RESPIRATORY MECHANICS AND DIAPHRAGMATIC FATIGUE DURING EXERCISE
IN MEN AND WOMEN

by

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

(Human Kinetics)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

February 2010

ABSTRACT

<u>Purpose:</u> The purpose of this thesis was to determine the underlying mechanisms associated with a higher total WOB in women (Study 1) and to determine if women experience greater levels of diaphragmatic fatigue relative to men (Study 2).

<u>Methods:</u>

Study 1: Sixteen endurance-trained subjects (8M:8F) underwent a progressive cycling test to exhaustion while esophageal pressure and lung volumes were measured. Modified Campbell diagrams were used to calculate the inspiratory and expiratory resistive and elastic components at 50, 75, 100 $l \cdot min^{-1}$ and maximal ventilations and also at standardized mass-corrected work-rates.

Study 2: Thirty-eight endurance-trained subjects (19M:19F) underwent a constant-load cycling test at 90% of peak work-rate until exhaustion. Pressure-time product of the diaphragm (PTP_{di}) was calculated during exercise. Trans-diaphragmatic pressure twitches ($P_{di,tw}$) were assessed using cervical magnetic stimulation before and 10, 30 and 60 minutes after exercise. Diaphragm fatigue was defined as a $\geq$ 15% reduction in $P_{di,tw}$ post-exercise.

<u>Results:</u>

Study 1: The inspiratory resistive WOB was higher in women at all absolute ventilations ($P<0.05$). The expiratory resistive WOB was higher in women at 75 $l \cdot min^{-1}$ ($P<0.05$). There were no sex-differences in the elastic WOB. However, the total WOB was significantly higher in men at relative percentages of maximal ventilation ($P<0.05$) but this sex-difference was reversed when the WOB was standardized for a given work-rate to body mass ratio.

Study 2: Diaphragm fatigue was present in 11 males and 8 females. The reduction in $P_{di,tw}$ at 10 and 30 min following exercise was significantly greater in men relative to women ($P<0.05$). Men consistently had higher absolute values for PTP_{di} during exercise but this sex-difference was reversed when body mass was taken into account. Over time, men continued to have a reduced contribution of the diaphragm to total inspiratory force output whereas diaphragmatic contribution in women remained relatively constant over time.

Conclusions: The higher total WOB in women is due to an increased resistive WOB which is likely attributable to their smaller airways. Despite a respiratory system that may have a higher mechanical cost of breathing, women appear to be more resistant to exercise-induced diaphragmatic fatigue.

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ACKNOWLEDGEMENTS

CO-AUTHORSHIP STATEMENT

A version of Chapter 2 has previously been published as:

Guenette JA, Querido JS, Eves ND, Chua R and Sheel AW. Sex differences in the resistive and elastic work of breathing during exercise in endurance trained athletes. *American Journal of Physiology; Regulatory, Integrative and Comparative Physiology.* 297(1):R166-175, 2009.

Guenette JA was the primary author and played the principle role in identification and design of the research program, performance of research, data analysis and manuscript preparation.

Querido JS assisted with data analysis.

Eves ND assisted in identification and design of the research program.

Chua R assisted with data analysis.

Sheel AW assisted in identification and design of the research program and manuscript preparation.

A version of Chapter 3 will be submitted for publication as:

Guenette JA, Romer LM, Querido JS, Chua R, Eves ND, Road JD, McKenzie DC and Sheel AW. Sex-differences in exercise-induced diaphragmatic fatigue in endurance-trained athletes.

Guenette JA was the primary author and played the principle role in identification and design of the research program, performance of research, data analysis and manuscript preparation.

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Sheel AW assisted in identification and design of the research program and manuscript preparation.

CHAPTER I: Introduction

A recent symposium (Sept. 2006) dealing with sex differences in exercise biology was held at the American College of Sports Medicine Integrative Physiology of Exercise Conference. Day (2008) introduced the symposium papers by stating that "the number of publications addressing sex differences and exercise physiology has increased from 12, before 1970, to as many as 1344 through October 2007." Clearly, sex-based physiological studies have become increasingly recognized as an important area of research among exercise physiologists. Our research group has been particularly interested in understanding sex differences in respiratory exercise physiology and how anatomical differences may have functional consequences during exercise in healthy women (Guenette *et al.*, 2004; Richards *et al.*, 2004; Sheel *et al.*, 2004; Guenette *et al.*, 2006; Guenette & Sheel, 2007a; Guenette *et al.*, 2007a; Guenette *et al.*, 2007b; Sheel & Guenette, 2008; Guenette *et al.*, 2009; Sheel *et al.*, 2009). The purpose of this introduction is four-fold. First, to discuss fundamental sex differences in respiratory anatomy with a focus on the airways. Second, to present a theoretical framework on how these anatomical sex differences influence resting pulmonary function and ventilatory mechanics during exercise. Third, to summarize the existing physiological literature that supports our theoretical framework and finally, to discuss sex differences in skeletal muscle fatigue.

Sex Differences in Respiratory Anatomy: There are a number of important sex differences in respiratory structure and resting pulmonary function that may influence the ventilatory response to exercise. Perhaps the most important sex difference in respiratory anatomy comes from studies suggesting that women have smaller lungs and airways relative to size-matched males. Direct anatomical evidence suggests that the pattern of airway-parenchymal growth is different between boys and girls. Thurlbeck (1982) obtained post-mortem lungs from boys and girls (6 weeks to 14 years) and showed that boys have larger lungs than girls starting at approximately 2 years of age. Even when lung volume was corrected for differences in body length, it appears

that with increasing age, boys continue to have larger lungs. If there is perfectly proportional growth of the airways and lung parenchyma, then the ratio of airway area to lung volume should be constant and independent of lung volume. However, the large variability in maximal expiratory flow rates in individuals with comparable lung volumes (Green *et al.*, 1974) suggests that their may be a dissociation between airway and lung size. This dissociation was termed "dysanapsis" (Mead, 1980). Mead (1980) indirectly assessed the relationship between airway size (estimated from maximal expiratory flow ÷ static recoil pressure at 50% vital capacity) and lung size (vital capacity) in men (n=21), boys (n=5) and women (n=7). He concluded that "the airways of men were approximately 17% larger in diameter than the airways of women," and speculated that "the adult sex difference in airway size develops relatively late in the growth phase." Additional evidence for dysanpsis comes from cross-sectional studies that have made estimates of tracheal area using acoustic reflectance in healthy men (n=26) and women (n=28) (Martin *et al.*, 1987). Martin *et al.* (1987) found that the tracheal cross-sectional area was 29% smaller in women even when matched for total lung capacity. These studies have given important insight into basic sex differences in airway anatomy but are limited in their indirect approaches to measuring airway size. Moreover, these studies and others (Griscom & Wohl, 1986; Hoffstein, 1986) have limited their assessment to areas above the tracheal carina. Accordingly, we recently used high resolution computed tomography (CT) to measure airway luminal areas in men and women (Sheel *et al.*, 2009) [see APPENDIX III for journal reprint]. Figure 1.1 shows a three-dimensional reconstruction of an airway tree in an individual subject and Figure 1.2 shows a similar airway tree with a highlighted section to demonstrate the various branches in which airway luminal area can be reliably determined based on resolution quality.
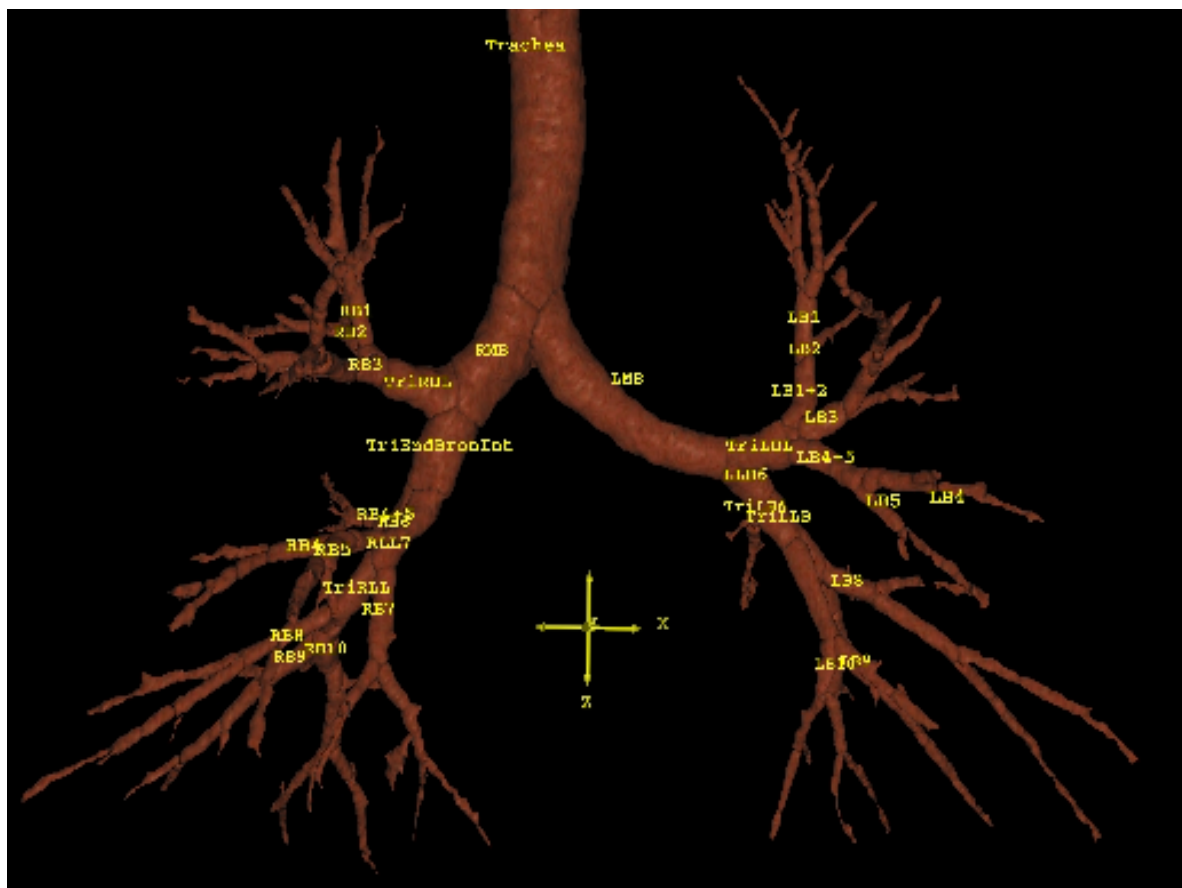
Figure 1.1: Three dimensional reconstruction of an airway tree using computed tomography.
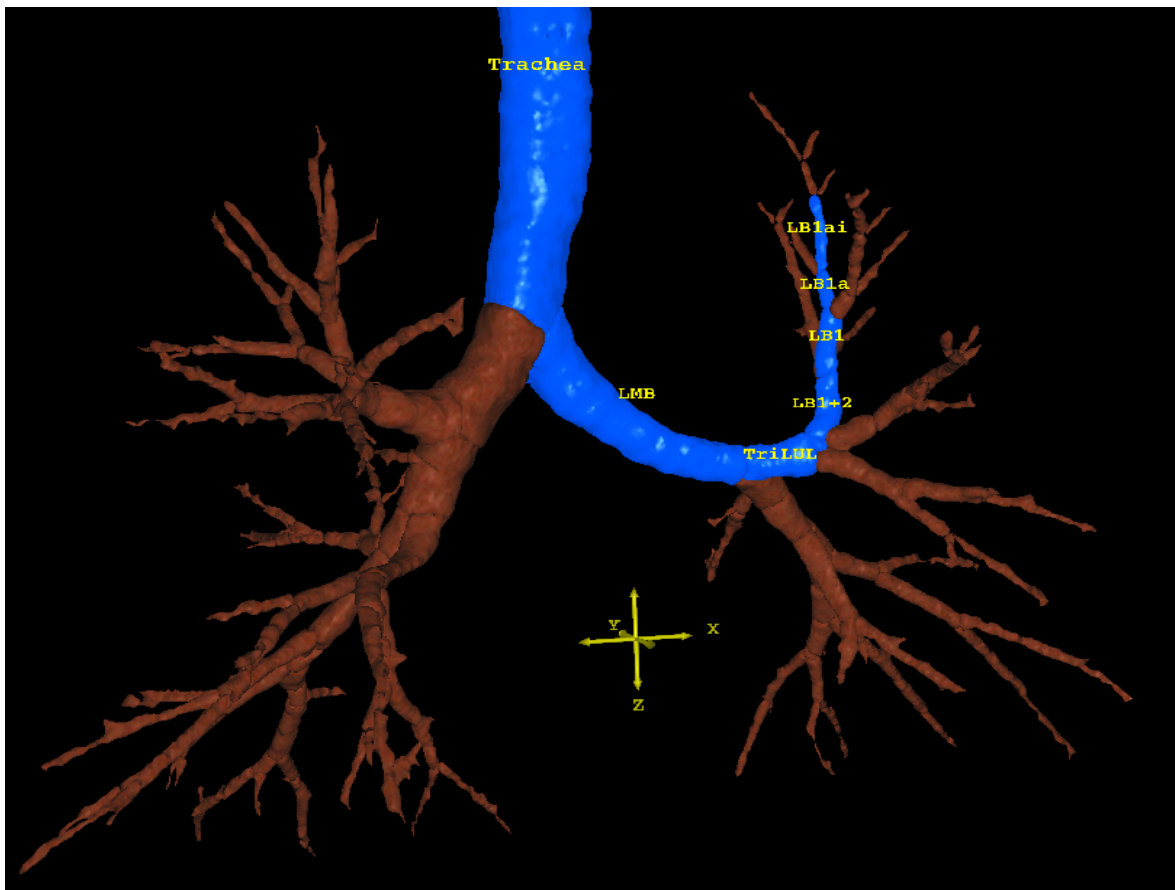
Figure 1.2: Three dimensional reconstruction of an airway tree using computed tomography with a highlighted section showing the branches that can be successfully measured.

We obtained images from 57 older ex-smokers (28 men and 27 women) that were being screened for lung nodules using CT scans. It was not possible to obtain such scans in healthy individuals due to risk of radiation exposure without diagnostic justification. Nevertheless, our relatively large sample size allowed us to compare airway luminal areas in all subjects, but more importantly, in a subset of subjects matched for lung capacity. We found that women had significantly smaller tracheal areas but also smaller diameter airways beyond the tracheal carina. Figure 1.3 shows the percentage difference in airway luminal area between men and women for specific airways.
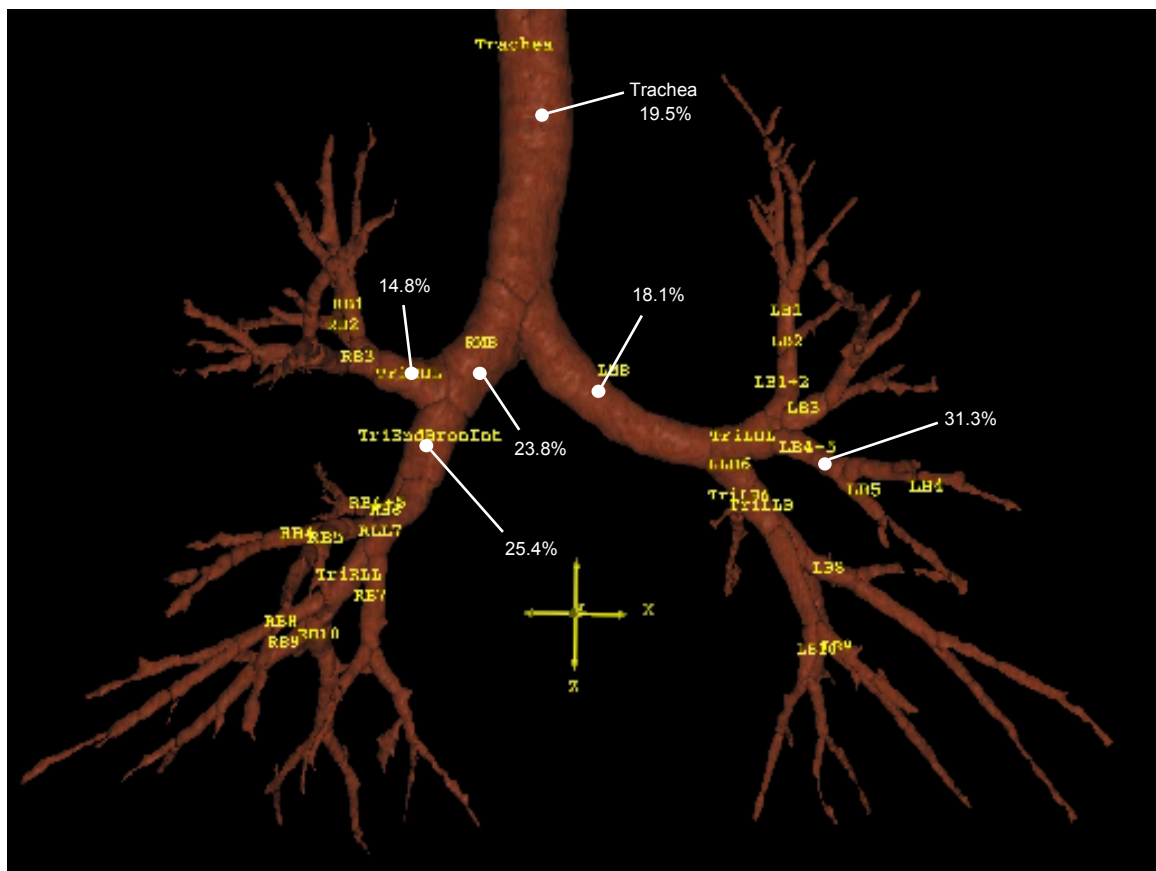
Figure 1.3: Percent differences in various airway areas between men and women matched for lung size.
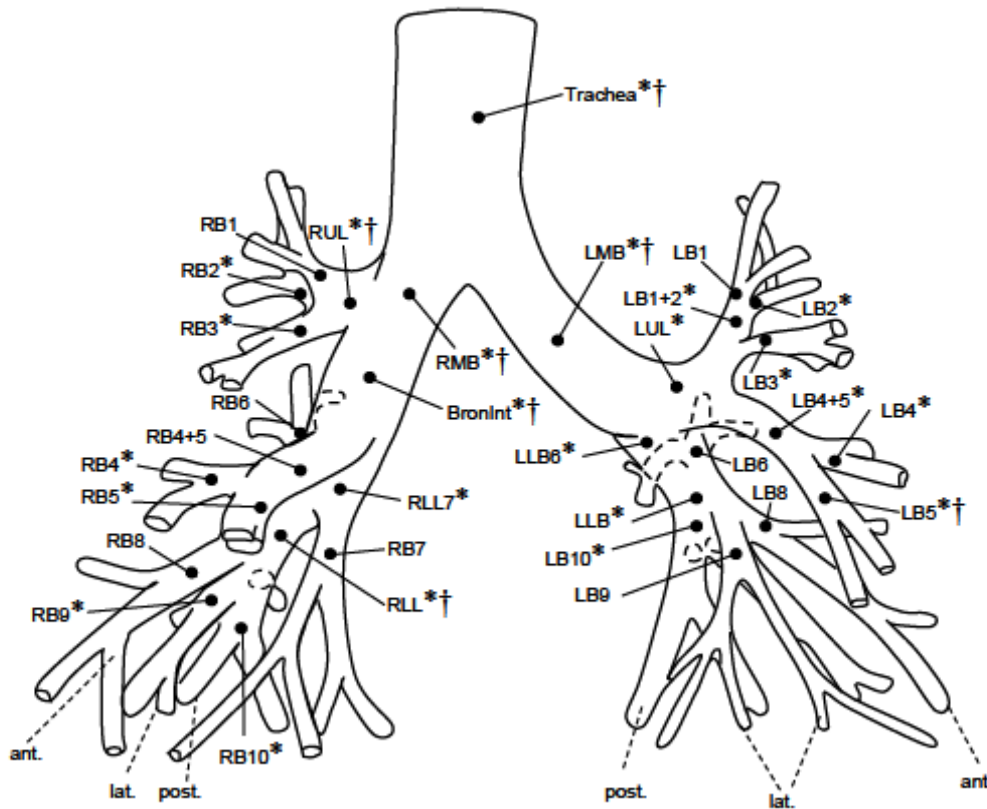
Figure 1.4: Airway tree with assigned labels. Labels refer to segments but are assigned to terminating branchpoint of respective segment. Drawing based on Boyden (1955). Post, posterior; lat, lateral; ant, anterior. * Significant differences between men and women of varying body size ($P < 0.05$). † Significant differences between subjects matched for lung size ($P < 0.05$). Reproduced with permission from Sheel *et al.* (2009).

This study confirmed previous reports that have shown women, matched for lung volume, have smaller tracheal areas than men. However, this study demonstrates that these sex differences persist beyond the tracheal carina which may have important implications for the flow resistive work of breathing (WOB), which will be discussed later. Collectively, these findings coupled with those of others (Mead, 1980; Thurlbeck, 1982; Martin *et al.*, 1987) suggest that women have smaller lungs and airways, even when matched for body size or lung capacity. How these anatomical differences influence ventilatory mechanics during exercise is discussed below.

Sex Differences in Breathing Mechanics: Given the aforementioned disparity in pulmonary structure, differences in resting pulmonary function between men and women are predictable. As previously described, women typically have smaller lung volumes and smaller diameter airways. Smaller airways mean that women will have lower maximal expiratory flow rates relative to men. Smaller lungs and lower flow rates result in a smaller maximum flow volume loop (MFVL) and thus, a reduced capacity to generate flow and volume during exercise. A smaller MFVL might make women susceptible to developing expiratory flow limitation (EFL) despite the fact that they achieve lower levels of minute ventilation during exercise relative to men. EFL is defined as the inability to increase expiratory flow despite increases in transpulmonary pressure and is quantified as the percent of the tidal volume that meets or exceeds the expiratory boundary of the MFVL (Johnson *et al.*, 1991a; Johnson *et al.*, 1991b). The presence of EFL may cause reflex inhibition of the hyperventilatory response and/or a significant alteration in breathing pattern.

Figure 1.5 provides a theoretical framework that illustrates the functional consequences of having smaller lungs and airways and how these factors may influence breathing during exercise.
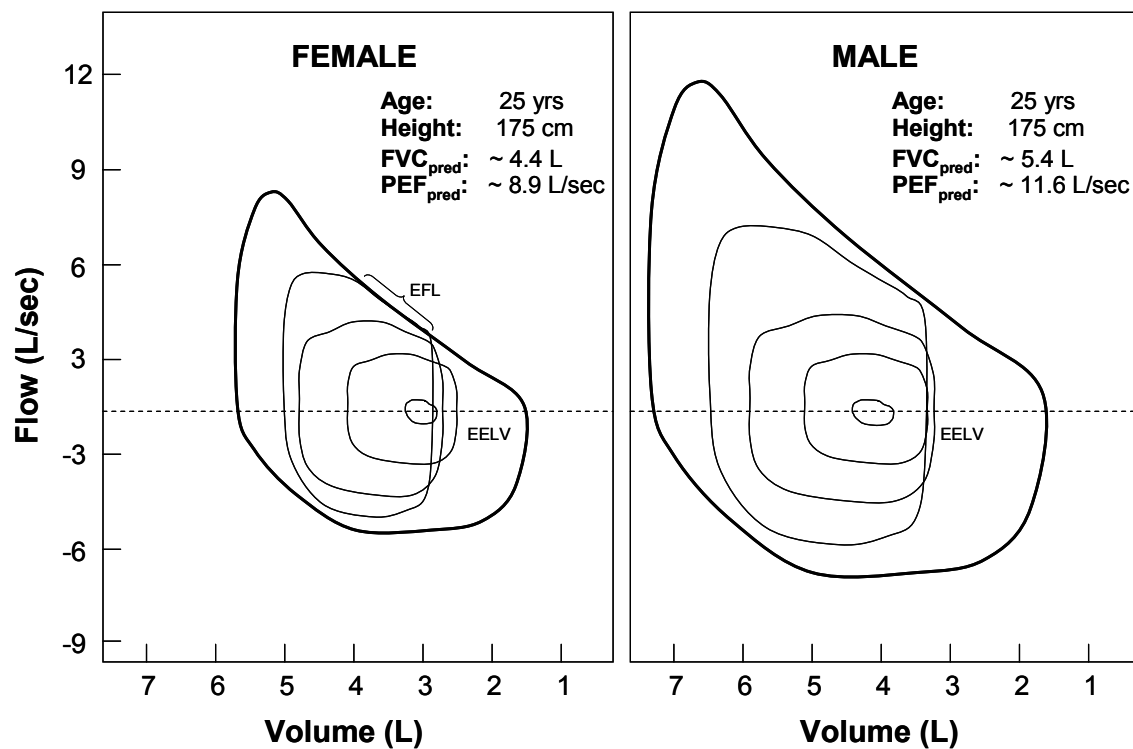
Figure 1.5: Theoretical response to progressive exercise in age and height matched men and women. Based on predictive equations, women have a smaller forced vital capacity (FVC) and peak expiratory flows (PEF). Shown are increasing tidal volumes and the presence of flow limitation in women when the expiratory tidal flow-volume loop intersects the volitional maximal flow-volume loop. At maximal exercise there is a greater increase in end-expiratory lung volume (EELV) in women relative to men. This leftward shift in EELV back towards resting values is indicative of dynamic hyperinflation. Reproduced with permission from Sheel and Guenette (2008).

Figure 1.5 shows a male and female matched for age and standing height. Tidal flow-volume loops are plotted within the MFVL. The forced vital capacity (FVC) and peak expiratory flow values are based on established prediction equations. It can be seen that the female has a smaller MFVL, lower maximal flow rates and thus, a smaller capacity to generate minute ventilation during exercise. With increasing exercising intensity, the female tidal flow-volume loop increases to the point of intersecting the boundary of the MFVL suggesting the presence of EFL. This response does not occur in an age and height matched male. It should be stressed however that Figure 1.5 is a theoretical schematic only and does not necessarily suggest that men do not

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develop EFL. The concepts in this schematic are supported by the work of McClaran et al. (1998) who were the first to demonstrate that healthy women develop significant EFL during heavy exercise because of their smaller lungs and lower maximal expiratory flow rates.

The few studies that have assessed EFL in women have used a technique whereby tidal flow volume loops are positioned within the MFVL based on a measurement of EELV. EFL is considered present if the tidal breath meets or exceeds the expiratory boundary of the MFVL. This technique provides an excellent visual representation of EFL and the regulation of lung volumes during exercise. However, this technique can overestimate the magnitude of EFL or even falsely detect its occurrence due to exercise-induced bronchdodilation and thoracic gas compression artefacts. An alternative method is to apply a negative expiratory pressure at the mouth, and compare the flow volume curve during the ensuing expiration with that of the preceding control breath. If the negative pressure does not increase expiratory flow then the subject is considered flow limited. This particular technique alleviates some of the limitations associated with the method of superimposing tidal breaths within a MFVL. We recently used the negative expiratory pressure technique to measure EFL in a group of highly trained male and female endurance athletes (Guenette *et al.*, 2007b). We found that 9 out of 10 women experienced significant EFL during maximal cycle exercise which is consistent with the work of McClaran *et al.* (1998), who observed EFL in 86% of their fit women during treadmill running to exhaustion. In contrast, we found evidence of EFL in only 43% of our male subjects. Although we had a relatively small sample size, the preliminary results of this study suggest that EFL may be more common in women.

With the onset of EFL, end-expiratory lung volume (EELV) may increase back to and sometimes beyond resting values resulting in dynamic hyperinflation of the lungs (Figure 1.5).

