (1997) suggested diaphragmatic muscle fibres could actually pull the ribs in and expiratory rather than inspiratory direction, when diaphragm flattens and the zone of apposition in part disappears.

It is possible that the ventilatory requirements of maximal exercise lead to hyperinflation of the lungs in some subjects. Mead (1980) showed that females have airways that are smaller relative to lung size than those of males. As mentioned previously in the hypotheses, McClaran and colleagues (1998) suggested that smaller lung volumes and maximal flow rates for women in general may cause hyperinflation and increased prevalence of expiratory flow limitation (EFL) during strenous exercise. This could have led them to use a greater percentage of ventilatory reserve during exercise. However, McClaran (1998) also showed that less-fit women showed minimal expiratory flow limitation during maximal exercise. Mota *et al.* (1999) showed that most well-trained male subjects did not reach EFL during maximal exercise. Since our sample population was moderately-trained and not highly-trained, these findings do little to fully explain why inspiratory muscle fatigue occurred to virtually the same degree in the male subjects. The associated increase in fb in females, that had been suggested by McClaran *et al.* (1998) to meet the ventilatory demand of maximal exercise. was not shown in our study.

Iandelli and colleagues (2002) artifically imposed expiratory flow limitation in six healthy male subjects by placing a Starling resistor on the expiratory port of the mouthpiece valve, limiting expiratory flow to approximately 1 L/min. This was completed in an attempt to simulate COPD. Intense difficulty in breathing at maximal exercise resulted. Borg ratings for breathing sensation ranged from 9-10, with subjects only able to perform at 65% of the maximal workload achieved by the control group. They quoted previous studies to cite shortening of the diaphragmatic fibre length, a decrease in lung compliance increasing the elastic work of breathing, as well as a decrease in $\dot{V}_E$, as possible factors involved.

In our study, no dramatic decrease in $\dot{V}_E$ was observed within the male subjects of this study. Comparisons of maximum values for $\dot{V}_E$ with $\dot{V}_E$ observed at $\dot{V}O_{2max}$ were not significantly different between the males and females. However, Śliwiński et al. (1996) showed that after induction of global inspiratory muscle fatigue (breathing against resistance), ventilatory response to heavy exercise included an increase in fb and VE, with minor changes in VT. The level of fatigue portrayed by the subjects within our study did not express these related changes in ventilatory response. Furthermore, Pearson correlation coefficients revealed no significant associations betweens post-exercise MIP and fb, $\dot{V}_E$, and VT.

Other factors, outside of the respiratory muscles themselves, must be considered to gather a greater understanding about fatigue. Babcock et al. (1995b) and Coast et al. (1999) showed that extended bouts of hyperpnea, mimicking levels attained during exercise, but without accompanying exercise did not alter MIP. Therefore, it has been suggested that the effects of exercise are independent of the work done by the muscles of respiration.

Johnson and colleagues (1996) have presented other possible mechanisms for inspiratory muscle fatigue. They suggested that reduced blow flow to the respiratory muscles may cause fatigue. Further, they proposed that a decrease in blood flow available to the respiratory muscles could result in a reduction in oxygen within these muscle cells, along with the amassing of metabolic by-products. Lactic acid accumulation is said to be associated with increased difficulty of the respiratory muscles to generate force, as well as in sensation of dyspnea (Johnson et al., 1996). Babcock et al. (1995b) further speculated that the fatiguing effect on the diaphragm brought on by whole body endurance exercise could be attributed to changes within the acid balance of the diaphragm, due to its uptake of

circulating lactate from the locomotor muscles under load. It would be important to measure blood lactate and evidence of metabolic by-products in future studies.

5.6   Inspiratory Muscle Fatigue (IMF) and Possible Implications for Performance

When severe EFL is observed in highly fit humans, the oxygen cost of breathing may approach 15% of total $\dot{V}O_2$ (Aaron *et al.*, 1992a). This number is slightly lower, 8 to 12%, in normally fit subjects who experience little or no airflow limitation (Aaron *et al.*, 1992a). Such results were found by mimicking the work of breathing (Wb) and respiratory muscle recruitment patterns experienced in maximal exercise through hyperpnea (Aaron *et al.*, 1992a, 1992b). It had been suggested that the associated metabolic costs, as well as the demand to meet this oxygen cost could potentially limit the blood flow available to locomotor muscles. This could lead to limiting their work output (Harms *et al*, 1997; Harms & Dempsey, 1999).

Johnson and colleagues (1996) assumed that the best method to determine whether IMF influenced human performance was to unload the respiratory muscles and determine whether performance improved. Harms *et al.* (1997) looked to determine whether competition for blood flow ($\dot{Q}$) and $\dot{V}O_2$ existed between the respiratory and locomotor muscles during maximal exercise. They showed that during maximal exercise, respiratory muscle work (Wb) compromised locomotor muscle perfusion and $\dot{V}O_2$ causing vasoconstriction in the locomotor muscles. By unloading the respiratory muscles, using a proportional assist ventilator (PAV), a decrease in leg vascular resistance (LVR) and increased leg blood flow ($\dot{Q}_{leg}$) and leg O_2 consumption ($\dot{V}O_2$) was observed. The PAV made mouth pressure positive in proportion to flow so that unloading of the respiratory

muscles occurred throughout the inspiratory cycle (Harms *et al.*, 1997). The opposite was seen when these muscles were loaded with respiratory resistance. They proposed that respiratory muscles competed more effectively than locomotor muscles for total $\dot{Q}$, at maximal exercise performance. They suggested that only during very heavy exercise (i.e. > 80-85% $\dot{V}O_{2max}$), is respiratory muscle work likely to influence performance. Their reasoning related to the fact that Wb is quite high and CO is limited in its ability to adequately dispense flow to both the respiratory and locomotor muscles during heavy exercise. Two effects of respiratory muscle work at maximal exercise include, 1) a substantial portion of CO redirected to the respiratory muscles, along with 2) a reduction in blood flow to the working locomotor muscles (Harms *et al.*, 1998).

Studies conducted at submaximal exercise intensities, where Wb was increased to 50 to 70% of normal, could not elicit changes in LVR or $\dot{Q}_{leg}$ (Wetter *et al.*, 1999). Sheel *et al* (2001) also showed that voluntary increases in inspiratory effort, in the absence of fatigue, had no effect on LVR or $\dot{Q}_{leg}$. Babcock *et al.* (2002) also confirmed that the workload tolerated by the respiratory muscles was critical to determining the extent of high- and low-frequency diaphragmatic fatigue from exhaustive whole-body exercise.

Induction of inspiratory muscle fatigue by voluntary hyperpnea against resistance has been shown to cause a gradual increase in muscle sympathetic nerve activity (MSNA) in the resting limb (St. Croix *et al.*, 2000). Sheel *et al.* (2001a, 2001b) attributed the resultant limb vasoconstriction to a metaboreflex initiated within the diaphragm. This metaboreflex would seek to correct the mismatch between blood supply and demand through an increase in CO.

Another study showed that a reduction in Wb, by unloading the respiratory muscle, consistently led to significantly longer exercise tolerance (Harms *et al.*, 2000). They suggested that the reflex sympathetic vasoconstrictor influences of respiratory muscle work on limb muscle fatigability, together with the effects of perceived effort could influence the system via feedback mechanisms.

These important findings within this field of research provide ample theories for the fatigue observed in our study. The ability to efficiently combine all of these methodologies into a study looking at gender differences in IMF could be intriguing.

The energy requirements of working muscles are usually proportional to their blood flow requirements. This puts forth the importance of sustaining effective and efficient performance of the respiratory muscles during exercise. There must be mechanisms within the body that assure that the needs of the respiratory muscles may be favoured to the locomotor muscles.

5.7   Baseline Inspiratory Muscle Strength and Fatigue

A study by Coast *et al.* (1990) claimed that highly trained subjects were completely guarded from exercise-induced respiratory muscle fatigue. However, McConnell *et al.* (1997) showed that subjects with the strongest inspiratory muscles displayed the least fatigue, though IMG was still portrayed. They examined 24 moderately-trained males, before and after an incremental, multi-stage shuttle run to volitional fatigue. Our study did not provide similar findings. We had no statistically significant correlation between training state ($\dot{V}O_{2max}$) and IMF.   The trend general in baseline MIPs was continued in post-exercise MIP measures, but the decline in MIP (presence of IMF) was still significant,

showing that unlike (Coast *et al.,* 1990) complete protection from IMF was not observed. These results also help to confirm those of Babcock and colleagues (1996), who showed that a high level of aerobic fitness failed to protect the muscles of inspiration from fatigue after exhaustive exercise.

5.8 Reliability

The variability in MIP between baselines was in agreement with previous reports of test-retest reliability (Larson *et al.,* 1985; Volianitis *et al.,* 1999). Most recently, Volianitis *et al.* (1999) showed a mean coefficient of variation (CV) for the baseline MIP measured on two occasions of 4.65%. Such results are consistent with the CVs observed in this study for resting (MIP$_{rest}$) measures; 4.9% in males, 3.5% in females, and 4.2% overall. The methodology involved in our study focused on giving the subjects ample opportunity to practice the required MIP maneuver. The inclusion of the familiarization day also helped to control any learning effect associated with the MIP task (Wen *et al.,* 1997).