This dynamic hyperinflation permits increases in flow rate (Pellegrino *et al.*, 1993) at the expense of an increased elastic work because lung compliance is reduced as lung volume increases. Hyperinflation may then lead to earlier fatigue of the respiratory muscles by requiring them to contract from a shorter length, which means that the muscular force required to ventilate the lungs is closer to the muscle's maximal capacity to generate force (Roussos *et al.*, 1979). This will further reduce inspiratory muscle length and may substantially increase the work and O_2 cost of breathing, thus decreasing inspiratory muscle endurance time (Tzelepis *et al.*, 1988). Secondary to the hyperinflation-induced fatigue, a relative ischemia to the diaphragm may further exacerbate diaphragm fatigue (Bellemare & Grassino, 1982). A potential consequence of diaphragm fatigue and a high work of breathing (WOB) is the sympathetically mediated vasoconstriction and reduction in locomotor muscle blood flow (Dempsey *et al.*, 2003). These effects were demonstrated by mechanically loading or unloading the respiratory muscles at maximum exercise (Harms *et al.*, 1997). Changes in leg blood flow were observed which indicate a competitive relationship between locomotor and respiratory muscles for a limited cardiac output and this may be associated with reductions in exercise performance.

Figure 1.6 shows sex differences in the regulation of lung volumes in trained men and women. EELV and end inspiratory lung volume (EILV) follow the same pattern during submaximal exercise. However, as the subjects approached maximal exercise, women increased EELV back towards resting values whereas men did not. EILV in these women approached 90% of their FVC indicating that there would be an increased elastic load on the inspiratory muscles relative to their male counterparts whose EILV was only 82% of FVC. When expressed as a percentage of FVC, women had significantly higher EELV and EILV compared with men at maximal exercise. Based on the higher relative values for EILV and EELV in women, it would be predicted that the WOB would also be higher in women compared with men. McClaran *et al.*

(1998) examined the relationship between EFL and operational lung volumes by having women breathe a low density gas mixture (i.e., helium) to increase the size of the MFVL. Increasing the size of the MFVL can eliminate EFL which resulted in subjects being able to maintain a lower EELV. This suggests an important association between EFL and the regulation of EELV and EILV during exercise.
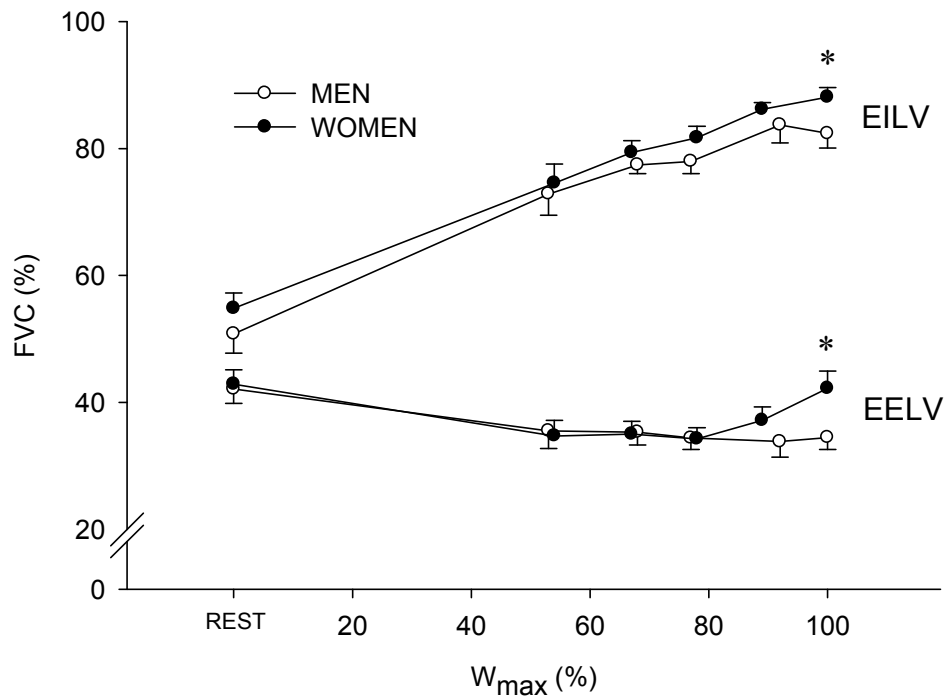
Figure 1.6: Regulation of lung volumes in men and women during progressive exercise to exhaustion (Guenette *et al.*, 2007b). Shown are end-inspiratory lung volume (EILV) and end-expiratory lung volume (EELV) expressed as % forced vital capacity (FVC) at rest and during progressive exercise to maximal workload (W_{max}) in men and women. Values are means ± SE. * Significantly different from men ($P < 0.05$). Reproduced with permission from Sheel and Guenette (2008).

Given the smaller lungs and airways in women, coupled with the finding of greater EFL and higher operational lung volumes during high intensity exercise, it may be predicted that the mechanical WOB would be higher in women. There have been few attempts to compare the WOB between sexes (Holmgren *et al.*, 1973; Guenette *et al.*, 2007b). Figure 1.7 shows the total

WOB in endurance-trained men and women across a range of ventilations measured during an

incremental cycle test to exhaustion.
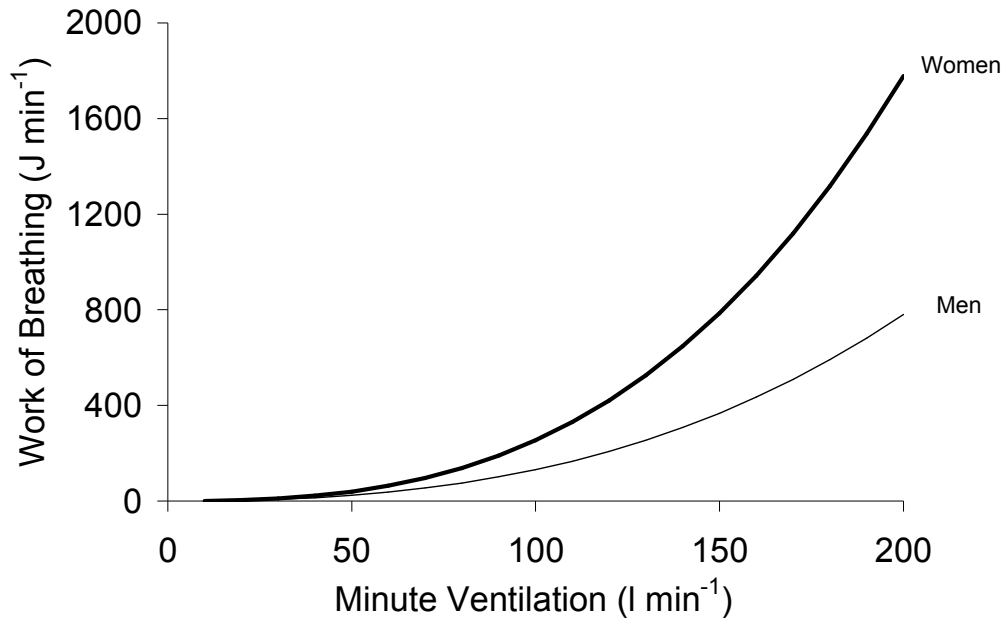
Figure 1.7: Work of breathing in men and women. Each curve represents a mean curve relating the work of breathing vs. minute ventilation. Each curve has been extrapolated to 200 l·min^{-1} for theoretical purposes. The work of breathing is essentially the same at rest and during very low levels of ventilation. As ventilation increases with increasing intensity, the work of breathing in women significantly increases out of proportion relative to men. Reproduced with permission from Guenette *et al.* (2007b).

The total WOB was not different at low intensity exercise up to 50 l·min^{-1}. However, the WOB

increased disproportionally in women as ventilation increased beyond 50 l·min^{-1}. When minute

ventilation exceeded 90 l·min^{-1} the WOB in women was approximately twice that of men.

Therefore, the work and presumably the O_2 cost of moving a given volume of air through the

lungs is substantially higher in women. It should also be noted that both curves in Figure 1.7

have been extrapolated to 200 l·min^{-1} for theoretical purposes only. In addition to the data

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presented in Figure 1.7, Guenette *et al.* (2007b) also assessed the relationship between minute ventilation and the WOB according to the following equation described by Otis *et al.* (1950):

$$\text{Work of breathing} = a\dot{V}_E{}^3 + b\dot{V}_E{}^2$$

The term $b\dot{V}_E{}^2$ represents the mechanical work done in overcoming the viscous resistance offered by the lung tissues to deformation and by the respiratory tract to the laminar flow of air whereas term $a\dot{V}_E{}^3$ represents the work done in overcoming the resistance to turbulent flow. A value for constant '*a*' and '*b*' was then determined for each individual subject. Constant '*a*' was significantly higher in women, suggesting that perhaps the higher WOB in women is associated with the additional work needed to overcome the resistance to turbulent airflow. This may explain why the magnitude of the difference between men and women increased out of proportion with increasing levels of minute ventilation, and thus airflow. It would be expected that subjects with larger lung volumes would have lower pulmonary resistance and thus a lower WOB for a given level of ventilation. The women in this study had significantly smaller lungs (and presumably airways) compared to the men which may be one of many major reasons for their higher WOB. Consistent with the concept of differences in lung volumes was the observation that when men and women were pooled together, there was a significant, albeit modest, correlation between FVC and constant '*a*' ($r = -0.54$, $P < 0.05$). Perhaps more importantly was the finding that constant '*a*' was significantly correlated with peak expiratory flow rates in women ($r = -0.76$, $P < 0.05$) and when all subjects were pooled together ($r = -0.68$, $P < 0.05$). Peak expiratory flow rates may serve as a crude surrogate for airway size, which may explain, in part, the increased work needed to overcome the resistance to turbulent airflow in women relative to men (Guenette *et al.*, 2007b).

Based on the aforementioned differences in respiratory anatomy and breathing mechanics, one may conclude that the respiratory muscles of females may be placed under greater mechanical stress relative to males. This is particularly the case if women are breathing at higher lung volumes. This would place the diaphragm in a sub-optimal contractile position along its length tension relationship resulting in a higher WOB for a given level of minute ventilation. We have hypothesized in previous articles that these sex differences might make the female diaphragm more prone to fatigue (Guenette & Sheel, 2007b; Guenette *et al.*, 2007b; Sheel & Guenette, 2008) but this continues to remain an untested hypothesis and is based solely on speculation. This hypothesis is also difficult to reconcile with previous studies showing that women are actually less susceptible to non-respiratory muscle fatigue. The following section will briefly summarize the literature as it pertains to sex differences in skeletal muscle fatigue.

Sex Differences in Skeletal Muscle Fatigue: The deleterious effects of skeletal muscle fatigue on exercise performance has been a topic of great interest to exercise and muscle physiologists over the last century. Muscle fatigue can be defined as a loss in the capacity for developing force and/or velocity resulting from muscle activity under load and which is reversible by rest (NHLBI, 1990) and can be further characterized as either peripheral or central fatigue. Peripheral fatigue occurs at or distal to the neuromuscular junction while central fatigue refers to a reduction in motor output from the central nervous system. The precise contribution of central or peripheral fatigue to the reduced force-generating capacity of the muscle remains controversial. Studies examining sex differences in skeletal muscle fatigue have typically shown that women have greater relative fatigue resistance compared to their male counterparts (Maughan *et al.*, 1986; Miller *et al.*, 1993; West *et al.*, 1995; Fulco *et al.*, 1999). The question germane to the present study is why would women be less susceptible to muscle fatigue compared with men and is this fatigue resistance a finding that can be extended to the muscles of

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respiration? Hicks *et al.* (2001) recently reviewed potential mechanisms of this apparent sex difference in muscle fatigue although none of the studies mentioned dealt specifically with the muscles of respiration. The most common mechanisms associated with greater fatigue resistance in women relative to men include differences in muscle mass, muscle morphology, substrate utilization and neuromuscular activation.

Sex Differences in Muscle Mass and Morphology: It is well known that women have smaller muscle mass compared with men and this fact has been proposed as one of the key contributors to explain the greater fatigue resistance found in women. Lower muscle mass translates directly into lower absolute force generation in females when performing at the same relative intensity as males. This lower absolute force production means there will be a decreased O_2 demand, a decrease in mechanical compression of the local vasculature and less intramuscular occlusion of blood flow. However, most studies have ignored the greater absolute force of submaximal contraction in men (Maughan *et al.*, 1986; Miller *et al.*, 1993) and therefore the proposed sex differences may simply be due to contraction conditions eliciting a greater degree of imbalance between muscle O_2 supply and demand in men (Fulco *et al.*, 1999). Fulco *et al.* (1999) addressed this issue by comparing muscle performance in men and women at the same absolute force development and matched subjects for maximal voluntary contraction of the adductor pollicis muscle. Despite matching for maximal muscle strength, these authors still found that females exhibit less fatigue than males.

In terms of muscle morphological differences, there is some evidence to suggest that there are sex differences in muscle fibre type composition such that women have more slow oxidative type I fibres (Nygaard, 1981; Simoneau *et al.*, 1985; Simoneau & Bouchard, 1989; Mannion *et al.*, 1997; Jaworowski *et al.*, 2002). Type I muscle fibres have a slower contraction

speed and thus a slower rate of energy utilization (Stienen *et al.*, 1996; Hamada *et al.*, 2003). This in turn means that type I fibres fatigue at slower rates compared to fast glycolytic type II fibres (Hamada *et al.*, 2003). These potential sex differences in muscle fibre type composition may explain, in part, why female muscles are more fatigue resistant than male muscles.

Sex Differences in Substrate Utilization: Sex differences in substrate utilitzation during exercise may also contribute to potential sex differences in muscle fatigue. It has been established that males have a higher glycolytic capacity and a greater reliance on glycolytic pathways than females (Hicks *et al.*, 2001). Muscle biopsy studies have also revealed that women have lower activities of common glycolytic enzymes, which in turn would translate into a decreased potential for anaerobic glycolysis (Tarnopolsky, 1999). As pointed out by Hicks *et al.* (2001), these differences may mean that women have a greater reliance on *β*-oxidation of fatty acids, thus prolonging endurance during certain types of exercise and perhaps improving their ability to resist fatigue.

Sex Differences in Neuromuscular Activation: One of the few studies to systematically examine sex differences in neuromuscular activation and its association with muscle fatigue was conducted by Häkkinen (1993). The purpose of this study was to examine acute neuromuscular fatigue and short-term recovery from fatigue in men and women following an intense resistance exercise protocol. The relative loading intensity and volume of exercise was kept the same for both sexes. Häkkinen (1993) found significant decreases in maximal voluntary EMG in males but not in the females, suggesting a greater impairment in neuromuscular activation in males relative to females after fatiguing resistance exercise. Despite this finding, the potential role of sex differences in neuromuscular activation as a mechanism for the increased fatigue resistance in women still requires additional research.

Summary: There are clear differences in respiratory anatomy, such that women have smaller lungs and airways, even when matched for body size. Smaller lungs and airways in women translate directly into a reduced capacity to generate flow and volume, and thus ventilation during exercise. Some have suggested that these anatomical factors may be associated with the notion that women may be more susceptible to pulmonary system limitations during exercise. For example, women may be more susceptible to EFL and have greater increases in operational lung volumes during high intensity exercise. Moreover, women have been shown to have a higher WOB during exercise for a given level of absolute minute ventilation. However, there are no studies that have examined possible mechanisms associated with a higher WOB in women. The aforementioned observations point to a female respiratory system that may be at a mechanical disadvantage relative to their male counterparts during exercise. This mechanical disadvantage might make the primary inspiratory muscle (i.e., the diaphragm) more susceptible to exercise-induced fatigue which has important physiological consequences in both health and diseased populations. However, there are no studies that have systematically compared the diaphragmatic fatigue response in women with that of men.

PURPOSE

1. To provide a comprehensive assessment of the mechanical WOB in men and women and to determine the mechanisms associated with a higher WOB in women.

2. To characterize diaphragmatic pressure production and recruitment during exercise and to determine if there are sex differences in the severity of exercise-induced diaphragmatic fatigue.

RESEARCH QUESTIONS

1. Which WOB component(s) is responsible for the higher total WOB in women for a given absolute ventilation?

2. Are there sex differences in the total WOB and its constituent components when comparisons are made at relative percentages of maximal ventilation?

3. Are there sex differences in the total WOB and its constituent components when comparisons are made at absolute mass-corrected workloads?

4. Is the severity of exercise-induced diaphragmatic fatigue different between men and women?

5. Are the absolute and mass-corrected esophageal and trans-diaphragmatic pressure-time products different between sexes during exercise?

HYPOTHESES

1. The higher total WOB during exercise for a given absolute ventilation will be due to a combination of inspiratory and expiratory resistive and elastic components.

2. There will be no sex differences in the WOB when comparisons are made at relative percentages of maximal ventilation.

3. The total WOB and its constituent components will be higher in women for a given mass-corrected workload.

4. The severity of exercise-induced diaphragmatic fatigue will be greater in women.

5. Absolute and mass-corrected esophageal and diaphragmatic pressure-time products will be higher in women during exercise

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CHAPTER II: Sex differences in the resistive and elastic work of breathing during exercise in endurance-trained athletes *

INTRODUCTION

A growing number of investigations aimed at characterizing the healthy female respiratory response to exercise have reported sex-based differences in pulmonary gas exchange (Harms *et al.*, 1998; Richards *et al.*, 2004) and respiratory mechanics (McClaran *et al.*, 1998; Guenette *et al.*, 2007). These studies show that young adult women free from respiratory disease may be more susceptible to pulmonary limitations during exercise which is likely associated with women having smaller lungs and airways relative to size-matched males (Mead, 1980; Thurlbeck, 1982).

There have been few attempts to systematically compare the work of breathing (WOB) between sexes (Holmgren *et al.*, 1973; Guenette *et al.*, 2007). We recently demonstrated that the WOB was higher in endurance trained women at moderate to high levels of minute ventilation compared with trained males with no differences in the total WOB when comparisons were made at different percentages of maximal aerobic capacity ($\dot{V}O_2max$) (Guenette *et al.*, 2007). On average, women had a WOB that was approximately twice as high as men at ventilatory rates above 50 l·min^{-1}. However, the analysis used in this previous study was limited in that it did not provide specific information regarding the individual components that make up the total WOB. The WOB can be sub-divided into the work of the respiratory muscles to overcome the elasticity of the lungs during inspiration, the work required to overcome airflow resistance during inspiration, the work of the expiratory muscles to overcome the elastic outward recoil of the chest wall and the work required to overcome airflow resistance during expiration. It is currently unknown which of these factors contribute to the higher total WOB in women compared with men.

Based on mechanical grounds, we hypothesized that the resistive WOB would be higher in women because of their inherently smaller diameter airways (Mead, 1980; Martin *et al.*, 1987). However, this hypothesis may be an oversimplification because it is unknown if women adopt a unique breathing pattern to minimize one WOB component at the expense of another. To this end, we re-analyzed data from our previous investigation (Guenette *et al.*, 2007) by partitioning the respiratory pressure-volume data into 4 distinct WOB components across a range of ventilations and also at 3 different body mass corrected work rates achieved by all subjects. The WOB was compared at different mass corrected work rates to determine if the WOB is higher for given level of external muscular work. Furthermore, we examined sex differences in breathing pattern to determine the effect of tidal volume and breathing frequency on the WOB.

METHODS

Subjects: Sixteen endurance trained athletes (8 men and 8 women) volunteered to participate in this study. Endurance trained athletes were used instead of untrained individuals because they are capable of generating higher levels of minute ventilation compared to their untrained counterparts. This permits physiological comparisons across a wider range of values. Moreover, the mechanical work of breathing appears to be independent of fitness level (Milic-Emili *et al.*, 1962). The subjects gave informed written consent [APPENDIX I] and all experimental procedures received institutional ethical approval and conformed to the Declaration of Helsinki. All subjects were healthy non-smokers and did not have any previous history of cardiopulmonary disease. Subjects with a forced expired volume in one second ($FEV_{1.0}$) to forced vital capacity (FVC) ratio of < 80% of predicted were excluded from the investigation.

Experimental Overview: Subjects participated in two testing sessions separated by a minimum of 48 hours. All women were tested during the early follicular phase (days 3 to 8) of the menstrual cycle as determined via a self-reported menstrual history/health questionnaire [APPENDIX II]. On the first day, subjects performed general spirometry to assess lung function and an incremental cycle test to exhaustion to determine $\dot{V}O_2max$. They also received extensive practice on how to perform inspiratory capacity maneuvers at rest and during exercise. The second day served as the primary testing day which included 10 min of seated quiet breathing followed by an incremental cycle test to exhaustion using the identical exercise protocol as used on Day 1.

Pulmonary Function: FVC, $FEV_{1.0}$, $FEV_{1.0}/FVC$ and peak expiratory flow were obtained using routine spirometry according to standardized procedures and expressed using prediction equations (ATS, 1995).

Maximal Cycle Exercise: Subjects performed an incremental test to exhaustion on a cycle ergometer using a step protocol. Men and women began cycling at 200 W and 100 W respectively, with the work rate increasing by 30 W every 3 min. Ventilatory and mixed expired metabolic parameters were assessed using a customized metabolic cart consisting of a calibrated pneumotachograph (model 3813, Hans Rudolph, Kansas City, MO) and calibrated CO_2 and O_2 analyzers (Models CD-3A and Model S-3-A/I respectively, Applied Electrochemistry, Pittsburgh, PA).

Flow, Volume and Pressure: Inspiratory and expiratory flow was measured using a heated and calibrated pneumotachograph (model 3813, Hans Rudolph, Kansas City, MO) attached to a mouthpiece. Inspiratory and expiratory volume was obtained through numerical integration of the flow signal. Esophageal pressure (P_{es}) was obtained by placing a 10-cm long latex balloon (no. 47-9005, Ackrad Laboratory, Cranford, NJ) ~45 cm down from the nostril (Milic-Emili *et al.*, 1964) after application of a local anesthetic. All air was removed from the balloon by having subjects perform a valsalva maneuver. The balloon was then inflated with 1-ml of air as per manufacturer specifications. P_{es} was measured using a calibrated piezoelectric pressure transducer ($\pm$ 100 cmH_2O; Raytech Instruments, Vancouver, BC, Canada).

End Expiratory Lung Volume: End expiratory lung volume (EELV) was determined by having subjects perform inspiratory capacity (IC) maneuvers at rest and during exercise as previously described (Guenette *et al.*, 2007). Two to three IC maneuvers were obtained near the middle

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and end of each 3 minute exercise bout and additional IC maneuvers were performed immediately prior to exhaustion. End-expiratory lung volume (EELV) was calculated as the difference between FVC and the IC volume. FVC was used to calculate EELV rather than total lung capacity because it was not possible to measure residual lung volume in our subjects. FVC maneuvers were performed before and immediately after exercise with the largest FVC value being used for the analysis.

Work of Breathing: The muscular WOB was determined using modified Campbell diagrams as described by Roussos and Campbell (1986) and using the technique of Yan *et al.* (1997). Flow, volume and pressures from several breaths (~5-20) corresponding to approximately 50, 75 and 100 l·min^{-1} and 25, 50, 75 and 100% of maximal ventilation were selected for each subject and ensemble averaged using a customized software program (Bibo, LabVIEW software V6.1, National Instruments). The same procedure was performed to compare the WOB at 3.0, 3.5 and 4.0 W·kg^{-1}. These ventilatory rates and workloads were selected because nearly all male and female subjects were successfully able to reach these values. Additional Campbell diagrams were also generated for each stage of exercise in order to determine the relationship between average inspiratory flow and the inspiratory resistive WOB. An example of the modified Campbell diagram in a representative male subject at approximately 75% of maximal minute ventilation (i.e., 115 l·min^{-1}) is shown in Figure 2.1.
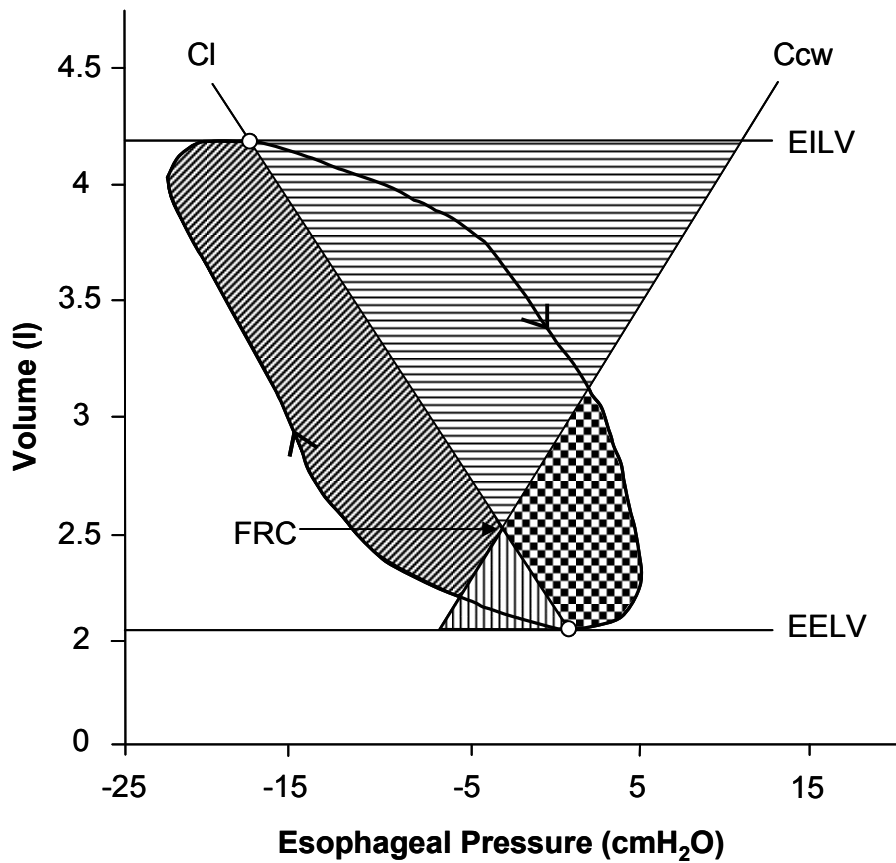
Figure 2.1: Example of a modified Campbell Diagram obtained from a male subject during exercise at approximately 75% of maximum ventilation (115 $l \cdot min^{-1}$). Oblique hatching represents the inspiratory resistive work of breathing. Horizontal hatching represents the inspiratory elastic work of breathing. Stippling represents the expiratory resistive work of breathing. Vertical hatching represents the expiratory elastic work of breathing. FRC, functional residual capacity; EELV, end-expiratory lung volume; EILV, end-inspiratory lung volume; Cl, dynamic lung compliance; Ccw, chest wall compliance. Upward arrow represents inspiration and downward arrow represents expiration. Small open circles represent zero flow points.

A line was drawn connecting the points of zero flow (i.e., EELV and EILV) representing the

dynamic compliance of the lung. The compliance of the chest wall was derived using previously

published data (Estenne *et al.*, 1985) based on both age and sex as done by others (Yan *et al.*,

1997; Sliwinski *et al.*, 1998; Butcher *et al.*, 2006; Eves *et al.*, 2006). The chest wall compliance

line was positioned through functional residual capacity (EELV at rest) and extended to EELV

and EILV. The muscular WOB was then partitioned into 4 separate components. The area

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inside of the P_{es}-loop to the left of the lung compliance line (oblique hatching) represents the work needed to overcome airflow resistance during inspiration (i.e., inspiratory resistive work). The area enclosed by the lung compliance and chest wall compliance lines (horizontal hatching) represents the work needed to overcome lung elasticity (i.e., inspiratory elastic work). The area to the right of the chest wall compliance line (stippling) represents the active muscular work needed to overcome airflow resistance during expiration (i.e., expiratory resistive work). Lastly, the area between the lung and chest wall compliance lines below functional residual capacity (vertical hatching) represents the work needed to overcome the outward elastic recoil of the chest wall to maintain EELV below functional residual capacity (i.e., expiratory elastic work). The sum of all four areas shown in Figure 2.1 represents the total WOB. All WOB values were multiplied by breathing frequency representing a unit of power (i.e., $J \cdot min^{-1}$). However, as conventionally used, we will refer to this throughout the manuscript as the WOB rather than the power of breathing.