The use of a portable unit to measure MIP may provide some insight into the highly reliable measures. It is only in recent years that research has utilized such portable units. Historically, the use of larger stationary units meant that there was a greater possibility of variability between measures upon completion of maximal exercise. Such variation would have been largely due to the variable time interval between the end of exercise and the completion of the first post-exercise MIP. Larger stationary units typically have more sophisticated steps involved in taking a single measure (e.g. the use of multiple software packages, checking for drift, etc.). The ease of use of the portable mouth pressure meter related to rapid measurement times post-exercise. The average time for measurement was

within 20 seconds of the completion of the maximal exercise task (volitional fatigue) (20.6±7.1s; M: 22.7±7.7; F: 18.3±5.6).

5.9   Limitations to the Study

The possibility of changes in residual volume and total lung capacity makes it necessary to correct for respiratory pressures (e.g. MIP). Through analysis of spirometric tracings, Loke *et al.* (1982) observed no change in inspiratory capacity. This suggests no change in total lung capacity. Similarly, they did not find an alteration in FVC, also suggesting that residual volume (RV) did not change. Unfortunately, the lack of spirometric measures in the present study meant that making such assumptions, was impossible since they were not backed by specific evidence (spirometry).

The exclusion of baseline spirometry also made it difficult to provide indication, through estimation, about whether the male and female subjects experienced the same ventilatory load that could possibly cause IMF. The ability to determine predicted $\dot{V}E_{max}$, by using FEV1 values and gender specific constants, would have allowed for such indications, with moderate confidence. By showing that the groups achieved approximately the same percentage of predicted $\dot{V}E_{max}$, it could be justified that both genders achieved roughly the same ventilatory load. A further study would need to make this an utmost priority.

Post-hoc estimation of ventilatory load was by using predicted equations to determine the predicted $\dot{V}E_{max}$. The results showed that males achieved a significantly higher percentage of predicted $\dot{V}E_{max}$ statistically compared to the females (100% vs. 85%). However, the use of a predicted value to predict another value meant that these values are of little use (Appendix III).

The subjects had no visual feedback when performing the MIP maneuvers. While a new optional software package (Puma®, Micro Medical, UK) that allows for comprehensive analysis and database functions has been developed, it did not exist at the time data of data collection. This software displays the pressure wave forms developed during testing with the MicroMPM (Micro Medical, UK). Additionally the measurements of the maximal rate of pressure development (MRPD) and maximal rate of relaxation (MRR). This software would have allowed for data analysis to be completed on PI_{peak} (peak inspiratory pressure) in addition to MIP. Furthermore, the visual feedback similar in the form of wave patterns of an oscilloscope may have helped to maximize effort of the subjects.

The ability to confer fatigue from a single measure of MIP is not possible. It may have been more realistic to conduct measurements similar to that of O'Kroy et al. (1992). The time intervals listed for their study were 5, 10, and 30 minutes post-test. Bye and colleagues (1994) presented their data in a similar fashion, because it allowed for the time intervals to be representative of multiple values. This was important in this study, because perhaps it gave a more realistic picture of the fatigue that took place. In hindsight, more measures should have been taken between 10 and 15 minutes to continue this strong view. Subsequent studies would include more stringent MIP measures up to 60 minutes post-exercise.

In summary, the data from the present study contributes further support to existing observations that show inspiratory muscle fatigue to occur following exhausting exercise to volitional fatigue. Additionally, this study has demonstrated that this incidence occured in both moderately trained, university-aged males and females, with no relationship between sex and fatigability of these muscles. In accordance with the modest amount of previous

studies that have looked at gender differences in respiratory muscle fatigue, the present data support the idea that there are no significant gender differences within inspiratory muscle fatigue following exercise to volitional fatigue. The exact mechanism by which inspiratory muscle fatigue occurs has yet to be elucidated.

CHAPTER VI: Conclusions

6.1 Conclusions

This study will add further findings to the increasing literature that has shown fatigue of the inspiratory muscles to occur following maximal exercise. The following was concluded based on our study:

1) The results demonstrated inspiratory muscle fatigue to occur in both males and females following exhaustive exercise.

2) Post-exercise MIP values were not significantly different between the male and female subjects at each of the time intervals tested. Thus, the pattern of recovery was also shown to be similar between the genders.

3) Frequency of breathing was not different between the males and females. Our results showed that females did not rely on a greater fb at any given $\dot{V}_E$ compared to males.

4) No statistically significant differences for Borg ratings of dyspnea and leg discomfort were seen between the two groups.

5) As hypothesized, the females had significantly lower V_T and $\dot{V}_E$ values compared to males.

Therefore, save the expected gender differences in V_T and $\dot{V}_E$ values, the initial hypotheses for the study were not confirmed. The results provide evidence against any gender differences with regards to inspiratory muscle fatigue following exhaustive exercise. Specifically, the data provides important evidence for the fatigability of inspiratory muscles in female subjects. These results, in particular, will add to a facet of this particular area of research that has been lacking, the inclusion of females subjects.