Data Processing: All raw data was recorded continuously at 200 Hz using a 16-channel data acquisition system (PowerLab/16SP model ML 795, ADI, Colorado Springs, CO) and stored on a computer for subsequent analysis (Chart v5.3, ADInstruments, Colorado Springs, CO).

Statistical Analysis: Descriptive characteristics were compared between sexes using unpaired t-tests. Pre-planned comparisons were used to compare men and women for the various WOB components and the ventilatory parameters at the target ventilations and work rates using unpaired t-tests. Linear regression analysis using Pearson correlations was performed to test for associations between specific WOB components and pulmonary function parameters. The α level was set *a priori* at 0.05 for all statistical comparisons. Values are presented throughout the manuscript as means $\pm$ SD unless otherwise stated.

RESULTS

Subject Characteristics: The subjects used in the present investigation participated in a study

that has been previously published (Guenette *et al.*, 2007) [see APPENDIX III for journal

reprint]. The present study has 8 males and 8 females while our previous manuscript reported

data on 8 males and 10 females. Two females were excluded from the present analysis because

they did not have P_{es} data (*n*=1) or were unable to correctly perform IC maneuvers (*n*=1). Males

and females were not different for age (25.9 ± 4.9 vs. 24.9 ± 3.1 years, respectively) but men

were taller (183.9 ± 6.6 vs. 168.8 ± 4.0 cm, *P* < 0.0001) and heavier (76.6 ± 9.8 vs. 64.3 ± 3.6

kg, *P* < 0.01). Table 2.1 summarizes the pulmonary function data for the present study and table

2.2 summarizes the maximal exercise data obtained on day 1. As expected, women had smaller

FVC, $FEV_{1.0}$ and peak expiratory flows compared with men. All subjects were within normal

values for all pulmonary function measures except peak expiratory flows which were typically

>120% of predicted. There were no significant sex differences in percent predicted values for

any pulmonary function parameters.

Table 2.1: Pulmonary function data

	Men (*n*=8)				Women (*n*=8)			
FVC (l)	6.0	±	1.0	(4.5-7.2)	4.5	±	0.5	(3.8-5.4) *
FVC (%predicted)	105	±	14	(84-125)	108	±	14	(92-134)
$FEV_{1.0}$ (l)	5.1	±	1.0	(3.5-6.4)	3.8	±	0.4	(3.0-4.5) *
$FEV_{1.0}$ (%predicted)	106	±	18	(80-133)	106	±	14	(80-131)
PEF ($l·s^{-1}$)	12.6	±	1.4	(10.4-14.8)	8.1	±	1.2	(6.5-10.0) *
PEF (%predicted)	124	±	11	(109-138)	118	±	19	(91-148)
$FEV_{1.0}$/FVC (%)	85.3	±	4.2	(78.3-88.6)	84.6	±	5.9	(74.3-92.4)
$FEV_{1.0}$/FVC (%predicted)	101	±	5	(92-106)	98	±	7	(86-109)

FVC, forced vital capacity; $FEV_{1.0}$, forced expired volume in 1 second; PEF, peak expiratory
flow. * Significantly different from men. Ranges are presented in parentheses.

Table 2.2: Maximal exercise data on day 1

	Men (n=8)			Women (n=8)			
$\dot{V}O_2$ (ml·kg^{-1}·min^{-1})	69.5	±	7.8	59.2	±	4.7	*
$\dot{V}O_2$ (l·min^{-1})	5.3	±	0.7	3.8	±	0.3	*
$\dot{V}CO_2$ (l·min^{-1})	5.7	±	0.6	4.1	±	0.4	*
RER	1.08	±	0.07	1.07	±	0.05	
F_b (breaths·min^{-1})	59	±	9	59	±	6	
V_T (l)	3.1	±	0.4	2.3	±	0.3	*
$\dot{V}E$ (l·min^{-1})	161.2	±	25.1	122.3	±	17.3	*
$\dot{V}E/\dot{V}O_2$	30.6	±	4.9	32.1	±	3.1	
$\dot{V}E/\dot{V}CO_2$	28.2	±	3.0	30.2	±	3.1	
HR (bpm)	189	±	8	191	±	13	
Exercise duration (s)	1273	±	189	1213	±	143	
Work-rate (W)	380	±	29	271	±	22	*

$\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; RER, respiratory exchange ratio; F_b, breathing frequency; V_T, tidal volume; $\dot{V}E$, minute ventilation; $\dot{V}E/\dot{V}O_2$, ventilatory equivalent for oxygen; $\dot{V}E/\dot{V}CO_2$, ventilatory equivalent for carbon dioxide; HR, heart rate.
* Significantly different from men.

Work of Breathing vs. Absolute Minute Ventilation: Figure 2.2 shows the total WOB (panel A) and the constituent components of the WOB (panels B-E) at comparable absolute ventilations and at maximal ventilation in men and women. The total WOB was significantly higher in women at 75 and 100 l·min^{-1} but not at 50 l·min^{-1}. The total WOB at 50, 75 and 100 l·min^{-1} was 23, 33 and 48% higher in women, respectively, but was 45% higher in men at maximal ventilations. The inspiratory resistive WOB was 67, 89 and 109% higher in women at 50, 75 and 100 l·min^{-1}, respectively ($P < 0.05$), while the expiratory resistive WOB was only significantly higher in women at 75 l·min^{-1}. The expiratory resistive WOB at 75 l·min^{-1} was 131% higher in women. There was no significant difference in the elastic WOB during inspiration or expiration at any absolute ventilation. However, the inspiratory elastic WOB was 42% higher in men at maximal ventilations ($P = 0.05$).
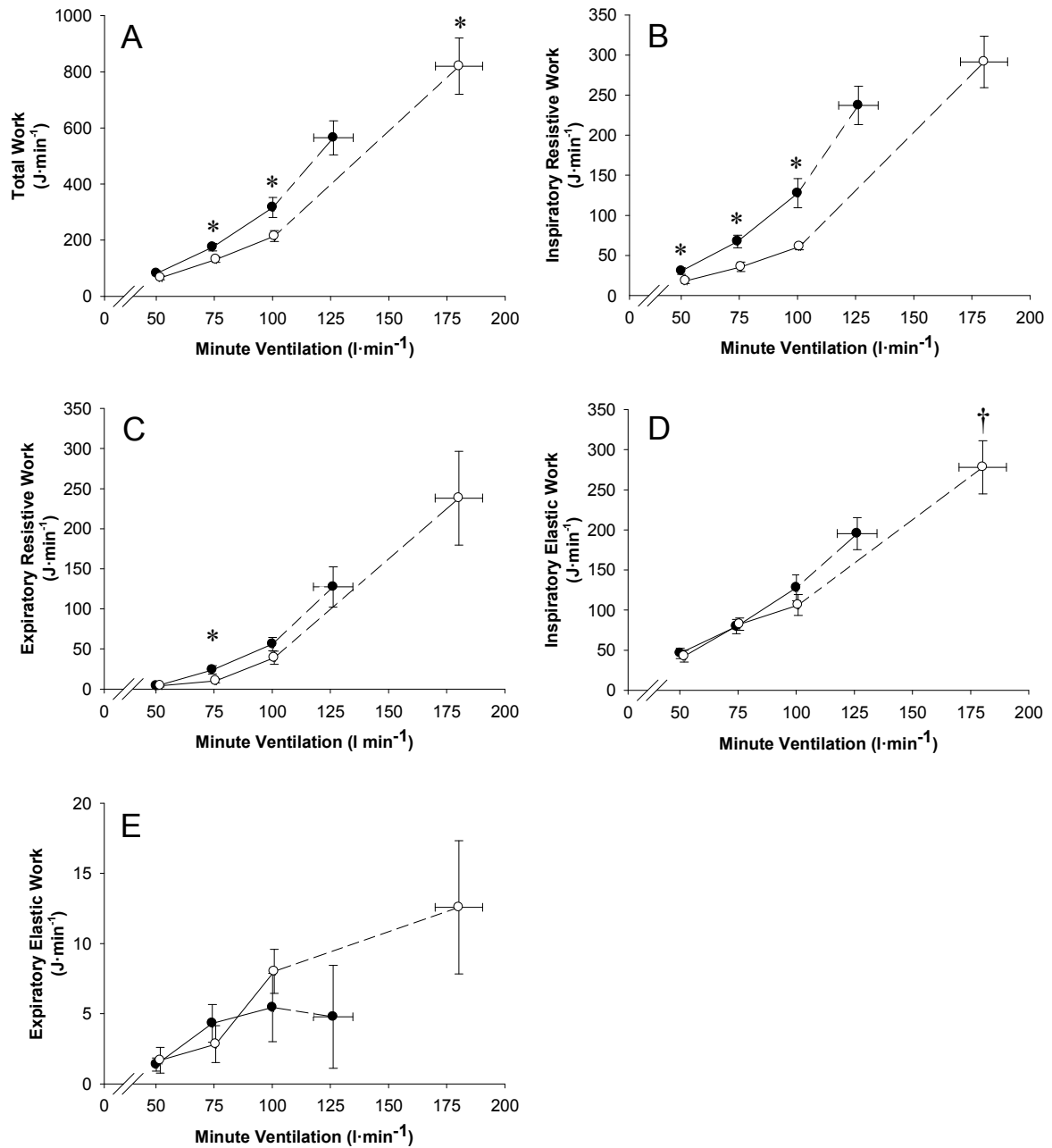
Figure 2.2: Total work of breathing (A), inspiratory resistive work (B), expiratory resistive work (C), inspiratory elastic work (D) and expiratory elastic work (E) versus minute ventilation in men (○) and women (●). The last data point represents maximal minute ventilation. Values are mean ± SE. * Significantly different between groups ($P < 0.05$); † $P = 0.05$.

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Figure 2.3 shows Campbell diagrams for an individual male and female subject matched for

minute ventilation, tidal volume and breathing frequency. This figure demonstrates the

significantly higher pressures needed to maintain the same ventilatory loads resulting in a much

higher inspiratory and expiratory resistive work with little difference in elastic work.
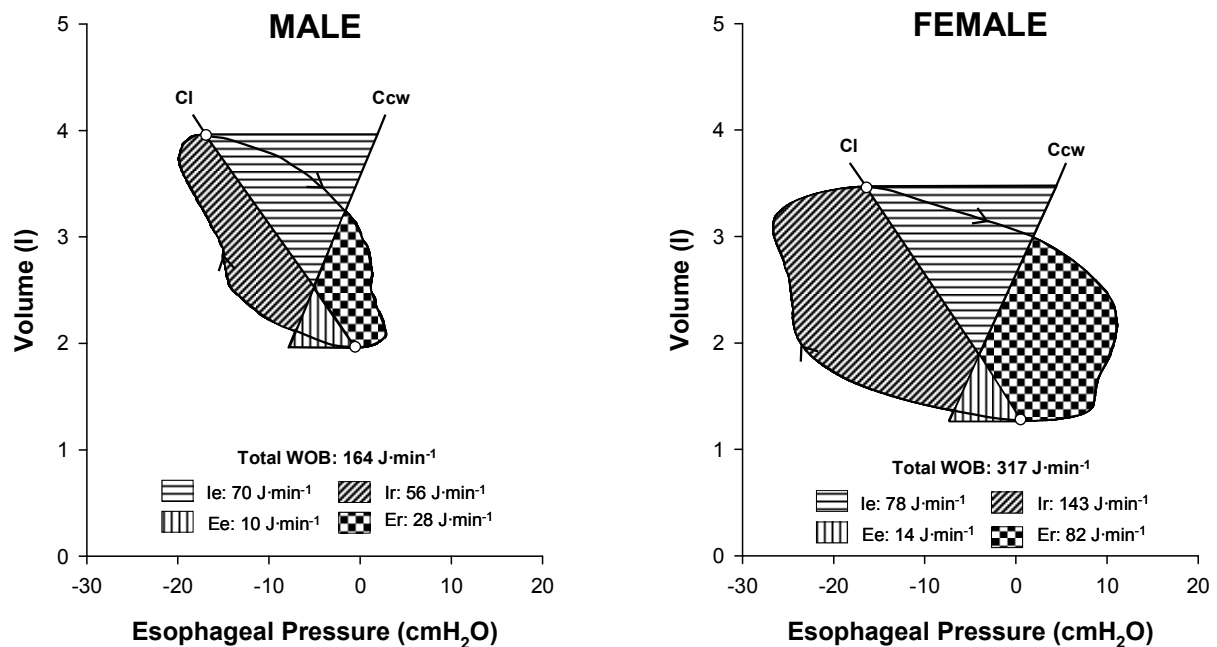
Figure 2.3: Modified Campbell Diagrams from an individual male and female subject matched approximately for absolute minute ventilation (100 vs. 101 $l \cdot min^{-1}_{(STPD)}$), tidal volume (2.1 vs. 2.2 liters), breathing frequency (52 vs. 49 breaths $\cdot min^{-1}$), age (24 vs. 25 years) and mass (64.6 vs. 64.2 kg), respectively. The male was slightly taller than the female subject (181 vs. 167 cm, respectively). Oblique hatching represents the inspiratory resistive work of breathing (Ir). Horizontal hatching represents the inspiratory elastic work of breathing (Ie). Stippling represents the expiratory resistive work of breathing (Er). Vertical hatching represents the expiratory elastic work of breathing (Ee). Cl, dynamic lung compliance; Ccw, chest wall compliance. Upward arrow represents inspiration and downward arrow represents expiration. Small open circles represent zero flow points.

Work of Breathing vs. Relative Submaximal Minute Ventilation: The total WOB plotted

against percentages of maximal minute ventilation is shown in figure 2.4. Men had a

significantly higher total WOB for any given percentage of maximal ventilation compared with

women. The total WOB was 120, 60 and 50% higher in men at 25, 50 and 75% of maximal

minute ventilations, respectively. While each component of the WOB was higher in men for a

given percentage of minute ventilation, the largest (and statistically significant) differences were

seen with the inspiratory elastic WOB (data not shown). The absolute ventilations in men versus

women corresponding to 25, 50 and 75 % of maximal ventilations were: 48.4 ± 6.5 vs. 32.8 ± 7.0

$l \cdot min^{-1}$, 90.3 ± 14.7 vs. 62.9 ± 12.2 $l \cdot min^{-1}$, and 134.9 ± 22.1 vs. 94.9 ± 18.4 $l \cdot min^{-1}$, respectively.

Thus, the absolute ventilations were, on average, 31% higher in men when comparing sexes at

the aforementioned relative minute ventilations. The tidal volumes were 75, 37 and 36% higher

in men at 25, 50 and 75% of maximal minute ventilations ($P < 0.05$), respectively with little to

no difference in breathing frequency.
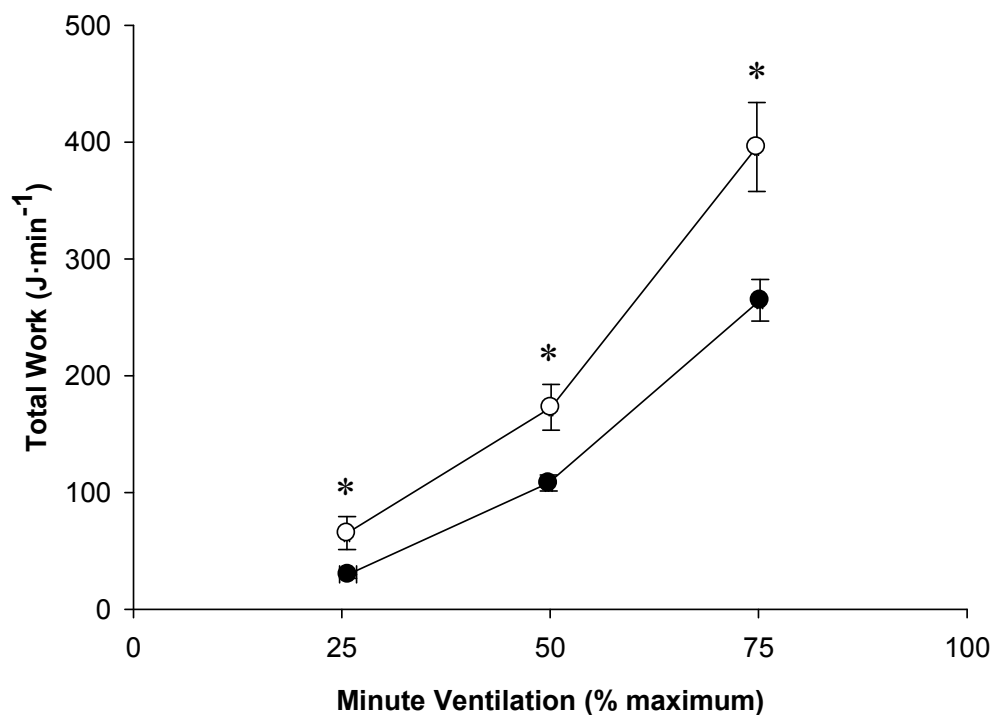
Figure 2.4: Total work of breathing versus relative percentages of maximal minute ventilation
in men (○) and women (●). Values are mean ± SE. * Significantly different between groups
($P < 0.05$).

Work of Breathing vs. External Muscular Work: Figure 2.5 shows the total WOB (panel A), inspiratory resistive WOB (panel B) and expiratory resistive WOB (panel C) versus work rate (corrected for mass) in men and women. Panel A demonstrates that the total WOB was significantly higher in women at approximately 3.5 $W \cdot kg^{-1}$ (239 ± 31 vs. 173 ± 12 $J \cdot min^{-1}$, $P <$ 0.05) and 4.0 $W \cdot kg^{-1}$ (387 ± 53 vs. 243 ± 36 $J \cdot min^{-1}$, $P < 0.05$). The inspiratory resistive WOB was higher in women at approximately 3.0 $W \cdot kg^{-1}$ (56 ± 8 vs. 41 ± 4 $J \cdot min^{-1}$, $P = 0.05$), 3.5 $W \cdot kg^{-1}$ (93 ± 15 vs. 56 ± 5 $J \cdot min^{-1}$, $P < 0.05$) and 4.0 $W \cdot kg^{-1}$ (162 ± 24 vs. 81 ± 2 $J \cdot min^{-1}$, $P <$ 0.05). The expiratory resistive WOB was higher in women at approximately 3.5 $W \cdot kg^{-1}$ ($44 \pm$ 11 vs. 20 ± 3 $J \cdot min^{-1}$, $P < 0.05$) and 4.0 $W \cdot kg^{-1}$ (67 ± 16 vs. 35 ± 8 $J \cdot min^{-1}$, $P = 0.05$). There were no significant differences in the inspiratory or expiratory elastic components at any work rate.
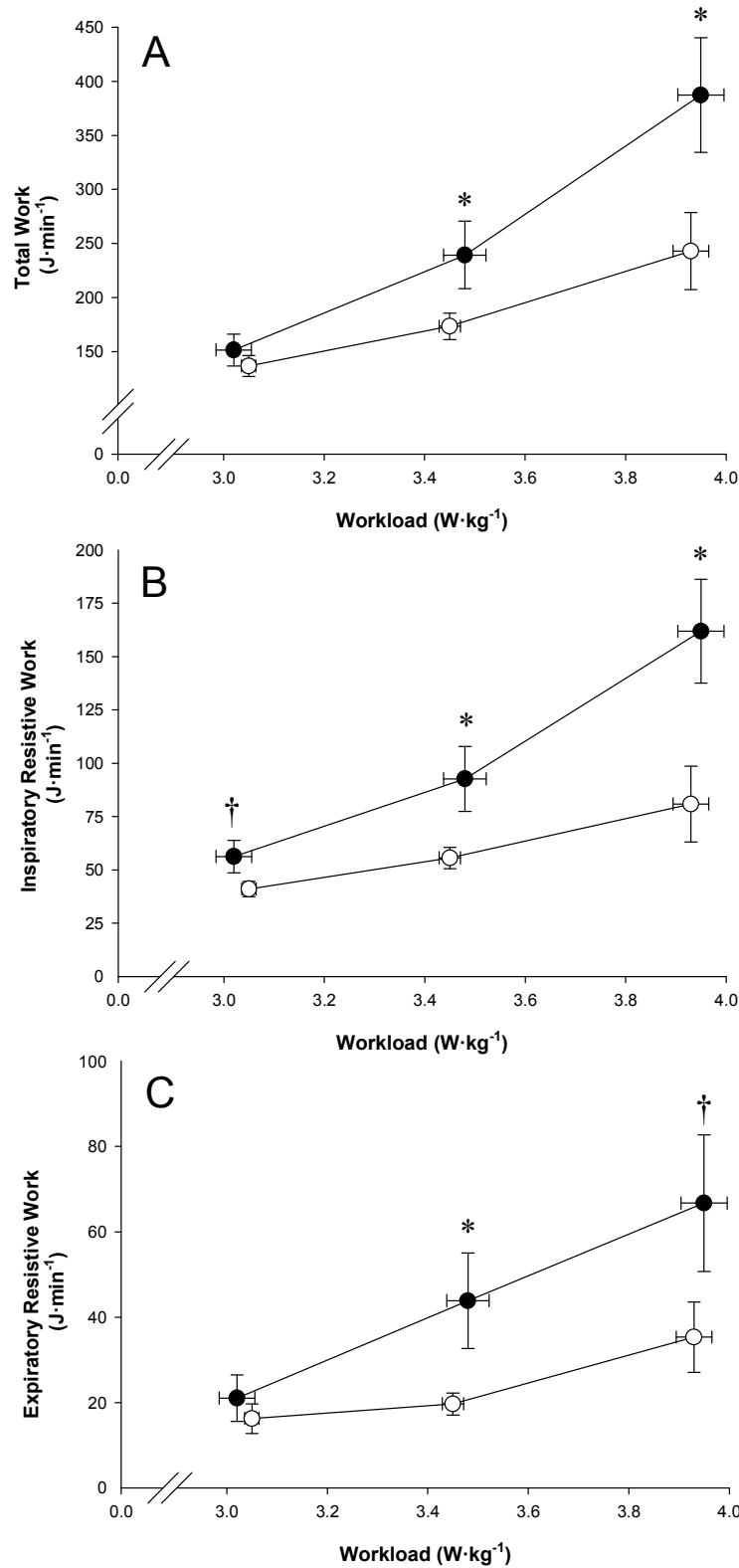
Figure 2.5: Total work (A), inspiratory resistive work (B) and expiratory resistive work of breathing (C) versus work rate in men (○) and women (●). Values are mean ± SE.
* Significantly different between groups ($P < 0.05$); † $P = 0.05$.

Breathing Pattern: Figure 2.6 shows the breathing frequency (panel A) and tidal volume (panel B) response to exercise. It can be seen that men achieved a maximal minute ventilation that was considerably higher than women (180.2 ± 28.7 vs. 126.2 ± 24.2 l·min^{-1}, respectively). Generally, women breathed with a significantly higher breathing frequency and lower tidal volume to achieve the same absolute minute ventilation as men. Breathing frequency was significantly different between sexes at 75 and 100 l·min^{-1} while tidal volume was attenuated in women at all ventilations above 50 l·min^{-1}. Figure 2.7 shows the effect of tidal volume (panel A) and breathing frequency (panel B) on the inspiratory elastic WOB at the 4 venitlatory points (i.e., 50, 75, 100 l·min^{-1} and maximal ventilation). For any given tidal volume, the inspiratory elastic WOB is considerably higher in women. However, the inspiratory elastic WOB is lower in women for any given breathing frequency. While these are physiologically significant observations, specific statistical procedures could not be performed on the data presented in Figure 2.7.
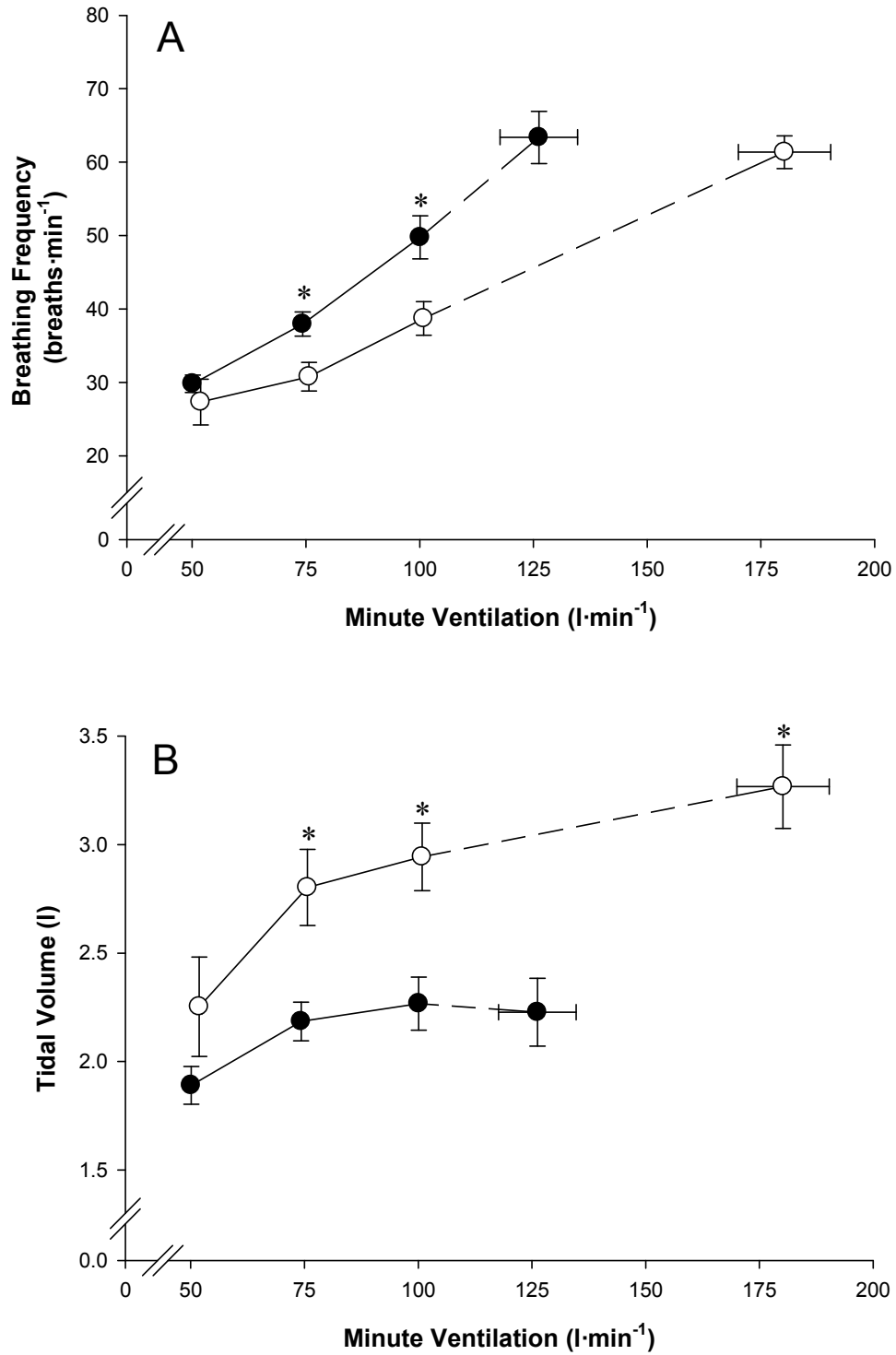
Figure 2.6: Breathing frequency (A) and tidal volume (B) versus minute ventilation in men (○) and women (●). The last data point represents maximal minute ventilation. Values are mean ± SE. * Significantly different between groups ($P < 0.05$).
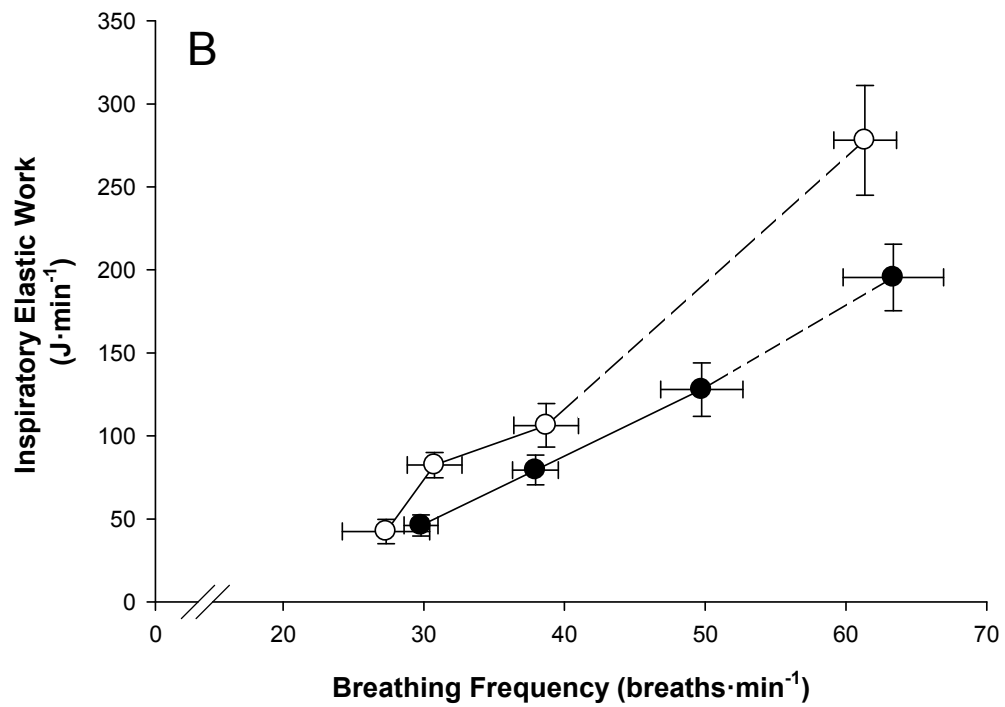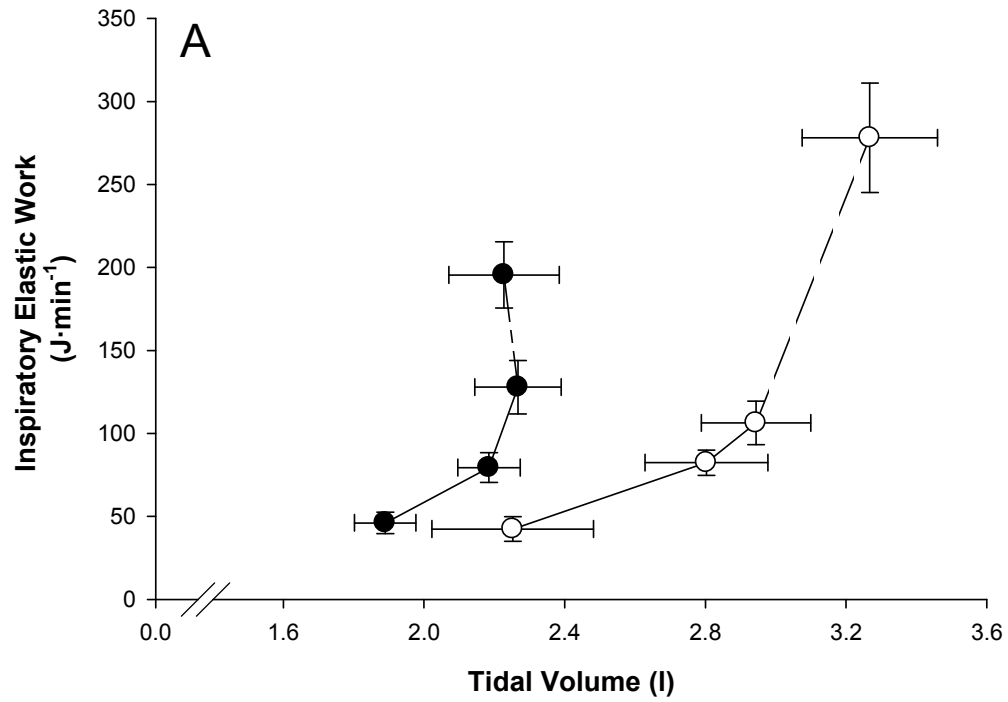
Figure 2.7: Inspiratory elastic work of breathing versus tidal volume (A) and breathing frequency (B) at the four ventilatory points (i.e., 50, 75, 100 l·min^{-1} and maximal ventilation) in men (○) and women (●). Values are mean ± SE.

Lung Size vs. Work of Breathing: Figure 2.8 summarizes the relationships between different components of the WOB and FVC with all subjects pooled together. The WOB values shown in this figure are from a minute ventilation corresponding to 100 l·min^{-1}. We chose to report data at 100 l·min^{-1} for the regression analysis for 3 reasons. Firstly, we wanted to report data at the highest range of absolute ventilations when the work and metabolic cost of breathing are highest. Secondly, this ventilation tended to show the largest sex-based difference in the total WOB and inspiratory resistive WOB. Finally, all subjects achieved 100 l·min^{-1} of ventilation during exercise. FVC was used as a surrogate for total lung capacity (less residual volume) in order to determine the relationship between lung size and the WOB. FVC was significantly and linearly related to the total WOB, the inspiratory resistive WOB and the expiratory resistive WOB. When partitioned into individual groups, the correlation coefficients relating FVC to the total WOB, the inspiratory resistive WOB and expiratory resistive WOB in women was 0.92 ($P = 0.001$), 0.78 ($P = 0.02$) and 0.73 ($P = 0.04$) whereas the men were 0.46 ($P = 0.25$), 0.27 ($P = 0.52$) and 0.75 ($P = 0.03$), respectively.
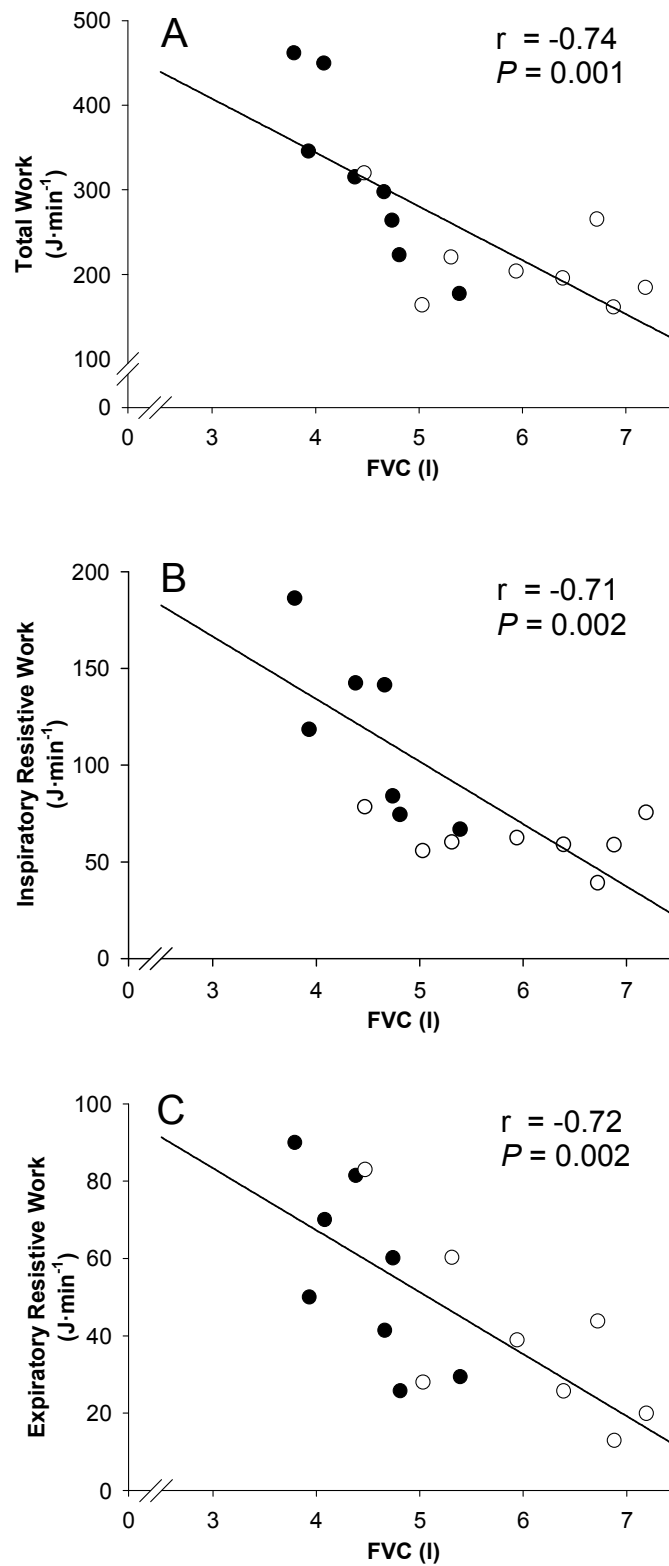
Figure 2.8: Regression analysis of the total work (A), inspiratory resistive work (B) and expiratory resistive work (C) versus forced vital capacity (FVC) in men (○) and women (●). Work of breathing values are obtained at a minute ventilation of 100 l·min^{-1}.

Flow vs. Work of Breathing: The inspiratory resistive WOB was plotted against the

corresponding average inspiratory flow throughout all exercise intensities in each individual

subject as shown in Figure 2.9 (panel A). All of the raw data points from panel A were fitted

with a 2nd order polynomial (mean r^2 for all subjects $= 0.99 \pm 0.01$) in order to produce a mean

curve for all men and women as shown in panel B. Panels A and B show that the inspiratory

resistive WOB was higher in women for any given flow rate above ~ 2 l·s^{-1} and the magnitude of

this difference increased disproportionally in women with increasing flow. Figure 2.9B also

shows the response of an individual female subject with FVC and peak expiratory flows that

were 134 and 148% above predicted values, respectively.
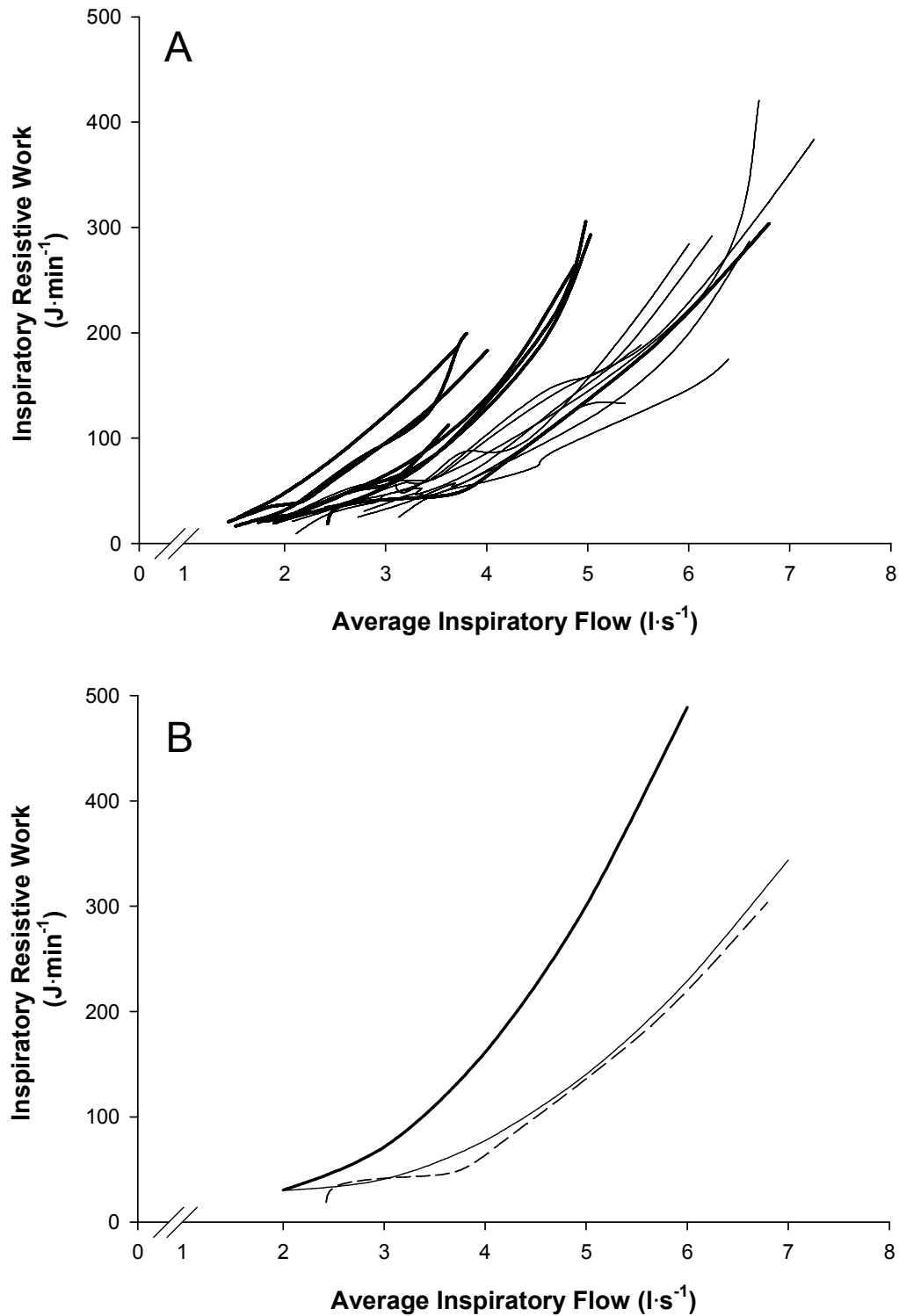
Figure 2.9: Inspiratory resistive work of breathing versus average inspiratory flow in individual male (thin lines) and female (thick lines) subjects (A). Panel B shows the mean inspiratory resistive work of breathing versus average inspiratory flow in women (thick line) and men (thin line) and an individual female subject (dashed line) with larger than average FVC and peak expiratory flow values.

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DISCUSSION

Our present understanding of the WOB during exercise is primarily based on studies conducted in males but a recent study by our group demonstrated significant differences in the total WOB between men and women for a given absolute minute ventilation (Guenette *et al.*, 2007). The present study adds to the previous literature by systematically measuring the elastic and resistive WOB in exercising women. The novel findings from this study are four-fold. First, the inspiratory resistive WOB was higher in women for any given absolute minute ventilation while the expiratory resistive WOB was higher in women at only 75 $l \cdot min^{-1}$. There were no sex differences in the inspiratory or expiratory elastic WOB across any absolute minute ventilation. However, the total WOB was actually higher in men when compared across relative percentages of maximal ventilations, due to their higher absolute tidal volumes and thus higher minute ventilations. Second, the total WOB and the inspiratory and expiratory resistive WOB were higher in women when performing the same relative external muscular work. Third, the WOB was inversely related to lung size and presumably airway size. Lastly, the inspiratory resistive WOB was considerably higher for a given level of inspiratory flow compared with men, demonstrating the importance of airway size in determining the mechanical cost of breathing. We interpret our findings to mean that the higher total WOB observed in exercising women at absolute ventilations is due to a higher resistive WOB which can be attributed to relatively smaller lungs and airways.

Resistive Work of Breathing vs. Minute Ventilation: In our previous work (Guenette *et al.*, 2007) we plotted the total WOB against a range of ventilatory rates and fit the data points to the following equation as originally described by others (Otis *et al.*, 1950; Margaria *et al.*, 1960):

$$\text{WOB} = a\dot{V}_E{}^3 + b\dot{V}_E{}^2$$

The term $b\dot{V}_E{}^2$ describes the mechanical work done in overcoming the viscous resistance offered by the lung tissues to deformation and by the respiratory tract to the laminar flow of air. The term $a\dot{V}_E{}^3$ represents the work done in overcoming the resistance to turbulent flow. We found that the constant a was significantly higher in women meaning that the higher total WOB in women is associated with the additional resistive work due to turbulent airflow. While this is an instructive analysis it does not permit a quantitative measure of the individual factors that make up the total WOB at specific time or physiological points. By using a more extensive approach we have now partitioned the total WOB into its individual components at specific values of minute ventilation and at standardized work rates. During progressive exercise we found that both inspiratory and expiratory resistive work were significantly higher in women over a range of ventilatory rates (Figure 2.2B and C). Interestingly, we observed significant differences in inspiratory resistive work at low levels of minute ventilation (50 $l \cdot min^{-1}$) and the magnitude of difference increased as ventilation increased up to 100 $l \cdot min^{-1}$. Figure 2.3 provides a compelling example of the high pressures that are needed in a female subject in order to achieve the same minute ventilation as a male subject. It is important to note that the male and female subject shown in Figure 2.3 have been matched for breathing frequency, tidal volume, age and body mass. Despite the fact that both subjects are breathing at the same volume and rate, the inspiratory and expiratory resistive work components are considerably higher in the female subject.

Elastic Work of Breathing vs. Minute Ventilation: The elastic work required to increase and decrease the volume of the lung is related to the elastic forces that develop in the tissues of the lung and chest wall. Unique to this study, we found that there were no sex differences in the

elastic WOB at any absolute ventilation. However, with further examination, it can be seen in Figure 2.7 (panel A) that the inspiratory elastic WOB is substantially higher in women for a given tidal volume. This can be attributed to the fact that women are breathing at a higher percentage of their total lung capacity for a given level of ventilation which reduces the compliance of the lungs. Perhaps more important is the observation that women adopt a higher breathing frequency for a given minute ventilation which acts to reduce the inspiratory elastic WOB (Figure 2.7B). This type of breathing pattern comes at the expense of an increased resistive WOB. This is an important observation if one is to consider how breathing patterns are regulated in humans in an effort to minimize the total WOB. The "principle of minimum effort" was first used to describe that for a given alveolar ventilation there is a breathing frequency that is optimal (Otis *et al.*, 1950). This pattern is adopted because if the breathing frequency is too low then large amounts of elastic work are required whereas if the breathing frequency is too high then respiratory muscle work is expended to ventilate dead space (i.e., wasted ventilation). During exercise the diaphragm generates most of the inspiratory driving force and appears to remain within the favourable part of its length-tension curve (Grimby *et al.*, 1976). This suggests that under spontaneously breathing conditions, the diaphragm tension or O_2 cost is what is being minimized during exercise. Based on the present study, it appears that women use a higher breathing frequency to minimize the elastic WOB which comes at the expense of a higher resistive WOB.

Total Work of Breathing vs. Minute Ventilation: The total WOB was higher in women at any absolute ventilation comparison above 50 l·min^{-1} which is consistent with our previous findings using a different analysis technique (Guenette *et al.*, 2007). In our previous work we also compared the total WOB between men and women at different percentages of $\dot{V}_{O_2}$max and found that the total WOB was modestly higher in men at $\dot{V}_{O_2}$max but there was no statistically

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significant difference (Guenette *et al.*, 2007). However, in the present study we have shown that the total WOB is significantly higher in men when compared at maximal ventilations (Figure 2.2) and also at submaximal relative percentages of maximal ventilation (Figure 2.4). We attribute this discrepant finding to the fact that the Modified Campbell Diagram technique takes into account the compliance of the chest wall which allows us to calculate the additional part of the inspiratory elastic WOB which extends beyond the area directly within the pressure-volume loop (see Figure 2.1). Indeed, it can be seen in Figure 2.2 that the only component to approach statistical significance at maximal ventilations was the inspiratory elastic WOB ($P = 0.05$). The primary driving force for the higher total WOB at submaximal relative ventilations was also the inspiratory elastic WOB (data not shown). This is due to the fact that for a given relative percentage of maximal ventilation, the tidal volume is considerably higher in men with little to no difference in breathing frequency. This will substantially increase the inspiratory elastic WOB and thus the total WOB when comparisons are made at relative intensities. We have purposely limited the majority of our analysis and interpretation in this study and in our previous work (Guenette *et al.*, 2007) to absolute ventilations in order to determine if the mechanical cost of moving a given amount of air in and out of the lungs is different between sexes. Examining the mechanics of breathing between sexes at relative ventilations is a difficult comparison because men are utilizing a much higher tidal volume and thus have higher minute ventilations than women. For example, the men in this study were breathing 54 l·min^{-1} higher than women at maximal exercise. Despite the fact that maximal ventilations were 43% larger in our male subjects, it is interesting to note that there were no significant differences in the resistive WOB components. This lends further support to the finding that women have a substantially higher resistive WOB than men.

Work of Breathing vs. External Muscular Work: Figure 2.5 shows the WOB required to perform the same relative external work on the cycle ergometer. Rather than using absolute work rate (i.e., power) in Watts, we have normalized the work rate by expressing it in Watts per kg of body mass which provides a physiologically relevant comparison because it minimizes the potential confounding effect of body size differences. Moreover, it allows us to compare the physiological cost of breathing betweens sexes for a given standardized external work load. Even when normalized for mass, the total WOB is higher at 3.5 and 4.0 $W \cdot kg^{-1}$ with the inspiratory and expiratory resistive WOB components accounting for the vast majority of this difference (Figure 2.5). At 3.5 and 4.0 $W \cdot kg^{-1}$, the inspiratory resistive WOB was 67 and 100% higher in women while the expiratory resistive WOB was 123 and 89% higher in women, respectively. It is important to note that these comparisons do not take into account lean body mass since body composition was not measured in these subjects. It has been suggested that sex differences are minimized or completely abolished in laboratory-based experiments when comparing sexes for a given power to lean body mass ratio (Stefani, 2006). Although all subjects were lean endurance athletes of similar training status, it would be expected that the women would still have a higher percentage of body fat and therefore less muscle mass. Therefore, it is important to acknowledge that this interpretation has its limitations because the female participants are still working at a slightly higher percentage of their maximum output relative to their male counterparts. This will certainly account for some of the differences observed in our WOB values. However, we cannot directly assess the impact of this limitation in our study without a measurement of lean body mass and therefore do not attempt to overstate these findings.

Sex vs. Size Differences: We observed statistically significant associations between the resistive WOB at 100 $l \cdot min^{-1}$ and FVC (see Figure 2.8). As would be expected, women had lower FVC

values than men, which were inversely related to a higher resistive WOB. Therefore, those with the smallest lungs and presumably the smallest airways had the highest resistive WOB. We do not have a direct measurement of airway size in our subjects but similar correlation coefficients were also observed when relating the resistive WOB components against peak expiratory flows which may serve as a crude surrogate for airway size. We are cognizant of the limits of correlative evidence and therefore do not attempt to overstate these findings. However, in an effort to provide a more mechanistic understanding of the higher resistive WOB in women, particularly on inspiration, we performed additional analyses as shown in Figure 2.9. In this analysis, panel A shows the inspiratory resistive WOB for a given level of inspiratory flow in individual subjects while panel B represents the group average. This data shows that for a given level of flow, the resistive WOB is higher in women and the magnitude of this difference increases disproportionally with increasing flow. Panel B includes one female subject superimposed with the mean curves. This subject had unusually large lungs and peak expiratory flows (> 130% predicted). In fact, her FVC and peak expiratory flows were relatively close to the group mean values for men. Interestingly, her inspiratory resistive WOB response for a given level of flow was nearly identical to the average curve for the male subjects. These observations in conjunction with correlative evidence points to an anatomical basis (i.e., smaller lungs and airways) for the WOB differences we observed during exercise. Additional physiological and performance based consequences of these anatomical differences in lung and airway size have been reviewed elsewhere (Sheel & Guenette, 2008).

Our sex-based comparisons were made between men and women of significantly different statures. It could be argued that our findings simply reflect size differences rather than a true male-female difference in lung and airway size. However, there is reason to suggest that our findings would be similar between men and women of comparable sizes (i.e., men and

women matched for total lung capacity). We make this claim based on two lines of anatomical evidence. First, in healthy young men and women matched for total lung capacity, women have significantly smaller tracheal areas (2.79 vs 1.99 cm^2) as assessed by acoustic reflectance (Martin *et al.*, 1987). As such, the reduced female tracheal area would result in a higher WOB for a given level of minute ventilation. Airflow is determined, in part, by Poiseuille's Law and the factors governing it are internal diameter, length, gas viscosity and airflow pressure where radius is raised to the fourth power. As such, even a small difference in airway radius is magnified and would have an effect on airflow resistance and the accompanying WOB. Second, the relationship between airway size (estimated from maximal expiratory flow ÷ static recoil pressure at 50% vital capacity) and lung size (vital capacity) shows that adult men have airways that are 17% larger than those of women (Mead, 1980). This has been termed "dysanapsis" to reflect the dissociation between airway size and lung parenchymal size (Green *et al.*, 1974). Given the brief summary presented above, coupled with the findings of the present study, it appears that the higher resistive WOB seen in women is due to inherently smaller airways.

Methodological Considerations: Our measures of the work done by the respiratory muscles do not take into account the distorting forces of the chest wall observed at high levels of minute ventilation. Volume displacement of the rib cage and abdomen can be independent of one another (Roussos & Campbell, 1986). Phrased differently this means that all of the respiratory muscles do not necessarily shorten during inspiration nor do all of the muscles of expiration shorten during expiration. We recognize this as a critique of the modified Campbell approach and that our measure of the WOB may be underestimates. However, it is unlikely that this systematic underestimation applied to all subjects equally would have had any substantive effect on our overall conclusion that women have a higher WOB during exercise owing to a greater resistive WOB. This is supported by optoelectronic plethysmography measures which suggest

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that men and women utilize muscles of the ribcage compartment and those of the abdomen to the same extent (Vogiatzis *et al.*, 2005).

The compliance of the chest wall is required to determine the various WOB components using the modified Campbell Diagram method. It is typically very difficult to reliably measure the compliance of the chest wall because naive subjects have a difficult time completely relaxing their respiratory muscles. Therefore, we based our chest wall compliance values on previously published data taking into account both age and sex (Estenne *et al.*, 1985) as done by others (Yan *et al.*, 1997; Sliwinski *et al.*, 1998; Butcher *et al.*, 2006; Eves *et al.*, 2006). There are limitations with this approach that warrant discussion. For example, the chest wall compliance values we used were obtained in healthy volunteers with normal static lung volumes and $FEV_{1.0}$ values and it is assumed that the values were measured in untrained individuals. Therefore, we are making the assumption that the compliance of the chest wall is similar between trained and untrained subjects. To our knowledge, there are no studies that have studied the effect of fitness on the compliance of the chest wall. While there are inherent limitations in using "normative" data and applying it to elite athletes, we do not think that this had an effect on our main conclusions regarding sex differences in the WOB. We base this assumption on several factors. Firstly, according to Estenne *et al.* (1985), there are no sex differences in the compliance of the chest wall (at least in untrained individuals). Our men and women were of similar relative fitness levels so it is reasonable to assume that this lack of a sex difference should persist along the fitness continuum and any potential errors in our chest wall compliance values would likely be a systematic error across all subjects. Secondly, the vital capacity values in our men (6.0 ± 1.0 l) and women (4.5 ± 0.5 l) were nearly identical to the age-matched men (6.0 ± 0.9 l) and women (4.3 ± 0.4 l) from whom we derived are chest wall compliance values. Finally, we have calculated that even a ± 10% change in the compliance of the chest wall would only affect the

elastic components by approximately ± 3-5%, the inspiratory resistive WOB by less than ± 0.5% and the expiratory resistive WOB by approximately ± 1.5-3%.