In addition, the results disseminate additional values regarding the reproducibility of MIP measures when using a portable mouth pressure meter. The device was able to confer reliable MIP recordings during recovery after maximal exercise, and above all for baseline MIP measures. Further research must be completed to determine the exact mechanisms for which no gender differences in inspiratory muscle fatigue was observed.

6.2 Recommendations for Future Research

Building upon the vast amount of evidence regarding possible mechanisms for IMF must continue within this field of research. Research must seek to make a breakthrough within the actual mechanisms to help answer past and future questions.

It is important that this field of research includes sample populations from both genders, various ages, exercise histories and even health backgrounds. Future studies must look to incorporate much larger sample sizes so that more adequate generalization to the population can be made. A more stringent methodological approach to the question of gender differences within inspiratory muscle fatigue could lead to important outcomes to many different populations. Gender-specific rehabilitation and training programs could be used by the athletic and clinical population. The ability to provide gender-specific treatment and training would be incredibly important.

6.2.1 Respiratory Muscle strength training with nonrespiratory maneuvers

DePalo *et al.* (2004) showed that nonrespiratory maneuvers (bicep curls and sit-ups) strengthened the inspiratory and expiratory muscles in healthy individuals. Conclusions were based on significant increases in pressures (transdiaphragmatic, gastric, static inspiratory and

expiratory mouth), as well as diaphragm thickness. They further suggested that such maneuvers may also strengthen the rib cage, abdominal wall, and upper extremities. Science continues to take large steps within this field of research. Recent studies continue to build upon the idea of IMF, only now they seek to elicit IMF with non-respiratory maneuvers.

The foundation for all future studies looking at fatigue of the respiratory muscles can be traced back to the likes of Black & Hyatt (1969), Roussos & Macklem (1977), and Moxham and colleagues (1980). These historic studies, laid the groundwork suggesting that these muscles could indeed become fatigued. It is now up to future generations of researchers to make new history by answering the questions regarding mechanism of respiratory muscle fatigue that has thus far eluded science.

The inability to replicate past research findings may in part be due to the small sample sizes and the extreme variability. Further insight must be reported into the results found in the clinical population, and the positive discoveries found within that specific population may become possible to generalize, or even test, in the healthy population. It is hoped that the future investigations continue to build and fortify the context in which the respiratory muscles are studied in relation to muscle fatigue, aerobic capacity and its effects on overall health.

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APPENDIX I: Hydrostatic Weighing

Densitometry: measure of whole body density (used to estimate % body fat)

Density = mass/volume

Archimedes' Principle:

Upthrust   = weight of fluid displaced
               = (volume of water)(density of water)
               = $(V + RV + GIV)d_w$

V= volume of water
RV = residual volume;

RV (litres)   = 0.01929(Ht)[cm] + 0.0115(Age)[yrs] – 2.24

GIV = gastro-intestinal volume;

GIV (litres)   = 0.200

w_{water} = w_{air} = upthrust
               = $w_{air} – (V + RV + GIV)d_w$
     V   = $w_{air} – w_{water} / d_w – RV – GIV$

     D   = $w_{air} / [(w_{air} – w_{water} / d_w) – RV – 0.200]$

     % Fat   = $(4.95/D_b – 4.50)$ x 100% *(Siri's Equation)*

     FFD   = 1.100g/ml

     FD   = 0.900g/ml

RV (litres)   = 0.01929(Ht)[cm] + 0.0115(Age)[yrs] – 2.24   (Siri WE, 1961)

Temperature Correction:

     C   = 1.004805 – 0.0003056T

APPENDIX II: Modified Borg Scale

MODIFIED BORG SCALE

Score for the sensation of breathlessness (dyspnea) <u>and</u> leg muscle (locomotor) discomfort.

0	**Nothing at all**	
0.5	**Very, very slight**	**(just noticeable)**
1	**Very slight**	
2	**Slight**	
3	**Moderate**	
4	**Somewhat severe**	
5	**Severe**	
6		
7	**Very severe**	
8		
9	**Very, very severe**	**(almost maximal)**
10	**Maximal**	

APPENDIX III: Predicted Measure of Ventilatory Load

	Males	Females
n	18	16
FEV_1 [predicted]	4.68 ± 0.1	3.61 ± 0†
$\dot{V}_E$ (L•min^{-1})	163.4 ± 6.2	3.61 ± 0.0†
$\dot{V}_E$ (L•min^{-1}) [predicted]	163.9 ± 2.3	126.3 ± 1.5†
$\dot{V}_E$ % predicted	99.6 ± 3.4	85.4 ± 3.9†

Values are means $\pm$ SEM; n, no of subjects; FEV_1, Forced expiratory volume end of 1 sec.

$\dot{V}_E$, minute ventilation; † Significantly different from male group ($p<0.05$)