The elastic WOB measurements that we and others (Yan *et al.*, 1997; Sliwinski *et al.*, 1998) have made may be underestimations because the calculations are based on tidal volume measured at the mouth which ignores gas compression (Otis, 1964). Gas compression is typically negligible in healthy individuals at rest or during exercise. However, under conditions where expiratory flow limitation is present, this could increase the magnitude of underestimation. We did observe expiratory flow limitation in many of our female and male subjects (Guenette *et al.*, 2007) during high levels of exercise but we do not believe that this had a major influence on our findings or overall interpretation of the present study, particularly at submaximal workloads. Specifically, we observed no demonstrable difference between men and women in the elastic WOB at low levels of ventilation (50 l·min^{-1}) when expiratory flow limitation is not present. This absence held true at higher levels of ventilation suggesting that any underestimation was most likely consistent across all ventilatory rates. Thus any underestimation due to gas compression likely had a negligible influence on our findings and conclusion that the elastic WOB is similar between men and women for a given level of ventilation. The current study design does not allow us to determine the direct role of expiratory flow limitation and the corresponding changes in operational lung volumes between sexes because flow limitation was typically observed at maximal to near-maximal intensities. As such, it would be difficult to isolate the effects of EFL on our WOB values in men and women because any potential differences would be masked by the fact that men have much higher tidal volumes and thus ventilations at maximal exercise. A novel study design would be required to assess sex-based differences in expiratory flow limitation and the direct corresponding effect on the WOB.

Conclusions: This study is the first to systematically assess the mechanisms of a higher WOB in women during dynamic exercise. Describing sex differences in breathing mechanics poses a significant challenge because of the inherent difficulties in comparing men and women due to known differences in body size and an incomplete understanding of the most appropriate allometric scaling factor to use. In the present study, we have made a number of unique physiological comparisons. First, comparing the WOB for a given level of ventilation allowed us to quantify the additional cost of breathing necessary to move a fixed amount of air through smaller lungs and airways in women. Second, comparing men and women at different percentages of maximal ventilations allowed us to determine the cost of breathing for a given relative intensity. Third, comparing sexes at a standardized size-corrected work rate enabled us to determine if there are differences in the WOB for a given level of external muscular work. The data from this study suggests that the higher overall WOB in women during dynamic exercise is due to a substantially greater resistive work during inspiration and expiration with no differences in the elastic WOB. However, much of these differences are reversed when comparisons are made at relative intensities. We conclude that sex-based differences in lung and airway size result in the higher work and thus O_2 cost of breathing in women during exercise for a given absolute level of ventilation or exercise intensity.

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CHAPTER III: Sex differences in exercise-induced diaphragmatic fatigue in endurance-trained athletes*

INTRODUCTION

The effects of skeletal muscle fatigue on the ability to perform muscular work has been a topic of interest to exercise and muscle physiologists for over a century. Muscle fatigue can be defined as a loss in the capacity for developing force and/or velocity resulting from muscle activity under load and which is reversible by rest (NHLBI, 1990). Studies examining sex differences in peripheral skeletal muscle fatigue have shown that women have greater relative fatigue resistance compared to their male counterparts (Maughan *et al.*, 1986; Miller *et al.*, 1993; West *et al.*, 1995; Fulco *et al.*, 1999). Commonly cited mechanisms associated with greater fatigue resistance in women include differences in muscle mass/morphology, substrate utilization and neuromuscular activation (Hicks *et al.*, 2001). The majority of studies examining sex differences in skeletal muscle fatigue have focussed on the muscles involved in moving the elbow, finger, knee, thumb, ankle, back and neck. To our knowledge, no study has systematically assessed sex differences in fatigue of the human diaphragm.

The diaphragm is embryologically, morphologically and functionally a striated skeletal muscle. However, it remains distinct from other skeletal muscles because it is under both voluntary and involuntary control and like the heart, contracts rhythmically across the entire life span. As such, the diaphragm has a primary role in breathing and in sustaining human life. Contraction of the diaphragm is important if we consider physiological conditions that require high levels of ventilation such as during strenuous exercise. However, the diaphragm is similar to other muscles in that it is susceptible to exercise-induced muscle fatigue (Johnson *et al.*, 1993; Vogiatzis *et al.*, 2008). Moreover, there are important hemodynamic and exercise performance consequences associated with fatigue of the human diaphragm as recently reviewed by Romer & Polkey (2008). While it appears that many non-respiratory skeletal muscles are more fatigue

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resistant in women, the diaphragm may be an exception due to known differences in respiratory structure and function that may predispose women to greater pulmonary limitations relative to men. We base this suggestion on four lines of evidence. First, there are a number of sex differences in respiratory structure and resting pulmonary function that may influence the ventilatory response to exercise in women. Compared to men, women have smaller lung volumes, lower maximal expiratory flow rates and a decreased capacity for lung diffusion even when corrected for age and standing height (Mead, 1980; Schwartz *et al.*, 1988; McClaran *et al.*, 1998). Second, women have smaller diameter airways even when matched for lung volume (Martin *et al.*, 1987; Sheel *et al.*, 2009). Third, these anatomical differences may explain, in part, why women have a higher work of breathing relative to men during exercise (Guenette *et al.*, 2007; Guenette *et al.*, 2009). Lastly, smaller lungs and airways may also predispose women to expiratory flow limitation during exercise (McClaran *et al.*, 1998; Guenette *et al.*, 2007). Based on the aforementioned differences, the respiratory muscles of exercising females may be placed under greater mechanical stress relative to males which may result in greater fatigue.

Previous work from our laboratory (Guenette *et al.*, 2007; Sheel & Guenette, 2008) and others (Harms, 2006) have postulated that these anatomical and functional sex differences in pulmonary physiology might make the female diaphragm more prone to fatigue. This hypothesis has not been directly tested, but is complicated by the findings of previous studies showing that women may be less susceptible to fatigue of the limb muscles (Hicks *et al.*, 2001; Hunter, 2009) and perhaps even cardiac muscle (Scott *et al.*, 2007). Therefore, the purpose of this study was to determine if there are sex differences in the magnitude of exercise-induced diaphragmatic fatigue. We hypothesized, that the magnitude of diaphragm fatigue following high-intensity cycling exercise would be greater in trained women relative to trained men.

METHODS

Subjects: Thirty eight (19 males and 19 females) healthy subjects gave informed written consent [APPENDIX I]. Thirty one subjects were competitive endurance-trained cyclists while the remaining 7 subjects were non-competitive endurance-trained individuals. "Competitive" was defined as regular participation in cycling and/or triathlon races. All of the women were tested randomly throughout the menstrual cycle. Subjects were excluded from participating if they were smokers or had a history of cardiopulmonary disease. Subjects with nasal septum deviation, esophageal ulcers, or allergies to local anaesthetics or latex were also excluded from participating. All procedures received institutional ethical approval and conformed to the *Declaration of Helsinki.*

Experimental Overview: The experiment was conducted on two separate days with a minimum of 48 h rest between each testing session. On day 1, subjects underwent basic anthropometric measures followed by pulmonary function testing, general experimental familiarization and an incremental cycle test to exhaustion to determine peak work-rate and associated variables (e.g., maximum oxygen uptake, $\dot{V}O_2max$). Day 2 served as the primary testing day, which involved the assessment of diaphragmatic fatigue in response to constant load cycling. On day 2, subjects were instrumented with diaphragmatic surface electromyography (EMG) electrodes, and balloon-tipped catheters were placed in the esophagus and stomach. They then underwent cervical magnetic stimulation (CMS) of the phrenic nerves following a 10 min period of quiet breathing. Upon completion of all baseline measures, subjects performed a self-selected warm-up on a cycle ergometer followed by steady-state cycling at 90% of peak work-rate as determined on day 1. CMS was then performed 10, 30 and 60 min after exercise.

Pulmonary Function: Forced vital capacity (FVC), forced expiratory volume in 1 second (FEV$_{1.0}$), FEV$_{1.0}$/FVC, peak expiratory flow (PEF) and forced expiratory flow between 25 and 75% of FVC (FEF$_{25-75\%}$) were measured using a portable spirometer (Spirolab II, Medical International Research, Vancouver, BC) according to ATS/ERS guidelines (2002). Actual values were compared to predicted normal values. Subjects with an FEV$_{1.0}$/FVC < 80% of predicted were excluded from participating in the investigation.

Incremental Exercise Test: Subjects performed a 10 min warm-up at a self-selected work-rate on an electromagnetically-braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands). Males and females started the test at 200 W and 100 W, respectively, with the work-rate increasing in a stepwise fashion by 30 W every 3 min until the subjects could no longer maintain cadence ≥ 60 rpm despite verbal encouragement. Peak work-rate was calculated as the sum of the final completed exercise stage and an extrapolated work-rate depending on the time spent in the final non-completed stage. To determine $\dot{V}O_2$max, subjects wore a nose clip and breathed through a mouthpiece connected to a non-rebreathing valve (model 2700B, Hans-Rudolph, Kansas City, MO). Mixed expired gases were measured using calibrated CO_2 and O_2 analyzers (Model CD-3A and Model S-3-A/I respectively, Applied Electrochemistry, Pittsburgh, PA) measured at a port located in a mixing chamber, while inspiratory flow was measured using a calibrated pneumotachograph (model 3813, Hans Rudolph, Kansas City, MO). Heart rate was recorded every 30 s using a commercially available heart rate monitor (Polar Electro, Kempele, Finland).

Pressure Measurements: Mouth pressure (P$_m$) was monitored at a port located in the mouthpiece and connected to a piezoelectric pressure transducer (Raytech Instruments,

Vancouver, BC). Esophageal pressure (P_{es}) and gastric pressure (P_{ga}) were measured using balloon-tipped catheters (no. 47-9005, Ackrad Laboratory, Cranford, NJ) attached to piezoelectric pressure transducers (Raytech Instruments Inc). The transducers were calibrated across the physiological range using a digital manometer (2021P, Digitron, Torquay England). Viscous lidocaine (2%) was applied to the nasal and pharyngeal passages to minimize discomfort during catheter placement. Both catheters were first inserted into the stomach. The esophageal catheter was withdrawn until a negative pressure deflection was observed during inspiration and then withdrawn an additional 10 cm to ensure that it was completely within the esophagus. After the balloons were inserted, all air was evacuated by subjects performing a Valsalva maneuver. One and two milliliters of air were injected into the esophageal and gastric balloons, respectively. Validity of the esophageal balloon position was verified using the occlusion technique (Baydur *et al.*, 1982). Trans-diaphragmatic pressure (P_{di}) was obtained online as the difference between P_{es} and P_{ga}.

Diaphragm Fatigue: The CMS technique was used to assess diaphragmatic fatigue following exercise (Similowski *et al.*, 1989). Both phrenic nerves were stimulated using a 90 mm circular coil attached to a magnetic stimulator (Magstim 200 Mono Pulse, MagStim, Whitland, Wales). With the subject seated in a chair, their neck was flexed and the coil was placed over the cervical spine. The site of optimal stimulation was determined by positioning the coil between C_5 and C_7 until the largest P_{di} was achieved. The position of the coil was then marked with indelible ink on the subject's neck to ensure the coil was in an identical position for all subsequent stimulations. Considerable care was also taken to ensure that subjects were seated in the same position throughout the experiment with the same level of neck flexion. Since lung volume can influence twitch amplitude, all twitches were performed at end-expiratory lung volume (EELV) with the glottis closed. EELV was verified by examining end-expiratory P_{es} prior to stimulation.

Supramaximal stimulation was assessed by charging the stimulator to progressively increasing levels of its maximal power output (i.e., 50, 60, 70, 80, 85, 90, 95, and 100%). Three twitches were performed at each power output with each twitch separated by 30 s of quiet breathing to avoid twitch potentiation. A plateau in mean twitch P_{di} ($P_{di,tw}$) with increasing power output was an indication that the phrenic nerves were maximally stimulated. Surface EMG of the right and left costal diaphragm was recorded using surface electrodes (Soft-E H59P: Kendall-LTP, Chicopee, MA, USA). The electrodes were placed on the anterior axillary line on the sixth to eighth intercostal spaces and re-positioned if necessary to optimize M-wave characteristics (Glerant *et al.*, 2006). Peak-to-peak amplitudes of the M-waves were measured for every twitch. EMG signals were amplified, band-pass filtered and the analog signals were A/D converted (PowerLab/16SP model ML 795, ADI, Colorado Springs, CO) and recorded simultaneously using PowerLab data acquisition software (Chart v6.1.3, ADInstruments, Colorado Springs, CO). Pressure and EMG signals were sampled at 1kHz.

The protocol for this experiment included a series of 5 potentiated twitches that were performed at 100% of the stimulator output before (baseline) and 10, 30 and 60 min after the exercise bout. Potentiated twitches involved a maximal inspiration for ~5 sec initiated at functional residual capacity against a device which incorporated a 2mm orfice to prevent glottic clousre. The phrenic nerves were then stimulated at the end of the second tidal expiration following the maximal inspiratory effort. The subject then immediately repeated this procedure a total of 5 times (Figure 3.1). We discarded the first two twitches from the analysis because twitch amplitude was still rising. Individual twitches were rejected if there was evidence from the raw P_{es} trace of deviation from relaxed FRC or esophageal contraction. The primary outcome variable for this study was the change in $P_{di,tw}$. Additional fatigue and contractility characteristics of the diaphragm included contraction time and half relaxation time. Contraction

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time was determined as the time interval between the initiation of twitch tension and peak

tension and half relaxation time was determined as the time for P_{di} to decrease to one-half of the

peak tension (Figure 3.1). Fatigue of the diaphragm was considered present if there was a $\geq 15\%$

reduction in $P_{di,tw}$ relative to the pre-exercise baseline values as used previously by others (Kufel

et al., 2002). This conservative definition of fatigue is based upon an approximate 2-3 fold

increase in the coefficient of variation of $P_{di,tw}$ as observed in the present study.
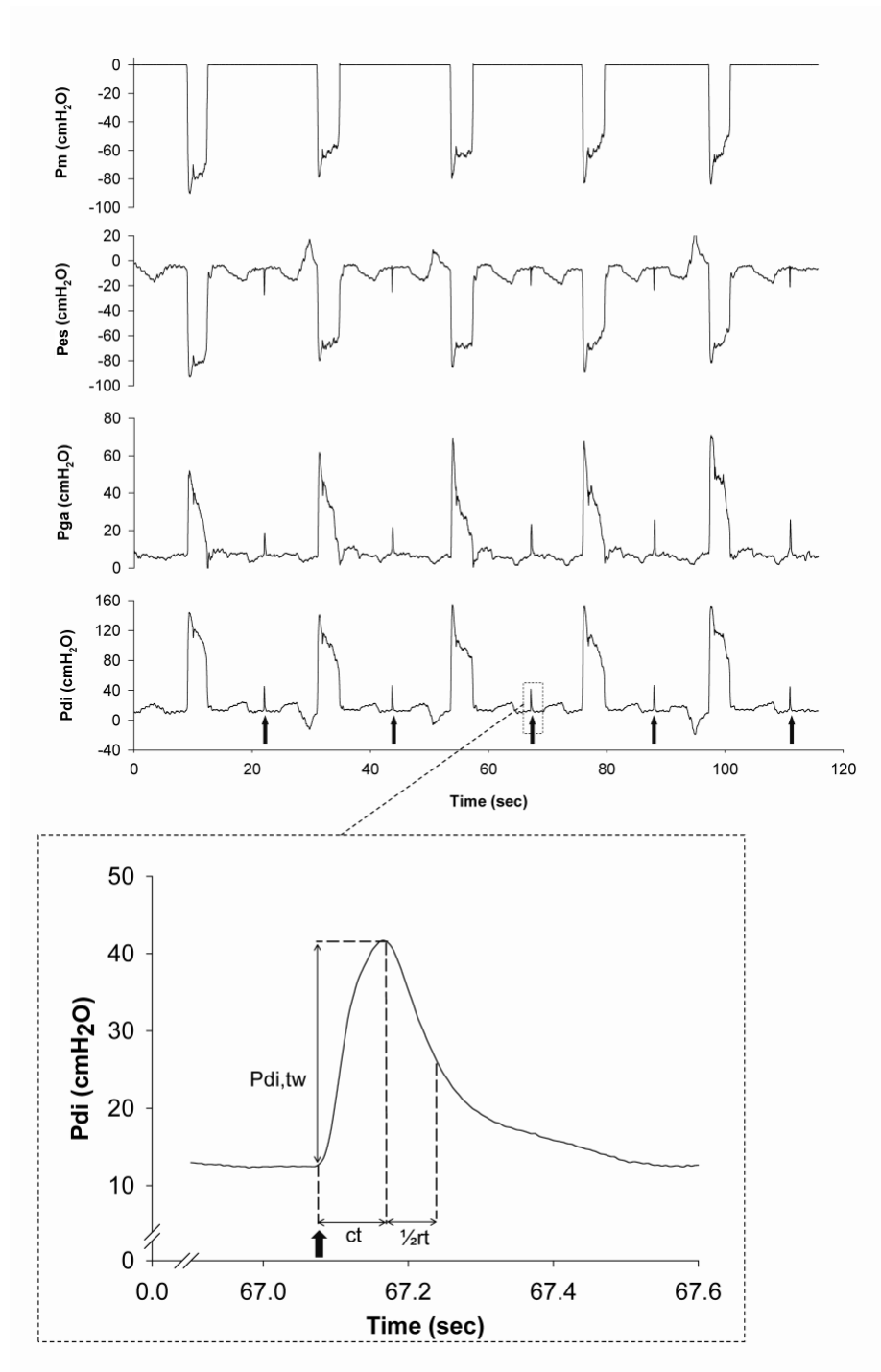
Figure 3.1: Twitch potentiation protocol. Example of the twitch potentiation protocol in an individual subject. Each of the 5 maximal inspiratory efforts is followed by a twitch performed at end-expiratory lung volume. The top 4 graphs include mouth pressure (P_m), esophageal pressure (P_{es}), gastric pressure (P_{ga}) and trans-diaphragmatic pressure (P_{di}). Bottom graph is an example of an individual trans-diaphragmatic pressure twitch ($P_{di,tw}$) using cervical magnetic stimulation to stimulate the phrenic nerves. ct, contraction time; ½rt, half-relaxation time; thick vertical arrow represents the onset cervical magnetic stimulation.

Exercise Breathing Mechanics: Subjects breathed through a mouthpiece connected to a bi-directional heated pneumotachograph (model 3813, Hans Rudolph, Kansas City, MO). Inspiratory and expiratory air flow was continuously monitored and integrated to obtain volume. Approximately 10 breaths during exercise were selected at various percentages of time to exhaustion (TTE) and ensemble averaged using customized software. The work of breathing against the lung was estimated from the area within the tidal P_{es}-volume loop with the addition of that portion of a triangle describing the work that fell outside the tidal P_{es}-volume loop representing part of the elastic work of breathing (McGregor & Becklake, 1961). The work of breathing was then multiplied by breathing frequency and converted into joules per minute. Diaphragmatic pressure-time product (PTP_{di}) and esophageal pressure-time product (PTP_{es}) were determined by integrating P_{di} and P_{es}, respectively during inspiration with respect to time and then multiplying these values by breathing frequency.

Symptom Evaluation: Ratings of perceived exertion for breathlessness and leg discomfort were measured each minute during exercise using Borg's 0-10 category ratio scale (Borg, 1982). The scale's endpoints were anchored such that '0' represented "no respiratory (or leg) discomfort" and '10' represented "the most severe respiratory (or leg) discomfort you have ever experienced or could ever imagine experiencing."

Statistical Analyses: Descriptive characteristics, pulmonary function and maximal exercise data were compared between groups using unpaired t-tests. Pre-planned comparisons were used to determine sex differences at each time point during the constant-load cycle test using unpaired t-tests with Bonferroni corrections for multiple comparisons. When 5 comparisons were made, a *P* value of < 0.01 was considered statistically significant and a *P* value of < 0.017 was considered statistically significant when 3 comparisons were made. *P* values ≤ 0.05 are also

reported throughout the manuscript. One way repeated-measures ANOVA with a Dunnet post hoc test was used to determine if there were significant differences in $P_{di,tw}$ at all submaximal stimulation intensities compared to maximal stimulation intensity (100%). Linear regression analysis was performed to determine the relationship between diaphragm fatigue and selected exercise parameters. Values are presented as mean ± S.E.M.

Subject Characteristics: Table 3.1 summarizes basic descriptive characteristics and pulmonary function data. Men and women were not different for age, but men were taller and heavier. As expected, men had larger absolute values for FVC, $FEV_{1.0}$, PEF and $FEF_{25-75\%}$ but there were no sex differences in percent predicted values for any of these parameters. Table 2 summarizes maximal exercise data for men and women on day 1. Men had a higher absolute and relative $\dot{V}O_2$max and higher peak work-rates compared with women. The larger minute ventilation in men was due exclusively to a higher tidal volume. At peak exercise, women reported lower levels of perceived exertion for both breathlessness and leg discomfort.

Table 3.1: Descriptive characteristics of the subjects

	Men	Women
Age (years)	27 ± 1	28 ± 1
Height (cm)	182 ± 2	167 ± 2*
Mass (kg)	77 ± 2	62 ± 2*
BMI (kg·m^2)	23.3 ± 0.6	22.3 ± 0.4
BSA (m^2)	1.98 ± 0.03	1.69 ± 0.03*
FVC (l)	6.0 ± 0.2	4.2 ± 0.1*
FVC (% predicted)	110 ± 3	113 ± 2
$FEV_{1.0}$ (l)	4.9 ± 0.1	3.7 ± 0.1*
$FEV_{1.0}$ (% predicted)	107 ± 2	111 ± 2
$FEV_{1.0}$/FVC (%)	81.8 ± 1.6	86.2 ± 1*
$FEV_{1.0}$/FVC (% predicted)	99 ± 2	103 ± 1
PEF (l·sec^{-1})	11.3 ± 0.3	8.1 ± 0.3*
PEF (% predicted)	111 ± 2	112 ± 4
$FEF_{25-75\%}$ (l·sec^{-1})	5.0 ± 0.3	4.4 ± 0.3*
$FEF_{25-75\%}$ (% predicted)	98 ± 5	108 ± 7

Values are mean ± S.E.M. BMI, body mass index; BSA, body surface area; FVC, forced vital capacity; $FEV_{1.0}$, forced expired volume in 1 second; PEF, peak expiratory flow; $FEF_{25-75\%}$, forced expiratory flow between 25 and 75% of FVC. * Significant difference between men and women.

Table 3.2: Maximal incremental exercise data on day 1

	Men	Women
$\dot{V}_{O_2}$ (ml·kg^{-1}·min^{-1})	64.0 ± 1.9	57.1 ± 1.5*
$\dot{V}_{O_2}$ (l·min^{-1})	4.9 ± 0.1	3.5 ± 0.1*
$\dot{V}_{CO_2}$ (l·min^{-1})	5.4 ± 0.1	3.8 ± 0.1*
RER	1.12 ± 0.01	1.10 ± 0.01
$\dot{V}_E$ (l·min^{-1})	154.7 ± 3.9	109.4 ± 3*
F_b (breaths·min^{-1})	62.0 ± 2.4	62.7 ± 2.3
V_T (l)	3.2 ± 0.1	2.2 ± 0.1*
HR (bpm)	190 ± 2	185 ± 2
Work-rate (W)	364 ± 10	269 ± 9*
Breathlessness (Borg scale)	9.1 ± 0.2	8.2 ± 0.4*
Leg discomfort (Borg scale)	9.3 ± 0.2	8.5 ± 0.3*

Values are mean ± S.E.M. $\dot{V}_{O_2}$, oxygen consumption; $\dot{V}_{CO_2}$, carbon dioxide production; RER, respiratory exchange ratio; $\dot{V}_E$, minute ventilation; F_b, breathing frequency; V_T, tidal volume; HR, heart rate. * Significant difference between men and women.

Supramaximal Stimulation: There was a proportional increase in $P_{di,tw}$ as the power output of the stimulator increased to 85-90% and then began reaching a plateau thereafter in men and women (Figure 3.2). Clear evidence of a plateau was observed in all of the female subjects and in 11 of the male subjects.
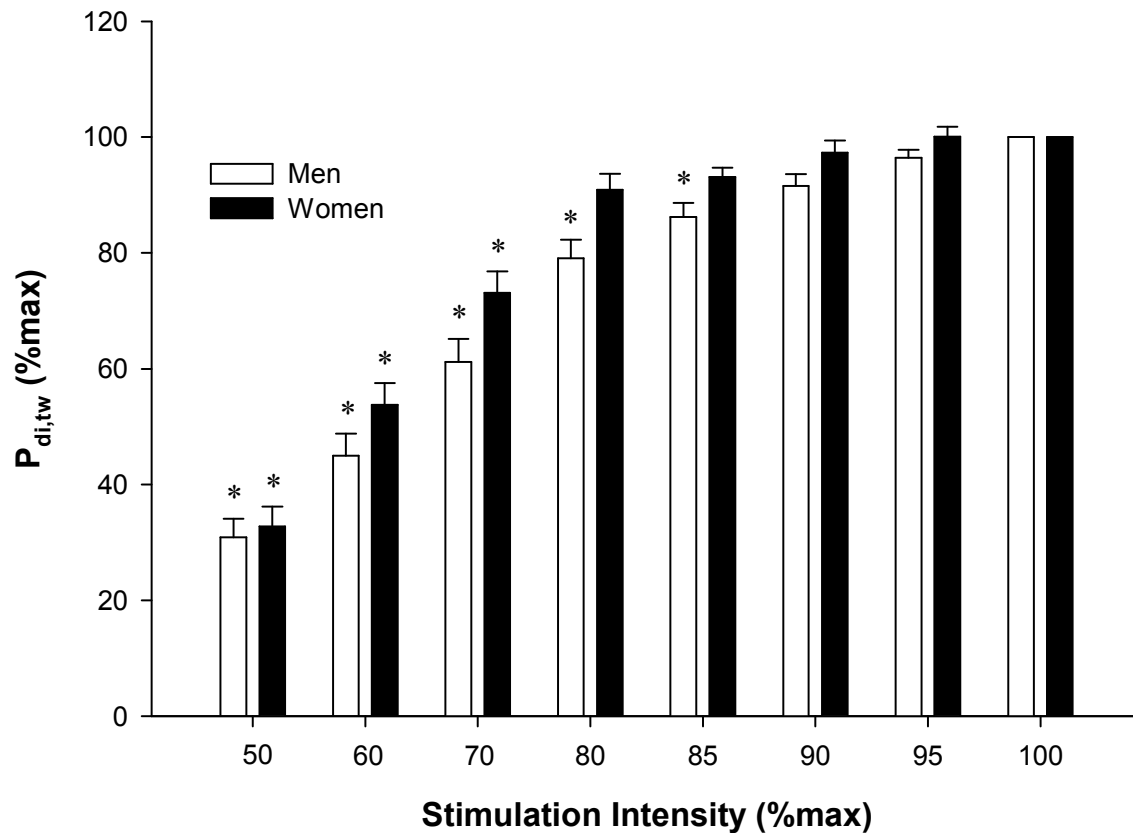
Figure 3.2: Trans-diaphragmatic pressure response to increasing cervical magnetic stimulation intensities in men and women. Values are mean ± S.E.M. * Significantly different from 100% stimulation intensity.

Diaphragm Fatigue: Diaphragm fatigue was present in 11 out of 19 males (58%) and 8 out of 19 females (42%). Figure 3.3A shows the reduction in potentiated $P_{di,tw}$ in all men and women. The percent drop in $P_{di,tw}$ at 10, 30 and 60 min following exercise in men (n = 19) was 20.4 ± 3.3, 12.0 ± 3.1 and 8.2 ± 3.2%, respectively. The drop in $P_{di,tw}$ in women (n = 19) at the same time points was 10.0 ± 2.3, 6.7 ± 2.2 and 4.5 ± 2.6% respectively. Figure 3.3B shows the reduction in

potentiated $P_{di,tw}$ in those subjects who developed diaphragm fatigue as defined by a $\geq 15\%$ reduction in $P_{di,tw}$ [for individual responses see APPENDIX IV]. The percent drop in $P_{di,tw}$ at 10, 30 and 60 min following exercise in these men were 30.6 ± 2.3, 20.7 ± 3.2 and $13.3 \pm 4.5\%$, respectively. The drop in $P_{di,tw}$ in the women that developed fatigue at the same time points was 21.0 ± 2.1, 11.6 ± 2.9 and $9.7 \pm 4.2\%$ respectively. Men consistently had greater reductions in $P_{di,tw}$ relative to women with the largest differences seen at 10 min post-exercise. The data presented in Figure 3.3 points to a female diaphragm that has greater resistance to exercise-induced diaphragmatic fatigue. Peak-to-peak M-wave amplitude for the right and left side of the diaphragm at 10, 30 and 60 min post-exercise were not different relative to baseline values in men or women (see also Figure 3.4). Mean coefficient of variation in $P_{di,tw}$ at baseline and 10, 30 and 60 min post-exercise was 5.5, 5.9, 5.5 and 5.5% in men ($P > 0.05$) and 6.7, 5.8, 5.9 and 6.5% in women, respectively ($P > 0.05$) with no differences between sexes. There were no significant sex differences for diaphragmatic contraction time or half-relaxation time at any time point following exercise.
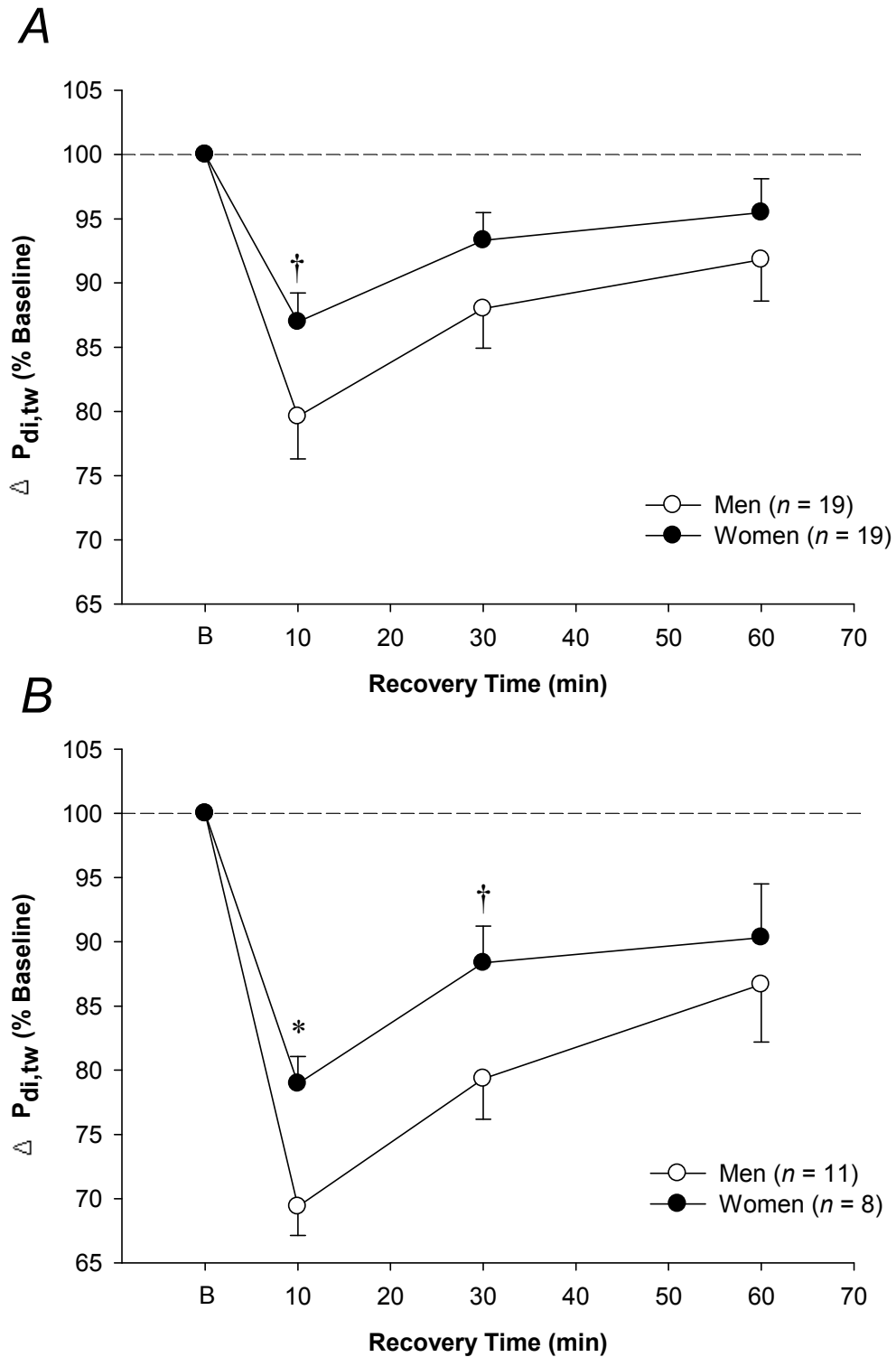
Figure 3.3: Response of twitch trans-diaphragmatic pressure ($P_{di,tw}$) during recovery in men and women. Values are mean ± S.E.M. Top graph shows all subjects. Bottom graph shows only those subjects that developed diaphragm fatigue (defined as a drop in $P_{di,tw}$ of ≥ 15%. B, baseline. * Significant difference between men and women ($P < 0.01$). † ($P < 0.05$)
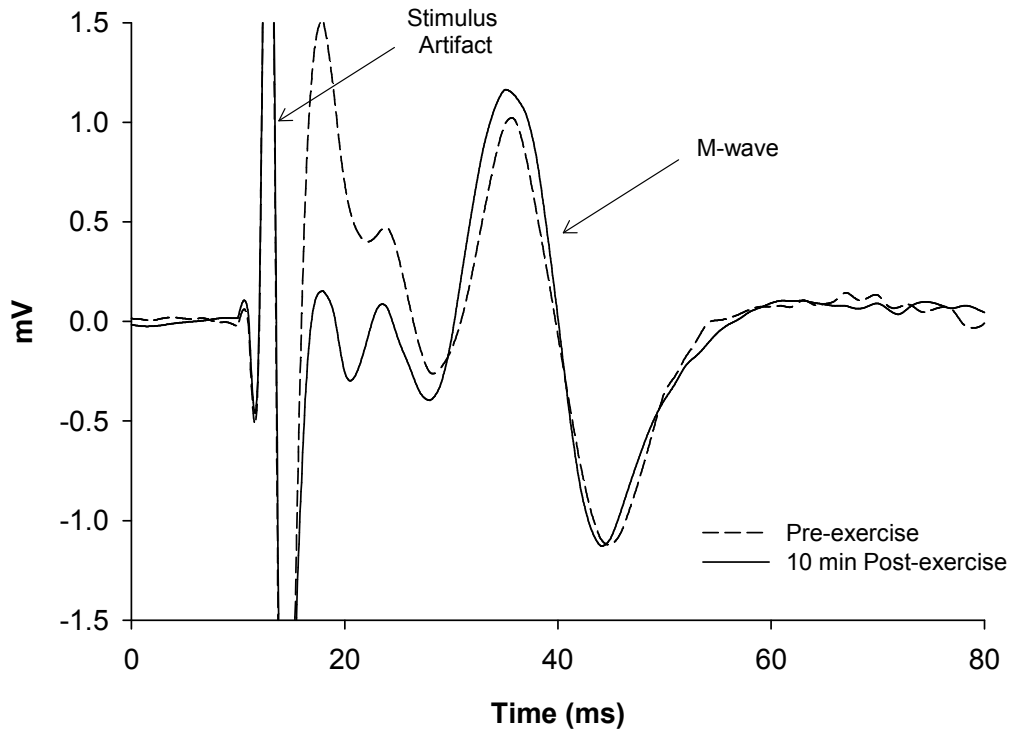
Figure 3.4: Example M-wave in an individual female subject at baseline and 10 min after exercise.

Time to Exhaustion Test: The work-rate for the time to exhaustion (TTE) test in men and women was 327 ± 9 W and 242 ± 8 W, respectively which corresponded to 90% of their peak work-rate as determined on day 1. Men cycled for 13.7 ± 0.9 min (range: 9.3-22.9 min) whereas women cycled for 11.4 ± 0.7 min (range: 7.8-18.0 min) ($P = 0.051$). For any given time (expressed as a percentage of TTE), women consistently cycled at a higher percentage of their maximum HR. During the latter half of the test, men averaged 95% and women averaged 97% of their maximum HR ($P < 0.01$).

Ratings of Perceived Exertion: Figure 3.5 shows the ratings of perceived leg discomfort and breathlessness for men and women during the TTE test. There were no significant differences in

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perceived exertion, despite women reporting lower levels of breathlessness and leg discomfort at maximal exercise during the incremental test on day 1 (Table 3.2).
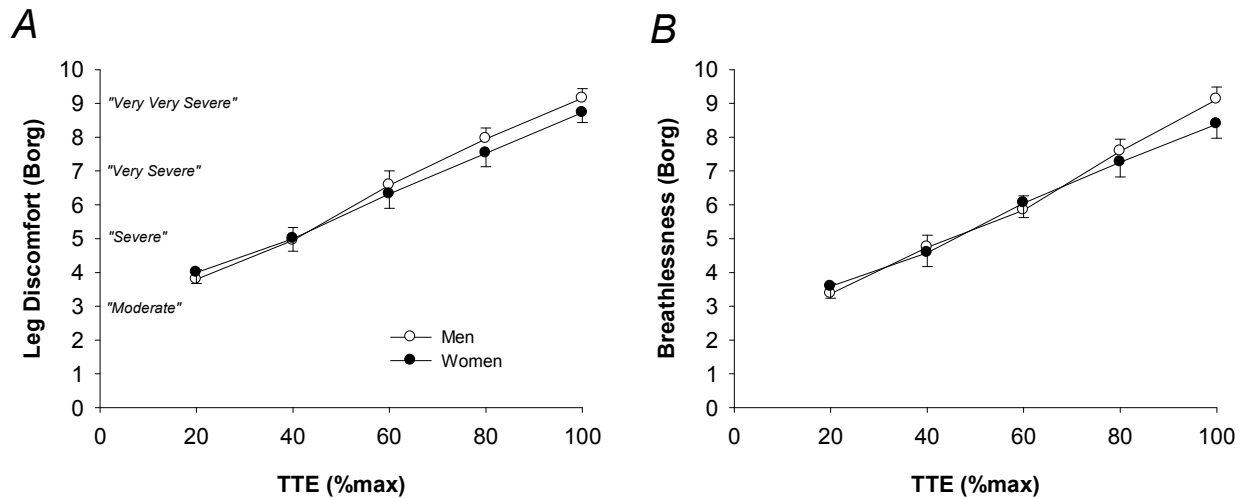
Figure 3.5: Perception of leg discomfort and breathlessness in men and women. Values are mean ± S.E.M. TTE, time to exhaustion. No differences in leg discomfort or breathlessness were detected at any time point between men and women.

Ventilation and Breathing Mechanics During Exercise: Breathing frequency, tidal volume, minute ventilation, minute ventilation expressed as a percentage of maximum minute ventilation (as determined on day 1) and the work of breathing are shown in Figure 3.6. Women relied on a higher breathing frequency during the first 60% of the exercise test with no sex differences over the last 40% of the test (Figure 3.6A). Men consistently had higher tidal volumes (Figure 3.6B) and ventilations (Figure 3.6C). Women utilized a larger fraction of their maximum ventilation ($\dot{V}_E/\dot{V}_{Emax}$) during the first 40% of the exercise test but utilized a lower fraction at the end of the exercise test relative to men (Figure 3.6D). The $\dot{V}_E/\dot{V}_{Emax}$ ratio at the end of the exercise test (i.e., 100%TTE) was significantly correlated with the magnitude of diaphragm fatigue in all subjects ($r = 0.52$, $P < 0.001$). Thus, those utilizing the largest fraction of their exercise ventilatory capacity at the end of the exercise test tended to demonstrate the greatest

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diaphragmatic fatigue. The mechanical work of breathing was consistently higher in men throughout the entire exercise test and rose disproportionally compared to women with increasing time (Figure 3.6E). The absolute- and mass-corrected pressure-time-products of the diaphragm and esophagus are shown in Figure 3.7. Men consistently had higher absolute values for PTP_{di} (Figure 3.7A). Women have a relative plateau in PTP_{di} whereas men continue to increase PTP_{di} towards exhaustion. Taking mass into account normalizes the PTP_{di} response such that there is no significant sex difference across time (Figure 3.7B). There were no differences in absolute PTP_{es} at any time point until exhaustion where PTP_{es} rose disproportionally in men relative to women (Figure 3.7C). However, this lack of difference in PTP_{es} throughout the majority of the test was reversed when body mass was accounted for (Figure 3.7D). Mass corrected PTP_{es} was, on average, approximately 15% higher in women across the entire TTE test. The relative contribution of the diaphragm to total inspiratory muscle force output (PTP_{di}/PTP_{es}) was higher in men at the start of the cycle test with the data converging upon reaching exhaustion (Figure 3.7E). However, over time, men continued to have a reduced contribution of the diaphragm to total inspiratory force output whereas diaphragmatic contribution in women changed very little over time. There was a 12% reduction in PTP_{di}/PTP_{es} from 20%TTE to 100%TTE in men and only a 3% reduction in women. To further characterize this response, mean slopes were calculated across the entire duration of exercise for all subjects (Figure 3.7F). The mean slope for men was significantly higher than for women ($P < 0.05$), suggesting a progressive decrease in diaphragmatic pressure contribution during exercise and an increase in accessory muscle recruitment in order to generate the increasing levels of ventilation shown in Figure 3.6C. Regression analysis on the mean values relating PTP_{di}/PTP_{es} to TTE (%max) showed a significant association for men ($r^2 = 0.89$; $P < 0.001$) but not for women ($r^2 = 0.37$; $P > 0.05$).
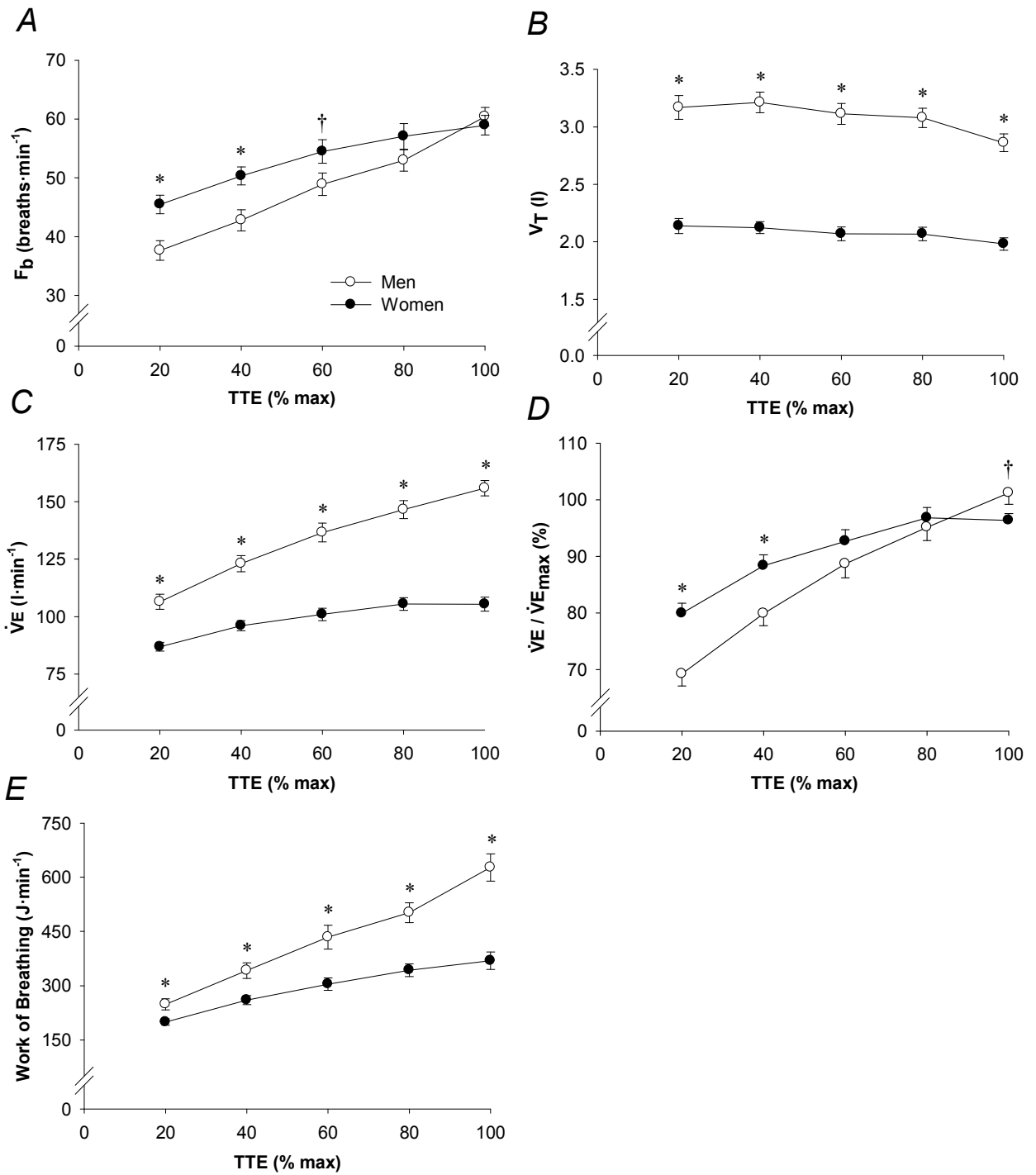
Figure 3.6: Ventilatory and work of breathing response to exercise in men and women. Values are mean ± S.E.M. TTE, time to exhaustion; F_b, breathing frequency; V_T, tidal volume; $\dot{V}_E$, minute ventilation, $\dot{V}_{E_{max}}$, maximal minute ventilation from the incremental cycle test to exhaustion (day 1). * Significant difference between men and women ($P < 0.01$). † ($P < 0.05$).
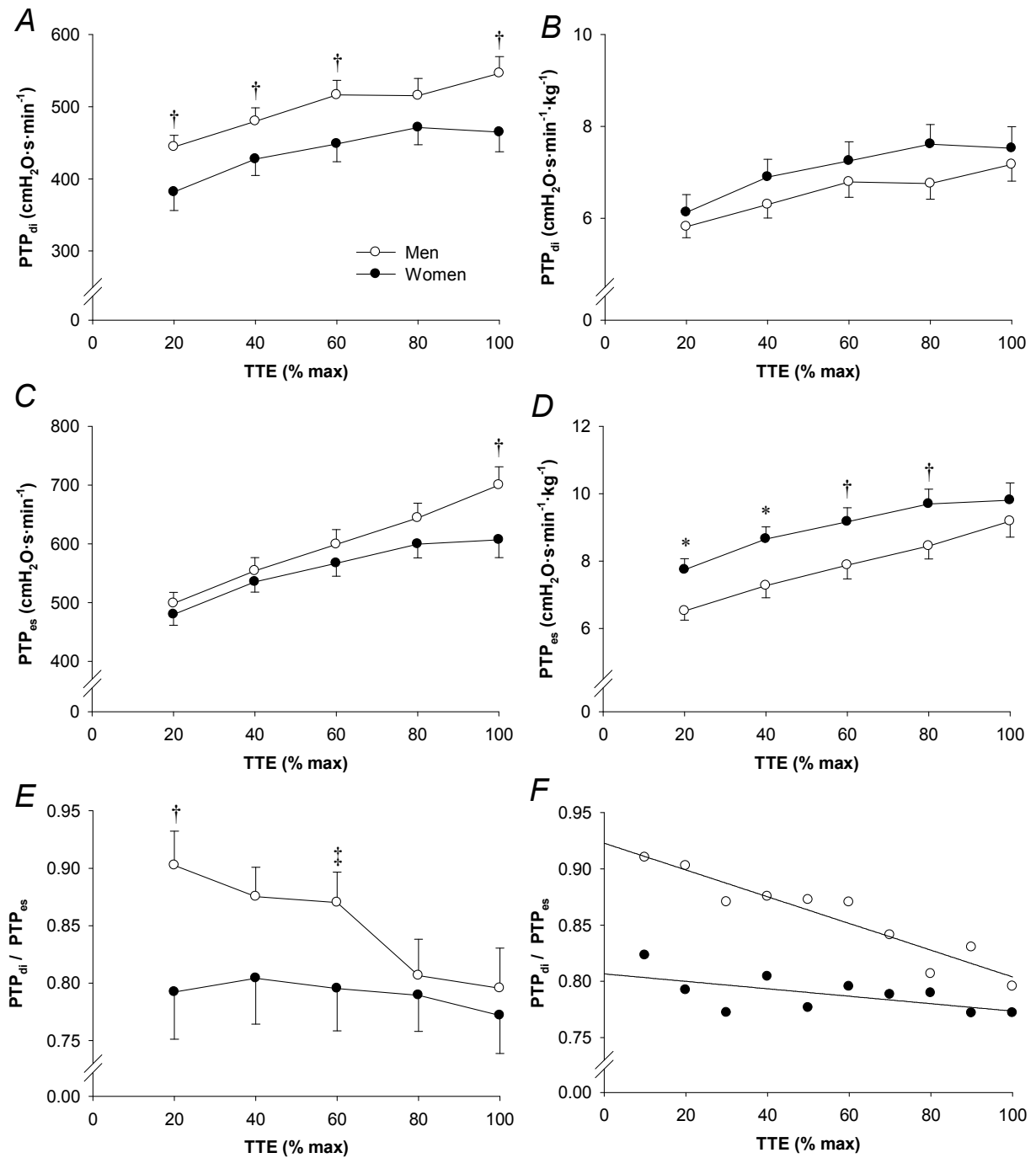
Figure 3.7: Diaphragmatic and esophageal pressure-time product response to exercise in men and women. Values are mean ± S.E.M. TTE, time to exhaustion; PTP_{di}, trans-diaphragmatic pressure-time product; PTP_{es}, esophageal pressure-time product. * Significant difference between men and women ($P < 0.01$). † ($P < 0.05$), ‡ ($P = 0.05$).

DISCUSSION

The purpose of this study was to determine if there are sex differences in the magnitude of exercise-induced diaphragm fatigue in healthy trained men and women. Our findings do not support our original hypothesis that women would develop more diaphragm fatigue than men. We have shown that fewer women developed diaphragmatic fatigue and that the magnitude of fatigue was significantly greater in men. To our knowledge, this is the first study to measure P_{di} during exercise in a large group of women and the first to assess diaphragm fatigue in women using phrenic nerve stimulation.

Diaphragm Fatigue: The reduction in $P_{di,tw}$ 10 min following exercise and the pattern of recovery at 30 and 60 min in our male subjects is consistent with previous studies (Babcock *et al.*, 1995; Babcock *et al.*, 1996; Vogiatzis *et al.*, 2007; Vogiatzis *et al.*, 2008). The time-course of $P_{di,tw}$ in response to phrenic nerve stimulation was nearly identical between men and women (Figure 3.3). That is, the greatest reductions in $P_{di,tw}$ were seen 10 min following exercise with $P_{di,tw}$ approaching baseline levels 60 min following exercise. However, the percent drop in $P_{di,tw}$ was lower in women, particularly at 10 and 30 min following exercise suggesting greater fatigue resistance in women. The literature concerning the prevalence of exercise induced diaphragmatic fatigue is controversial due to the wide range of methodologies used, the large variation in subject characteristics and the relatively small sample sizes used. Thus, it is difficult to determine the prevalence of exercise induced diaphragm fatigue. In the present study, we show that the fatigue response of the diaphragm is variable with only about half of the subjects showing evidence of diaphragm fatigue (as defined by a $\geq$ 15% reduction in $P_{di,tw}$). It is difficult to determine why some subjects in a relatively homogenous population develop diaphragm fatigue while others do not. Nevertheless, the present study suggests that the magnitude of

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fatigue appears to be greater in men. Potential mechanisms for this apparent fatigue resistance in the female diaphragm are discussed below.

Fatigue Resistance Mechanisms: The men and women in this study were exercising at the same relative intensity (90% of peak work-rate) but the absolute work-rate was higher in men. Therefore it would be expected that the absolute load on the male respiratory system would be higher to accommodate their higher levels of absolute ventilation. Indeed, the total work of breathing was higher in men across the entire duration of the constant load cycling test (Figure 3.6D) which is an observation that is consistent with our recent data (Guenette *et al.*, 2009) showing a higher total work of breathing in men compared to women at the same relative levels of minute ventilation. Women generally have less muscle mass compared to men and this has been proposed as one of the key contributors to explain the greater fatigue resistance found in women (Hicks *et al.*, 2001). Lower muscle mass translates directly into lower absolute force generation in females when exercising at the same relative intensity as males. This lower absolute force production in women means there will be a decreased O_2 demand, a decrease in mechanical compression of the local vasculature and less intramuscular occlusion of blood flow. The question relevant to the present study is whether or not we observed greater levels of diaphragmatic fatigue in men because they had higher absolute diaphragmatic force production during exercise. Thus our proposed sex difference may simply be due to contractile conditions eliciting a greater degree of imbalance between muscle O_2 supply and demand in men. While we cannot completely rule out this possibility, we do not believe that this mechanism alone can fully explain our findings. First, the absolute PTP_{di} in men was only modestly higher than women (~14%) during exercise. Second, when we corrected the PTP_{di} for body mass in an attempt to normalize for size differences, we found that men and women were actually well matched for PTP_{di} (Figure 3.7B). Third, we were able to match men and women for maximal

voluntary contraction of the diaphragm by removing some males with the highest maximal P_{di} values (data not shown) and we still show that men have greater levels of fatigue 10 min following exercise. Finally, Fulco *et al*. (1999) compared muscle performance in men and women at the same absolute force development and matched subjects for maximal voluntary contraction of the adductor pollicis muscle. Despite matching for maximal muscle strength, these authors still found that females exhibit less fatigue than males. Other potential mechanism to explain our findings include differences in muscular recruitment, muscle morphology and substrate utilization (see below).

Diaphragmatic Force Production and Respiratory Muscle Recruitment: A close examination of Figures 3.6 and 3.7 point to some important sex differences in the ventilatory response to exercise that may explain why men develop greater levels of diaphragmatic fatigue than women. These potential differences stem, in large part from the response of minute ventilation as shown in Figure 3.6C. Men have a progressive and linear increase in minute ventilation with increasing time spent at the same absolute work-rate. The male ventilatory response to this constant load cycling test is nearly identical to what has been previously reported by Johnson *et al*. (1993) in fit males exercising at 95% of their $\dot{V}O_2$max to exhaustion. The percent increase in minute ventilation from the early stages of exercise (20%TTE) to exhaustion (100%TTE) in our male subjects was 46%. This contrasts sharply to the modest increase in minute ventilation of 21% in women from 20%TTE to 100%TTE. In fact, the minute ventilation rises only 4% from 60%TTE to 100%TTE and is not different between 80 and 100%TTE. Thus women have a plateau in minute ventilation despite performing the same muscular task and therefore use a smaller fraction of their maximal exercise ventilatory capacity at 100%TTE (Figure 3.6D) compared with men. In fact, the $\dot{V}_E/\dot{V}_E$max ratio at 100%TTE was significantly, albeit modestly related to the magnitude of diaphragmatic fatigue. Thus the higher ventilatory requirements in men during

exercise might make the diaphragm more susceptible to fatigue and compromise its role as the primary inspiratory muscle. Why do men increase ventilation linearly over time whereas women do not? There are several possible explanations for this. Firstly, the women may have been mechanically constrained due to the potential presence of expiratory flow limitation. While expiratory flow limitation was not assessed in the present study, we (Guenette *et al.*, 2007) and others (McClaran *et al.*, 1998) have shown that women may be particularly susceptible to mechanical ventilatory constraints which may be related to their smaller lungs and airways (Sheel & Guenette, 2008). However, this is unlikely to fully explain our findings since women appeared to have a slightly larger ventilatory reserve at 100%TTE compared with men. Alternatively, it is possible that the blunted ventilatory response in women during exercise was due to a reduced chemical drive to breathe. We have previously shown that chemosensitivity is not different between healthy men and women (Guenette *et al.*, 2004) under resting conditions but it is currently unknown if there are sex differences in chemosensitivity during exercise. Finally, it is entirely possible that the female ventilatory response was perfectly adequate for gas exchange and therefore did not need to increase over time. Unfortunately, we do not have the required data to address this hypothesis.

What effect does this difference in the ventilatory response have on respiratory mechanics and muscle recruitment? Figure 3.7A demonstrates a continual rise in PTP_{di} across time for men whereas women plateau between 80 and 100%TTE. Figure 3.7E demonstrates the relative contribution of the diaphragm to total inspiratory muscle force output and Figure 3.7F shows the slopes of these relationships for men and women. In relative terms, men rely less on the diaphragm over time suggesting an increased recruitment of accessory inspiratory muscles in order to enable them to continue increasing minute ventilation. Women on the other hand show very little change in diaphragmatic contribution to total inspiratory force output shown by the

slope in Figure 3.7F. This is likely a function of their reduced ventilatory requirement. This change in recruitment pattern in men may be a physiological response to the onset of diaphragm fatigue which persists well into recovery.

The potential role that PTP_{di} and respiratory muscle recruitment has on diaphragmatic fatigue remains difficult to determine. While these potential sex differences in breathing mechanics are an attractive hypothesis to explain our findings, there are some important factors that must be considered. For example, diaphragmatic work is partially related to exercise-induced diaphragmatic fatigue because fatigue was prevented when diaphragmatic work was reduced during exercise using a mechanical ventilator (Babcock *et al.*, 2002). However, diaphragmatic work alone cannot fully explain diaphragm fatigue during exercise because Babcock *et al.* (1995) found that fatigue did not occur when resting subjects mimicked the magnitude and duration of diaphragmatic work incurred during exercise. Fatigue only occurred when pressures developed by the diaphragm were voluntarily increased to levels that were twice as high as those required during whole-body exercise. The lower fatigue threshold for diaphragmatic force production during exercise compared with rest suggests an important contribution from mechanisms directly related to the whole-body exercise itself. For example, recent work by Vogiatzis *et al.* (2009) suggests that intercostal muscle blood flow increases linearly with the work of breathing during voluntary hyperpnea but decreases at the same work of breathing during whole body exercise at intensities above 80% of maximal work-rate. Thus the circulatory system is unable to meet the demands of both locomotor and respiratory (intercostal) muscles during heavy exercise, which likely contributes to respiratory muscle fatigue. While we have shown differences in diaphragmatic force production between sexes and differences in respiratory muscle recruitment, we recognize that there are other crucial factors such as blood flow competition that may explain our findings. Recent work has utilized near

infrared spectroscopy and a light absorbing tracer (indocyanine green) to measure respiratory muscle blood flow during voluntary hyperpnea and exercise (Guenette *et al.*, 2008; Vogiatzis *et al.*, 2008; Vogiatzis *et al.*, 2009) but no such measurements have been made in women. Future work in this area is required to help explain the mechanisms underlying the greater fatigue resistance in women.

Muscle Morphology and Substrate Utilization: Some evidence suggests that there are sex differences in muscle fibre type composition such that women have more slow twitch oxidative fibres (Miller *et al.*, 1993). Slow twitch oxidative fibres fatigue at slower rates compared to fast twitch glycolytic fibres (Hamada *et al.*, 2003). These potential sex differences in muscle fibre type composition may explain, in part, why female muscles are more fatigue resistant than male muscles (Hicks *et al.*, 2001). In the present investigation, we are interested in the primary muscle of inspiration. The human diaphragm is composed of 76% high-oxidative fibres (55% slow twitch and 21% fast twitch) and 24% low-oxidative fast twitch fibres (Lieberman *et al.*, 1973). However, we are unaware of any studies that have looked specifically at sex differences in diaphragmatic fibre type composition in healthy humans, particularly in endurance-trained individuals. There are also sex differences in substrate utilitzation during exercise which may also contribute to potential sex differences in muscle fatigue. It has been established that males have a higher glycolytic capacity and a greater reliance on glycolytic pathways than females with muscle biopsy studies revealing that women have lower activities of common glycolytic enzymes, which in turn would translate into a decreased potential for anaerobic glycolysis (Tarnopolsky, 1999). As pointed out by Hicks *et al.* (2001), these differences may mean that women have a greater reliance on β-oxidation of fatty acids, thus prolonging endurance during certain types of exercise and perhaps improving their ability to resist fatigue.

Consequences of Diaphragm Fatigue: There are numerous studies pointing to a female respiratory system that may be more susceptible to specific pulmonary limitations such as expiratory flow limitation (McClaran *et al.*, 1998; Guenette *et al.*, 2007) and exercise induced arterial hypoxaemia (Harms *et al.*, 1998; Richards *et al.*, 2004; Guenette & Sheel, 2007). However, the data from the present study points to a female respiratory system that has a distinct advantage over their male counterparts. That is, women are more resistant to exercise-induced diaphragmatic fatigue. There are several important physiological and performance based consequences of diaphragmatic fatigue which have recently been reviewed by Romer and Polkey (2008). We will briefly discuss some of these consequences in the context of the present data.

The greater reliance on accessory inspiratory muscles in men with progressive exercise may result in chest wall distortion (Goldman *et al.*, 1976; Grimby *et al.*, 1976) and reduce the mechanical efficiency of breathing (Hart *et al.*, 2002). The reliance and recruitment of accessory inspiratory muscles may lead to an increase in sensory input to the central nervous system resulting in an increased sensation of breathlessness (Romer & Polkey, 2008). However, despite the greater levels of fatigue in men, there appeared to be no sex differences in ratings of perceived exertion for either breathing or leg discomfort. While respiratory muscle fatigue may increase the sensation of breathlessness (Gandevia *et al.*, 1981; Supinski *et al.*, 1987; Ward *et al.*, 1988), it is likely that this effect is specific to the accessory muscles because diaphragm fatigue does not increase neural respiratory drive as assessed by esophageal diaphragm EMG (Luo *et al.*, 2001).

Another potential consequence related to diaphragm fatigue is a sympathetically mediated metaboreflex that originates from fatiguing inspiratory muscles. Fatigue inducing inspiratory contractions result in a time-dependent increase in muscle sympathetic nerve activity

(St Croix *et al.*, 2000) and a reduction in arterial blood flow to the resting limb (Sheel *et al.*, 2001). This metaboreflex response may also become active during whole body endurance exercise. Harms *et al.* (1997) found that reducing the inspiratory work of breathing using a mechanical ventilator causes vascular conductance and blood flow in the exercising limb to increase. Thus the sympathetically mediated vasoconstriction of locomotor limb muscle vasculature may lead to an exacerbation of peripheral fatigue, increase effort perceptions and ultimately limit exercise performance (Dempsey *et al.*, 2002). It is possible that men have a more pronounced inspiratory muscle metaboreflex response relative to women given that they tend to exhibit greater levels of diaphragm fatigue. A sex-based comparison of the inspiratory metaboreflex is required to fully address this hypothesis.

Methodological Considerations: The CMS technique is considered to be as effective as electrical stimulation for detecting diaphragmatic fatigue (Laghi *et al.*, 1996). Moreover, it has been shown to be reproducible, safe and painless (Similowski *et al.*, 1989). We chose the CMS technique over electrical stimulation for two main reasons. First, magnetic stimulation does not activate cutaneous pain receptors and is therefore less painful for subjects than electrical stimulation. Second, CMS is easier than electrical stimulation in terms of finding the optimal stimulation site, which is important for consistency and reproducibility between pre- and post-stimulations. However, the technique does have certain limitations that require attention. It is more difficult to demonstrate supramaximal stimulation with CMS compared with electrical stimulation. Supramaximal stimulation can be confirmed by showing a plateau in $P_{di,tw}$ with increasing power output of the stimulator. Figure 3.2 shows, that on average, men and women demonstrated a levelling off in $P_{di,tw}$ at 90-95% of stimulator output. However, fewer men reached a plateau in $P_{di,tw}$ compared with women, likely because the men were generally taller with thicker necks. However, we do not believe that submaximal stimulation in some of our

men would influence our primary finding of greater fatigue resistance in women for several reasons. First, Verges *et al*. (2006) found that only 4 out of 11 subjects showed evidence of a plateau when comparing $P_{di,tw}$ at 94% of the stimulator output to 100%; however, a plateau was seen in 8 out of 11 subjects when comparing $P_{di,tw}$ at 98% of the stimulator output to 100%. We made our measurements at larger increments on the stimulator output than Verges *et al*. (2006). Our more conservative approach probably led to an underestimation of the number of subjects showing a plateau in $P_{di,tw}$, and thus suggesting that stimulations may have been submaximal. Had we performed our stimulations at 98% of the stimulator power output, we believe we would have demonstrated a plateau in most of our male subjects. Second, M-wave amplitude was unchanged following exercise in both men and women, suggesting that the reduction in $P_{di,tw}$ was not due to decrecruitment of muscle fibres or to transmission failure. Third, the stimulator output was set at 100% of maximal power output for all stimulations and we paid particular attention to ensure that the subjects were in the same position before and after exercise. In addition to visual inspection of the subject while seated, we also marked the coil position on the back of the neck enabling us to reposition the coil in the exact location for all stimulations. The consistency of our stimulations before and after exercise can be seen by the lack of change in the coefficient of variation in $P_{di,tw}$. Fourth, any twitches that were not initiated from resting EELV were discarded from analysis. Therefore, while supramaximal stimulations were not obtained in all men, we are confident that our stimulations were constant and that this limitation would not influence our primary finding regarding sex differences in diaphragmatic fatigue. Finally, when the male subjects that did not reach supramaximal stimulation were removed from the analysis, we found that the magnitude of fatigue was still greater in males compared with females (data not shown).

Conclusions: This study is the first to measure diaphragm function during exercise in healthy trained women and the first to use objective measures to investigate diaphragm fatigue in a large group of women. The data from the present study points to a female diaphragm that is more resistant to exercise-induced fatigue compared with men. We have also shown sex differences in the ventilatory response to high-intensity constant load cycling exercise that may be related, in part, to the greater fatigue resistance observed in women.

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CHAPTER IV: Conclusions

Overall Summary: For many years, human physiology research was almost exclusively focussed on understanding the male response to exercise. Only recently have scientists recognized the importance of studying sex-based physiological differences. This is particularly the case when dealing with the respiratory response to exercise. Studying sex differences in respiratory exercise physiology is a discipline that is still in its infancy and before we can understand sex differences in clinical populations, it is important to first characterize the "normal" responses to exercise in healthy women. The work in this thesis, coupled with previous work from our laboratory (Guenette *et al.*, 2004; Richards *et al.*, 2004; Guenette *et al.*, 2007a; Guenette *et al.*, 2007b) and others (Harms *et al.*, 1998; McClaran *et al.*, 1998; Harms *et al.*, 2000; Olfert *et al.*, 2004) provides an important first step in characterizing the normal respiratory response to exercise in healthy women. This thesis provides unique insight into the mechanisms associated with a higher mechanical work of breathing (WOB) in women. Specifically, we have shown that women have a higher WOB for a given absolute ventilation due to a higher inspiratory and expiratory resistive WOB rather than differences in the elastic WOB. This is presumably due to the inherently smaller diameter airways in women (Sheel *et al.*, 2009). This study and others (McClaran *et al.*, 1998; Guenette *et al.*, 2007b) point to a female diaphragm that may be at a mechanical disadvantage relative to their male counterparts.

The findings of greater expiratory flow limitation, higher operational lung volumes and a higher WOB in women during exercise led to the subsequent hypothesis that women would be more susceptible to diaphragmatic fatigue. This thesis presents the first study to systematically measure diaphragmatic function during exercise and its ensuing fatigue in exercising women. Contrary to our original hypothesis, the female diaphragm was actually more resistant to fatigue than the male diaphragm. We have also demonstrated clear sex differences in the ventilatory

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response to constant load cycling which directly influenced diaphragmatic recruitment during exercise. These sex differences may explain, in part, why women were able to preserve the diaphragm's force generating ability, despite having higher body mass-corrected esophageal pressure-time products during exercise. Not only was the relative load on the respiratory muscles greater in women, but they were also exercising at a higher percentage of their maximum heart rate, even though they were all exercising at the same relative external work-rate. This makes the finding of greater diaphragmatic fatigue resistance in women even more impressive. Is this an evolutionary or physiological adaptation to a respiratory system that may be at a mechanical disadvantage (i.e., smaller lungs, smaller airways, etc.) during strenuous exercise? Perhaps the diaphragm is simply behaving like any other skeletal muscle that shows greater fatigue resistance in women. The answers to these questions remain unknown.

Significance: Recent review's by Hicks *et al.* (2001) and Hunter (2009) provide a comprehensive list of studies that support the idea that women have greater skeletal muscle fatigue resistance relative to males. The majority of these studies have focussed on muscles involved in moving the elbow, finger, knee, thumb, ankle, back and neck. These studies have provided important insight into sex differences in skeletal muscle fatigue. The data presented in this thesis adds to the existing literature by studying the single most important and complex skeletal muscle in the human body; the diaphragm. The diaphragm is the primary muscle involved in breathing and is required to contract for the entire duration of one's life. As such, its ability to resist fatigue may have important clinical implications, particularly in the intensive care unit where individuals are undergoing mechanical ventilation and where weaning success depends on the ability of the diaphragm to maintain a given force production.

This research is also significant because it is the first to comprehensively assess respiratory mechanics and diaphragmatic function during an actual bout of high-intensity exercise. Despite performing the same external muscular work (i.e., 90% of peak work-rate), women had a very different ventilatory response to exercise with different respiratory muscle recruitment patterns. This may give some initial insight into why women have greater diaphragmatic fatigue resistance relative to men.

Pulmonary Limitations in Women: Three of the primary pulmonary limitations that occur in healthy athletes during exercise include: exercise induced arterial hypoxaemia, expiratory flow limitation and diaphragmatic fatigue (Dempsey *et al.*, 2008). Studies have shown that women may be more susceptible to exercise-induced arterial hypoxaemia (Harms *et al.*, 1998; Richards *et al.*, 2004) and that it occurs in relatively untrained women, a phenomenon that is not typically observed in men (Harms *et al.*, 1998; Richards *et al.*, 2004). Furthermore, there is evidence to suggest that expiratory flow limitation may also be more common in women (McClaran *et al.*, 1998; Guenette *et al.*, 2007b). These studies have led some to conclude that women are more susceptible to pulmonary system limitations during exercise relative to men, which may be related, in part, to their smaller lungs and airways and their reduced surface areas for diffusion. However, the present study suggests that diaphragmatic fatigue is not a pulmonary system limitation that is more common in women. The force preserving ability of the female diaphragm may be a physiological adaptation to counteract the potential deleterious effects of hypoxaemia and expiratory flow limitation on diaphragm fatigue and or exercise performance.

Strengths and Limitations: The primary strengths of the studies presented in this thesis relate to study design and the methodological approaches to answering our research questions. Accurately quantifying respiratory mechanics and diaphragmatic fatigue often requires highly

invasive and uncomfortable procedures for the research participants. All of our primary

outcomes were measured using the most objective and validated methods. An additional

strength to this research, particularly the diaphragm fatigue study (Chapter 3) is the large sample

size used. To our knowledge, 38 subjects is one of the largest sample sizes used for any

respiratory mechanics or diaphragm fatigue study in the current literature. This gave us

excellent statistical power and allowed us to partition our data to look at responses to various

sub-groups (i.e., those that did and those that did not develop diaphragm fatigue).

The studies comprising this thesis have certain limitations beyond those discussed in

detail within chapters 2 and 3. Specifically, we made our measurements on very well-trained

endurance athletes with a high aerobic capacity. We used athletes because they are able to stress

their cardio-respiratory system well-beyond their untrained or diseased counterparts. Thus,

athletes provide an excellent model to study the limitations of the human respiratory system

during exercise. However, endurance athletes represent a small fraction of the general

population and thus the generalizability of our findings are limited to the population from which

we derived our data. How our findings fit to other populations such as healthy ageing, obesity

and those with chronic diseases remains unknown. Another limitation to this research is that we

did not assess the functional consequences or the non-mechanical mechanisms associated with

respiratory muscle fatigue (e.g., perfusion). These ideas are discussed below in "future

research."

Future Research: There are several unanswered questions related to this thesis that will require

unique experimental designs and methodologies. For example, what are the functional

consequences of expiratory flow limitation, diaphragm fatigue and a high WOB? Unloading the

respiratory muscles using proportional assist ventilation or breathing low density gas mixtures

such as heliox may provide unique insight into the exercise performance-based implications of these pulmonary limitations during exercise in men and women.

Perfusion to a muscle is also an important factor involved in the fatigue process. Are the respiratory muscles in women receiving adequate perfusion to avoid fatigue? Perhaps there are sex differences in respiratory muscle blood flow regulation which may provide some mechanistic data to support the finding of greater fatigue resistance in women. Are there sex differences in blood flow competition between respiratory and locomotor muscles for a limited cardiac output during exercise? All of these unique questions have not been tested because a method for measuring respiratory muscle blood flow in conscious humans was not available. However, we recently developed a method to measure blood flow to superficial respiratory muscles using near-infrared spectroscopy and a light absorbing tracer (Guenette *et al.*, 2008). This innovative method may provide insight into some of the underlying mechanism associated with pulmonary system limitations during exercise in humans.

As highlighted above, the data from the present thesis can only be generalized to healthy humans with a relatively high aerobic capacity. Therefore, future research is required to expand on these findings to different populations such as chronic obstructive pulmonary disease, obesity, chronic heart failure and healthy ageing, among others. For example, population studies in patients with cardiopulmonary diseases have shown that women experience greater levels of respiratory discomfort, greater exercise intolerance and poor perceived health status relative to men when matched for disease severity (Watson *et al.*, 2004; de Torres *et al.*, 2005; Han *et al.*, 2007). Recent work also suggests that women experience greater levels of dyspnea with advancing age (Ofir *et al.*, 2008). These findings point to the importance of studying sex-based physiological differences since biological sex can play a direct role in disease. Finally, future

research is required to determine how greater fatigue resistance in women influences athletic performance. Perhaps the findings of the present study may explain, in part, why women tend to outperform men in ultra-endurance events (Speechly *et al.*, 1996).

Overall Conclusion: The purpose of this thesis was to provide a comprehensive assessment of respiratory mechanics and diaphragm fatigue in men and women during exercise. We found that women have a higher WOB for a given level of ventilation due to their smaller diameter airways which act to increase the inspiratory and expiratory resistive WOB. We also observed that that the female diaphragm is more resistant to fatigue following high intensity exercise, which may be related, in part, to sex differences in respiratory muscle recruitment and differences in the ventilatory response to exercise. Future work is required to expand on these observations to different patient populations.

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APPENDIX I: Informed consent forms

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INFORMED CONSENT FORM

Title of Project: Ventilatory responsiveness and the work of breathing in men and
women with exercise induced arterial hypoxaemia.

Principal Investigator: William Sheel, Ph.D.

Co-Investigators: Donald McKenzie, Ph.D., M.D.
Mike Koehle, M.D.
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Meaghan McNutt, Ph.D. candidate
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Background:
Some athletes develop low oxygen content in their blood during exercise (hypoxaemia) and
investigators have suggested that this is due to insufficient breathing during exercise. Some
researchers have suggested that there is a gender difference, however, there is limited scientific
information available to make accurate conclusions.

Purpose:
The purpose of this study is to investigate how breathing changes in response to low oxygen
content in the blood during rest and exercise and how breathing mechanics influences these
responses. Specifically, we are concerned with gender differences between elite male and
female cyclists and triathletes.

<u>Procedures</u>:

You are being invited to participate in one data collection test day and your participation in the study is entirely voluntary. The session will take place at the

Health and Integrative Physiology Laboratory at the Osborne Center (Unit 2, Room 202) on the University of British Columbia campus. The study will require approximately two (2) hours of your time. Before any measurements are taken, a physical activity readiness questionnaire (PAR-Q) will be administered. The experiment is divided into three parts. First, your height and weight will be measured. You will then undergo a simple, non-invasive breathing test to ensure that you do not have any obstructive lung disease (i.e., asthma). This requires you to breathe deeply and exhale quickly through a mouthpiece. Another simple, non-invasive test of your wrist will be performed by a physician to ensure adequate circulation. If your circulation is deemed inadequate, you will not be able to participate in the study. If you are currently using anti-inflammatory medication or have a history of bruising easily or having blood clotting problems, you will be excluded from the study.

You will then be required to lie comfortably on a bed while listening to music and wearing a nose clip in which you will breathe through a two-way valve so that expired gases and flow can be monitored. A small plastic clip will be attached to your ear. This will permit us to measure the amount of oxygen in your blood. After 10 minutes of breathing normal air, experimenters will slowly and progressively add nitrogen gas to the air you are breathing. We will measure the amount that your breathing (rate and depth) increases in response to this. This experiment will simulate high altitude exposure and will take approximately 15-20 minutes.

You will then perform a warm-up exercise session on a bicycle (5-10 minutes). After you have warmed up and stretched you will then perform a maximal cycling test. The test will progressively become more difficult and will last approximately 10-15 minutes until you are tired and must stop. It will be necessary that you breathe through a mouthpiece while wearing a nose clip so that expired gases and flow can be monitored.   A thin and flexible tube (catheter) will be placed in the brachial or radial artery of your arm to allow the experimenters to take multiple blood samples throughout the test. Approximately 10 samples will be taken throughout the test with each sample containing 3mL of blood for a total of 30mL. To minimize discomfort, a local anesthetic will be applied. In order to measure temperature in the body during exercise, a temperature measuring probe will be placed through your nose into the lower one-third of the esophagus. The esophagus is the tube that connects your mouth to your stomach. To continually measure the amount of oxygen in your blood, a small clip will be attached to your ear and finger. Also, two additional thin flexible tubes will be inserted through nose into the esophagus and stomach so that breathing pressures can be obtained. A local anesthetic will be applied in your throat to minimize discomfort. If you have any diseases such as an ulcer in your esophagus or a tumor you will be excluded from the investigation. You will also be excluded if you have had recent nasopharyngeal surgery.

Risks:

Due to the unpredictable response of some individuals to exercise, unforeseen difficulties may arise which would necessitate treatment. You are asked to report any unusual symptoms during the test. You may stop the test when you wish to because of personal feelings of discomfort and tiredness. Every effort will be made to conduct the tests in such a way as to minimize discomfort and risk. However, there exists the possibility of

Potential risks from maximal exercise such as vomiting (5%), abnormal blood pressure (less than 1%), fainting (less than 1%), disorders of heartbeat (less than 0.1%), and very rare instances of heart attack (less than 0.001%). If you experience any of these, you will receive immediate care from a physician (co-investigator Dr. McKenzie or Dr. Koehle) at no cost. If you feel that you are experiencing any side effects as a result of any procedures you should immediately report this to the principal investigator. You may also experience mild discomfort due to the catheter in your arm and the catheters in your oesophagus.   Potential risks associated with the catheter in your arm include bleeding (less than 1%), bruising (14%), and infection (less than 1%). Other potential risks associated with an arterial line include artery aneurysms, blood clotting, brief tightening of a blood vessel, death of skin tissue over the catheter site, and line disconnection. When collecting blood and measuring temperature, the utmost care will be taken to ensure your comfort. Catheter placement in your arm will be performed by a trained physician.

There are no risks associated with decreased oxygen supply simulating mild high altitude exposure (approximately 10,000 feet).

Benefits:

As a result of your participation in this study, you will receive detailed fitness assessments that can be used to help you with your personal training program.

Confidentiality:

Your rights to privacy are protected by the Freedom of Information and Protection of Privacy Act of British Columbia. This Act lays down rules for the collection, protection, and retention of your personal information by public bodies, such as the University of British Columbia and its affiliated teaching hospitals. Further details about this Act are available upon request. Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records and medical records identifying you may be inspected in the presence of the Investigator or his or her designate by representatives of the UBC Research Ethics Board or Health Canada for the purpose of monitoring the research. However, no records which identify you by name or initials will be allowed to leave the Investigators' offices. You are encouraged to ask for an explanation or

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clarification of any of the procedures or other aspects of this study before signing this consent form or at any time during your participation in the study.

YOU MAY DECLINE TO ENTER THIS STUDY OR WITHDRAW FROM THE EXPERIMENT AT ANY TIME.

If you have any concerns or questions about your rights or experience as a research subject, you may contact the Research Subject Information Line in the UBC Office of Research Services at (604) 822-8598.

Consent:

In signing this form you are consenting to participate in this research project and acknowledge receipt of a copy of this form. Signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else.

_____   _____
Signature of Subject   Date

_____
Printed Name of Subject

_____   _____
Signature of Witness   Date

_____
Printed Name of Witness

_____   _____
Signature of Investigator   Date

_____
Printed Name of Investigator

THE UNIVERSITY OF BRITISH COLUMBIA

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INFORMED CONSENT FORM

Title of Project: Respiratory muscle fatigue in men and women

Principal Investigator: A. William Sheel, Ph.D.

Co-Investigators: Jordan A. Guenette, B.H.K., M.Sc. PhD candidate
Donald McKenzie, Ph.D., M.D.
Jeremy Road, M.D.
Meaghan McNutt, Ph.D. candidate
Jordan Querido, Ph.D. candidate
Simone Tomczak, M.Sc. candidate

Institution: School of Human Kinetics
The University of British Columbia

Contact Person: Jordan Guenette (office phone)   (604) 822-4384
24 hour emergency contact: (604) 617-4644

Background:
It is well known that women, on average, have smaller lungs and airways compared to men of similar stature. There is recent evidence to suggest that these anatomical differences may predispose women to certain breathing limitations and that the energy cost of breathing may be higher in women compared to men. The higher energy cost of breathing may cause the breathing muscles to become fatigued during exercise to a greater degree in women relative to men. However, there are no research studies that have measured fatigue of the breathing muscles in women and compared their responses to men after exercise.

Purpose:
The purpose of this study is to determine if the breathing muscles (diaphragm and abdominal muscles) are more prone to fatigue in women compared to men following high intensity cycling exercise.

Procedures:

You are being invited to participate in two data collection testing days and your participation in the study is entirely voluntary. Both testing days will take place at the Health and Integrative Physiology Laboratory at the Osborne Center (Unit 2, Room 202) on the University of British Columbia campus. The study will require approximately six to seven (6-7) hours of your time. Before any measurements are taken, a physical activity readiness questionnaire (PAR-Q) and medical history questionnaire will be administered. Female participants will also be required to fill out an additional menstrual cycle history questionnaire. You are not required to answer any questions that make you feel uncomfortable.

Day 1: This testing day is divided into two parts. First, your height and weight will be measured. You will then undergo a simple, non-invasive breathing test to ensure that you do not have any obstructive lung disease (i.e., asthma). This requires you to breathe deeply and exhale quickly through a mouthpiece. You will also be required to perform some additional breathing exercises which consist of taking maximal inspirations (breathing in) and additional exercises which require maximal expirations (breathing out).

The second part of this day requires you to perform exercise on a stationary bicycle. You will perform a warm-up exercise session on the bicycle (15 minutes). After you have warmed up you will then perform a maximal cycling test. The test will progressively become more difficult and will last approximately 10-20 minutes until you are tired and must stop. It will be necessary that you breathe through a mouthpiece while wearing a nose clip so that expired gases and air flow can be monitored. We will also place a clip on your ear and finger to measure the amount of oxygen in your blood.

The entire testing session will take approximately 2 hours.

Day 2: A trained respiratory physiologist (Jordan A. Guenette) will insert two thin flexible tubes through your nose into your oesophagus and stomach. The oesophagus is the tube that connects your mouth to your stomach. This will allow us to measure breathing pressures. A local anesthetic will be applied to your nose and throat to minimize discomfort. If you have any diseases such as an ulcer in your oesophagus or a tumor you will be excluded from the investigation. You will also be excluded if you have had recent nasopharyngeal surgery or if you have a nasal septum deviation. We will then place sticky electrodes on your stomach and chest in order to monitor muscle activity of your breathing muscles. Once the catheters and electrodes are secured and positioned correctly, you will then rest for 20 minutes. After this rest period, we will place a circular coil at the back of your neck while you are seated comfortably in a chair. This coil will be activated which uses a magnet to non-invasively stimulate the nerve that attaches to your diaphragm. This will cause you to take a quick inspiration and it will feel like a hiccup. We will also perform the same test but this time the coil will be placed in the

middle of your back while you lay face down on an incline bench. You will be excluded if you have any metal inside of your body or if you have a cardiac pacemaker.

Upon completion of several stimulations, you will then be required to perform a cycling test at a constant workload until you can no longer continue. The resistance on the bicycle will correspond to approximately 90% of the resistance you achieved on the first day of testing. You will then undergo the same stimulations 10, 30, 60 and 90 minutes after the exercise test.

The entire testing session will take approximately 4-5 hours.

Risks:

Due to the unpredictable response of some individuals to exercise, unforeseen difficulties may arise which would necessitate treatment. You are asked to report immediately any unusual symptoms during the test. You may stop the test when you wish to because of personal feelings of discomfort and tiredness. Every effort will be made to conduct the tests in such a way as to minimize discomfort and risk. Potential risks from maximal exercise include vomiting (5%), abnormal blood pressure (less than 1%), fainting (less than 1%), disorders of heartbeat (less than 0.1%), and very rare instances of heart attack (less than 0.001%). If you experience any of these, you will receive immediate care from a physician (co-investigator Dr. McKenzie) at no cost. If you feel that you are experiencing any side effects as a result of any procedures you should immediately report this to the principal investigator.

You may feel mild discomfort or soreness in the nostrils and upper airway during the placement of the tubes in your oesophagus and stomach. You may also experience slight discomfort as a result of "gagging" while swallowing the tubes and during the removal of the tubes. This will resolve once the tubes are in position. A numbing gel called Lidocaine will be used to minimize the discomfort. Adverse reactions to Lidocaine are extremely rare but include light-headedness, blurred/double vision, euphoria, confusion, dizziness, convulsions, sensations of heat, cold or numbness. You will not be allowed to participate in the study if you are known to be sensitive to local anaesthetics or if you have allergies to latex. We are unaware of any laboratory that has experienced any of the aforementioned adverse reactions to such a small amount of lidocaine. There is also a small risk that the catheters in your oesophagus may be placed in the wrong position. In some extremely rare cases, the catheter can enter your trachea (wind pipe). You may experience mild discomfort in the back of your throat and you may gag. When this occurs the catheter will be pulled out and re-positioned.

Although extremely rare, you may feel nausea, headache, mild discomfort and annoyance with the magnetic stimulation procedure. You may also experience muscular contractions, involuntary movements (such as the arms) and a mild tingling sensation in your arms.

Benefits:

As a result of your participation in this study, you will receive detailed fitness assessments that can be used to help you with your personal training program. You will receive an explanation of your test results and recommendations will be given on how this information can be used to assist you in your training.

Remuneration:

You will receive a $100 honorarium for your participation in this study. The honorarium will be based on your participation on the second day of testing.

Confidentiality:

Your rights to privacy are protected by the Freedom of Information and Protection of Privacy Act of British Columbia. This Act lays down rules for the collection, protection, and retention of your personal information by public bodies, such as the University of British Columbia and its affiliated teaching hospitals. Further details about this Act are available upon request. Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records and medical records identifying you may be inspected in the presence of the Investigator or his or her designate by representatives of the UBC Research Ethics Board or Health Canada for the purpose of monitoring the research. However, no records which identify you by name or initials will be allowed to leave the Investigators' offices. You are encouraged to ask for an explanation or clarification of any of the procedures or other aspects of this study before signing this consent form or at any time during your participation in the study.

YOU MAY DECLINE TO ENTER THIS STUDY OR WITHDRAW FROM THE EXPERIMENT AT ANY TIME WITHOUT PROVIDING ANY REASONS FOR YOUR DECISION AND WITHOUT AFFECTING YOUR MEDICAL CARE IN ANY WAY.

Should you have any questions about the procedures or your involvement in this study, please contact Dr. A.W. Sheel at 604-822-9451. If you have any concerns or questions about your rights or experience as a research subject, you may contact the Research Subject Information Line in the UBC Office of Research Services at (604) 822-8598.

Consent:

In signing this form you are consenting to participate in this research project and acknowledge receipt of a signed and dated copy of this form. Signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else.

_____   _____
Signature of Subject   Date

_____
Printed Name of Subject

_____   _____
Signature of Witness   Date

_____
Printed Name of Witness

_____   _____
Signature of Investigator   Date

_____
Printed Name of Investigator

APPENDIX II: Physical activity readiness and health questionnaires

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?
☐	☐	2.	Do you feel pain in your chest when you do physical activity?
☐	☐	3.	In the past month, have you had chest pain when you were not doing physical activity?
☐	☐	4.	Do you lose your balance because of dizziness or do you ever lose consciousness?
☐	☐	5.	Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?
☐	☐	6.	Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
☐	☐	7.	Do you know of <u>any other reason</u> why you should not do physical activity?

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to <u>all</u> PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:
- if you are not feeling well because of a temporary illness such as a cold or a fever — wait until you feel better; or
- if you are or may be pregnant — talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

<u>Informed Use of the PAR-Q</u>: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____   DATE_____

SIGNATURE OF PARENT _____   WITNESS _____
or GUARDIAN (for participants under the age of majority)

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

Supported by: Health   Santé
Canada   Canada

Menstrual History Questionnaire

1. Are you having regular periods?   YES/NO

2. How long is your cycle length?   _____ (days)

3. How many days long is your flow?   _____ (days)

4. Can you usually tell, by the way you feel, that your period is coming?   YES/NO

5. Do you usually experience the following symptoms?

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

6. How many times did you menstruate in the past year?   _____

7. How many periods have you missed in the last five years?   _____

8. Are you currently taking oral contraceptives? YES/NO

- If yes, for how long?   _____

- What is the name of the oral contraceptive pill which you are taking?

        _____

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

Medical History

1. Are you currently taking any medications (excluding oral contraceptives)?

        Please List: _____

2. Do you currently smoke? YES/NO

3. Are you a past smoker? YES/NO

4. When was the last time you had a cold? _____

5. Do you have asthma, other lung problems or significant illness? Please List:
_____

6. Do you have a cardiac pacemaker or any metal inside of your body? YES/NO

7. Have you had recent nasopharyngeal surgery? YES/NO

8. Do you have an ulcer or tumour in your oesophagus? YES/NO

Physical Activity History

Type of Physical Activity: _____
Average Duration: _____
Average Frequency: _____

APPENDIX III: Reprints of selected publications

Evidence for dysanapsis using computed tomographic imaging of the airways in older ex-smokers

A. William Sheel,[1] Jordan A. Guenette,[1] Ren Yuan,[2,3] Lukas Holy,[2,3] John R. Mayo,[2] Annette M. McWilliams,[4] Stephen Lam,[4] and Harvey O. Coxson[2,3]

[1]*School of Human Kinetics, University of British Columbia;* [2]*Department of Radiology, Vancouver General Hospital;* [3]*The James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research at the Heart and Lung Centre of St. Paul's Hospital; and* [4]*British Columbia Cancer Agency, Vancouver, British Columbia, Canada*

Submitted 25 May 2009; accepted in final form 11 September 2009

Sheel AW, Guenette JA, Yuan R, Holy L, Mayo JR, McWilliams AM, Lam S, Coxson HO. Evidence for dysanapsis using computed tomographic imaging of the airways in older ex-smokers. *J Appl Physiol* 107: 1622–1628, 2009. First published September 17, 2009; doi:10.1152/japplphysiol.00562.2009.—We sought to determine the relationship between lung size and airway size in men and women of varying stature. We also asked if men and women matched for lung size would still have differences in airway size and if so where along the pulmonary airway tree would these differences exist. We used computed tomography to measure airway luminal areas of the large and central airways. We determined airway luminal areas in men ($n = 25$) and women ($n = 25$) who were matched for age, body mass index, smoking history, and pulmonary function and in a separate set of men ($n = 10$) and women ($n = 11$) who were matched for lung size. Men had greater values for the larger airways and many of the central airways. When male and female subjects were pooled there were significant associations between lung size and airway size. Within the male and female groups the magnitudes of these associations were decreased or nonsignificant. In males and females matched for lung size women had significantly smaller airway luminal areas. The larger conducting airways in females are significantly smaller than those of males even after controlling for lung size.

airway luminal area; airway-parenchymal dysanapsis; three-dimensional computed tomography

THE CONCEPT that airway size is not necessarily related to lung size was first proposed by Green et al. (12). The wide variation in maximal expiratory flow rates between individuals with similar lung size was interpreted to mean that there is no consistent association between lung and airway size. Subsequent to this, the term "dysanapsis" has been used to reflect unequal growth and express the physiological variation in the geometry of the tracheobronchial tree and lung parenchyma due to different patterns of growth. Mead (27) determined the association between airway size (estimated using maximal expiratory flow/static recoil pressure at 50% vital capacity) and lung size (estimated using vital capacity) in adult women and men. It was found that healthy adult men have airways that are 17% larger in diameter than are the airways of women. Moreover, it was concluded that women and boys have airways that are smaller relative to lung size compared with men; therefore, the apparent sex-based differences occur late in the growth period. Additional support for the concept of dysanapsis comes from studies that have made acoustic reflectance estimates of tracheal area in young healthy men and women (23). In a subset of subjects matched for total lung capacity, Martin et al. (23) found that the tracheal cross-sectional area was 29% less in women compared with men. As such, there are significant male-female differences in tracheal size that do not appear to be explained by lung size.

While previous studies have provided insight into potential sex-based differences in the relationship between airway size and lung size, they have been limited either by the use of indirect estimates of airway size (12, 27) or by the use of an examination of the airways above the tracheal carina (13, 14, 19, 23). In addition, the majority of these studies have not made comparisons between men and women of equal size, making interpretation of potential airway differences difficult. As such, the purpose of this study was twofold. First, we sought to determine the relationship between lung size and airway size in men and women of varying stature. Second, we asked if men and women matched for lung size would still have differences in airway size and if so where along the pulmonary airway tree would these differences exist. To this end, we used computed tomography (CT) in men and women to provide quantitative measures of airway luminal areas. The larger conducting airways are defined as generations 0 through 16 (18). In this study we reported data for airways up to segmental bronchi (generation 3), which we have defined as the large and central airways.

METHODS

Subjects. A total of 57 (28 male, 27 female) subjects for this study were selected from the British Columbia Cancer Agency "Lung Health Cohort" (26). The clinical ethics review boards of the British Columbia Cancer Agency and the University of British Columbia approved the study. All subjects provided informed consent to allow their spirometry and CT images to be used for research. This is a cohort of heavy smokers who have been screened for the presence of lung nodules using CT scans. All subjects were former smokers at the time of the study.

Spirometry. Spirometry was conducted according to American Thoracic Society recommendations. The forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) were recorded in liters and expressed according to predicted values (9).

Study overview. The relationships between airway size, biological sex, and lung size were assessed in two ways. First, we measured airway luminal area (Ai) in men ($n = 25$) and women ($n = 25$) who were matched for age, body mass index (BMI), smoking history, percent predicted FEV_1, FEV_1/FVC, and CT measured total lung volume [expressed as % of predicted total lung capacity (TLC)]. The purpose of this approach was to examine subjects of a broad range of body heights to extend the range of lung volumes in each group. Second, we examined a separate set of men ($n = 10$) and women ($n = 11$) who were matched for lung size (estimated from FVC). The

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purpose of this analysis was to compare airway luminal areas in males and females with similar lung volumes to remove the independent effect from lung size and therefore reveal any sex-based difference in the relationship of airway size to lung parenchyma size.

CT scans. All CT scans were acquired in the volume scan mode at suspended full inspiration with subjects in the supine position. No intravenous contrast media was used. These CT scans were acquired using a Siemens scanner (Siemens Medical Solutions, Erlangen, Germany, Siemens Sensation 16 multislice scanner, 120 kVp, 215 mA, 1.0-mm slice thickness, and a low spatial frequency reconstruction kernel, i.e., B35f) The effective radiation dose for this protocol was <1.5 mSv, providing minimal risk in this cohort of men and women aged 50–75 yr.

All CT scans were analyzed using Pulmonary Workstation 2.0 software (Vida Diagnostics, Iowa City, IA), and the airway tree was reformatted into three-dimensional images (Fig. 1). Briefly, lungs were segmented from the thorax wall, the heart, and main pulmonary vessels. The airways were segmented using a region-growing algorithm starting in the trachea and projecting to the smallest airway visible on the CT scan as previously described (8, 32). The lumen of the airway (Ai, in mm^2), was measured for all identified airway segments at the midpoint between airway branches.

Quantitative densitometric analysis was performed, and areas of CT emphysema were defined as low attenuation areas (LAA) less than −950 Hounsfield units (6). The percentage of LAA was used to estimate the amount of emphysema.

Statistical analyses. Descriptive characteristics were compared between sexes using unpaired *t*-tests. Analysis of variance procedures were used to compare Ai values. To test for associations between selected airways and pulmonary function parameters, simple linear regression analysis using Pearson correlations were performed. A *P* value of <0.05 was considered statistically significant. Values are presented as means ± SD.

RESULTS

Subjects. Subject characteristics are summarized in Table 1. Female and male subjects were matched for age, BMI, smoking history, and spirometry measurements. Males were significantly taller, heavier, and had larger lung volumes. Table 2 summarizes descriptive characteristics for those subjects matched for lung size. Males were slightly, but significantly, older than females. However, indexes of lung size showed that men and women were well matched. Males had a significantly lower FEV_1 %predicted as we purposely selected males who were on average smaller in stature and therefore lung size.

Airway area. Figure 2 shows an airway tree diagram with assigned labels of airway segments. Significant differences were observed between men and women matched for age, BMI, smoking history, and %predicted values for spirometry. The mean values for Ai are shown in Table 3. These data show that men had significantly larger lumen areas compared with women for the larger central airways (trachea, generation 0 through lobar, generation 2) and many of the segmental (generation 3) airways. The association between tracheal lumen area and FVC, as an index of lung size, is shown in Fig. 3, while the remaining correlation coefficients for all the airways measured are summarized in Table 4. The correlation coeffi-

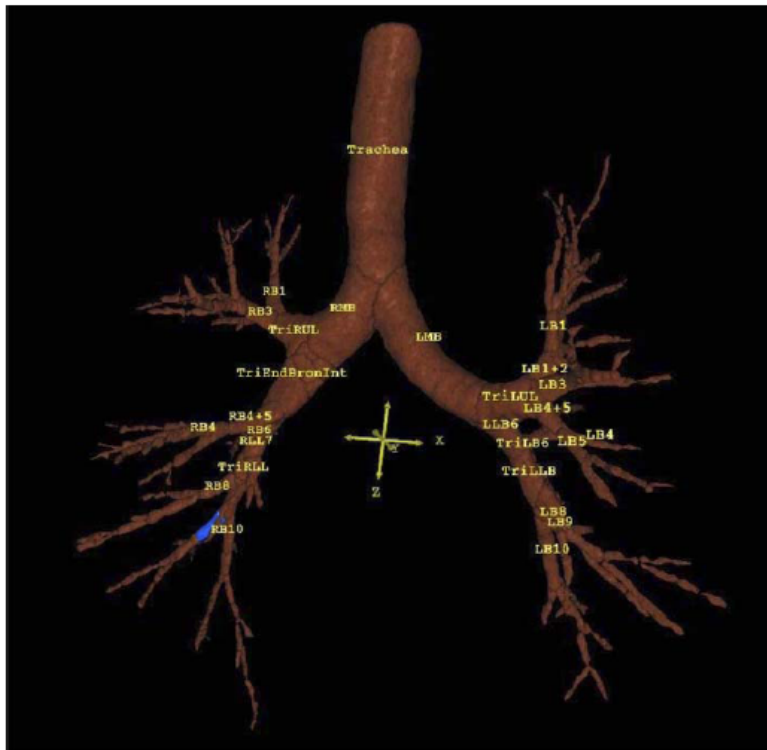Fig. 1. Three-dimensional reconstruction of an airway tree in a 56-yr-old ex-smoking male. Blue region represents the segment that was measured. LMB, left main bronchus; LUL, left upper lobe; LB, left bronchus; LLB, left lower lobe; RMB, right main bronchus; RUL, right upper lobe; RB, right bronchus; BRONINT, intermediate bronchus; RLL, right lower lobe; Tri, trifurcation of an airway into 3 segments.

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Table 1. *Descriptive characteristics of subjects of varying body size*

	Men ($n = 25$)	Women ($n = 25$)
Age, yr	56.6±4.9	58.3±3.4
Height, cm	178.4±5.0*	164.2±6.3
Weight, kg	88.3±11.4*	72.3±8.7
BMI, kg/m²	27.8±3.6	26.9±3.3
Smoking, pack-yr	51.8±18.8	45.8±12.8
$FEV_{1.0}$, liters	3.92±0.77*	2.48±0.45
$FEV_{1.0}$, %predicted	102.1±14.7	96.8±12.6
$FEV_{1.0}/FVC$	78.4±3.4	78.0±4.5
FVC, liters	4.99±1.00*	3.16±0.46
Lung volume at CT scan, %predicted TLC	72.7±10.3	75.4±12.0
Lung volume at CT scan, ml	6008±938*	4549±692
LAA%	2.7±2.0	3.0±1.6

Values are means ± SD. BMI, body mass index; $FEV_{1.0}$, forced expiratory volume in 1 s; FVC, forced vital capacity; TLC, total lung capacity; LAA%, percent low attenuation areas. *Significantly different from female ($P < 0.05$).

cients ranged from 0.53 to 0.76 for the largest of the airways when male and female subjects were pooled together. However, within the male and female groups the magnitudes of these associations were decreased. The more distal airways had less (or no) association with lung size. There were significant associations between Ai and lung size (FVC) for some airways for men and women, but the strength of these relationships can be considered weak to modest. We performed correlational analyses using FVC as an index of lung size to facilitate comparisons with other investigations. We also performed these analyses using lung volume obtained during the CT scan. The relationships we observed were the same regardless of which measure of lung volume was used. Mean lung volumes during supine CT imaging are shown in Tables 1 and 2. These values were in excellent agreement with values obtained during pulmonary function testing, and no systematic changes due to posture were observed between men and women.

Also indicated in Fig. 3 are the specific airway comparisons between men and women matched for lung size. The mean values for Ai are shown in Table 5. These data show that women had significantly smaller lumen areas than men in only the largest airways ($P < 0.05$).

DISCUSSION

In this study we used CT scans of the lungs of men and women to determine if there were differences in the airway size between the sexes. The most important finding of this study is that there are significant male-female differences in the luminal areas of the larger and central airways (trachea generation 0 through lobar generation 2 and many of the segmental airways) that are not accounted for by differences in lung size. When matched for lung size, men and women have similar luminal areas of the more distal airways. Our collective findings provide new evidence for sex-based dysanapsis.

Our findings build on previous studies in several important ways. There have been very few published values for Ai beyond the trachea, and even fewer providing a direct comparison in lumen area between men and women. Those studies that have sought to directly compare airways between the sexes have principally used acoustic reflectance. Acoustic reflectance permits assessment only of the trachea rather than any of the other airways (4, 5, 7, 21, 23). In addition, acoustic reflectance

estimates are made of a tracheal "region" rather than a single anatomic point. With the advancement of high-resolution CT scanning methods we have been able to overcome these limitations and appreciably expand on earlier observations and demonstrate the Ai of the larger and central airways are significantly smaller in women compared with men even when matched for lung volume.

Correlational analyses showed that there were statistically significant but moderate associations between lung size and Ai for the trachea and for the largest airways when male and female subjects were pooled together. However, when subjects were partitioned into separate groups the strength of these associations was reduced or was nonsignificant. We interpret these observations to mean that there is a modest-to-weak relationship between airways and lung parenchymal size. Collectively, our findings are in agreement with the concept of airway-parenchymal dysanapsis (19, 27). Our experimental approach does not provide insight into the potential mechanism(s) of dysanapsis, but there is evidence that there are sex differences in the maturation and physiological function of the lungs in early childhood that persist throughout adolescence and into adulthood (for review see, see Ref. 2).

We compared men and women with overlapping lung volumes to remove the effect of size per se and determine the relationship between airway size and lung parenchyma size. The larger airways were significantly larger in men relative to lung volume-matched women. The magnitude of difference (14–25%) we observed is consistent with previous reports that have shown that the tracheal areas of males are significantly larger than those of females after controlling for lung size (23). Our results confirm those previous findings and further extend their observations by showing that some, but not all, of the airways distal to the trachea are still smaller in women who have the same sized lungs as men. These are important observations if we consider the principles of airflow. Flow through the airway tree depends on the driving pressure and airway resistance. Airway resistance to airflow is affected by several factors: viscosity of the gas, length of the airway, gas density, and radius of the airway. Whether airflow is laminar or turbulent it is strongly dependent on the radius of a given airway as resistance to flow is inversely proportional to airway radius to the fourth power. This relationship, also called Poiseuille's equation, emphasizes that the radius of the airway is the major determinant of airway resistance when airflow is laminar and is typical in airways < 2 mm. In most of the bronchial tree

Table 2. *Descriptive characteristics of subjects matched for lung size*

	Men ($n = 10$)	Women ($n = 11$)
Age, yr	69.2±2.2*	67.0±1.8
Height, cm	175.6±5.7*	170.1±4.6
Weight, kg	88.8±16.5*	69.3±9.2
BMI, kg/m²	28.7±4.7*	24.1±3.2
Smoking, pack-yr	54.6±22.7	42.7±13.2
$FEV_{1.0}$, liters	3.13±0.22	2.98±0.31
$FEV_{1.0}$, %predicted	88.4±9.0*	107.4±9.2
$FEV_{1.0}/FVC$	77.0±3.6	77.0±5.6
FVC, liters	4.08±0.20	3.88±0.35
Lung volume at CT scan, ml	5351±273	5116±922
LAA%	3.9±2.7	3.1±1.9

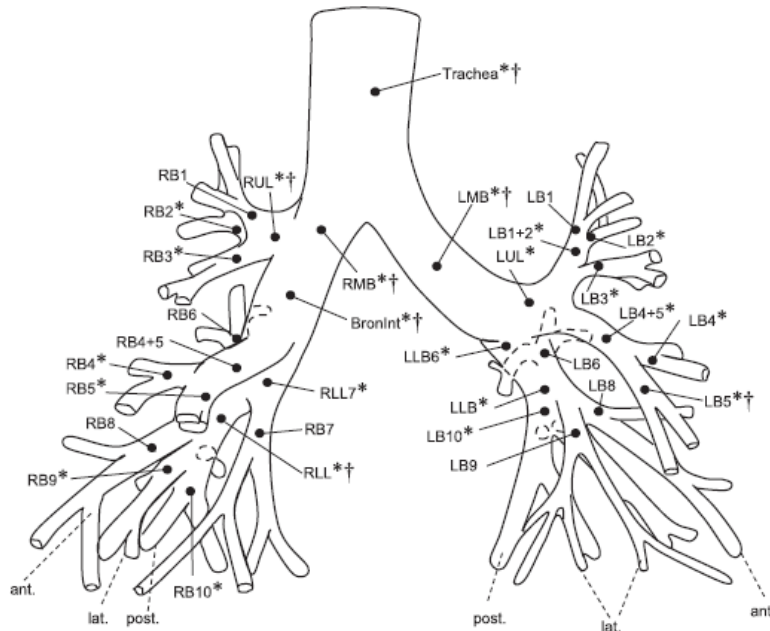Values are means ± SD. *Significantly different from female ($P < 0.05$).

Fig. 2. Airway tree with assigned labels. Labels refer to segments but are assigned to terminating branchpoint of respective segment. Drawing based on Boyden (3). post, posterior; lat, lateral; ant, anterior. *Significant differences between men and women of varying body size ($P < 0.05$). †Significant differences between subjects matched for lung size ($P < 0.05$).

airflow is either transitional or turbulent and is also dependent on the Reynolds number (Re) and is given by:

$$Re = \frac{2rv\text{d}}{n}$$

where d is density, v is average velocity, r is radius, and n is viscosity. Turbulence is most likely to occur when flow is high and airway diameter is relatively large. Importantly, when turbulent airflow occurs, the driving pressure is not proportional to flow and for a given flow the driving pressure must be much greater than that in laminar flow. As described by West (33), turbulence and the Re are higher in the trachea and other larger airways especially during conditions when airflow velocities are high. The main sites of airway resistance ($\sim$80%) are the larger airways (up to 7th generation) whereas the smaller ($<$2 mm) airways contribute $<$20%. Within this context of the findings of the present study and the principles that govern airflow, we would predict that a woman with the same sized lungs as a man would have higher airway resistance and more turbulent airflow. This may be important under physiological conditions where ventilation is high such as dynamic exercise.

The findings of this study yield important insight into sex-based differences in pulmonary structure. The purpose of this

Table 3. Airway luminal cross-sectional area (mm²) for subjects of varying body size

Airway:	Men	Women	%Difference	Airway:	Men	Women	%Difference
Trachea	305.3±46.5	213.7±44.8	30.0*	RMB	196.5±27.3	141.5±26.0	28.0*
LMB	131.8±24.8	93.7±22.8	28.9*	RUL	87.8±20.2	60.6±13.4	30.9*
LUL	92.0±51.1	64.5±11.5	29.9*	RB1	28.1±13.2	23.1±13.9	17.7
LB1 + 2	29.8±7.0	23.1±6.8	22.4*	RB2	26.9±8.6	19.9±6.2	26.2*
LB1	15.8±5.6	12.8±4.8	19.0*	RB3	31.7±10.0	24.9±7.4	21.5*
LB2	13.6±4.2	10.6±5.3	21.4*	BRONINT	119.9±25.5	82.8±12.8	31.0*
LB3	31.8±12.7	24.6±7.7	22.9*	RB4 + 5	33.7±8.2	30.1±7.5	10.8
LB4 + 5	39.9±13.3	26.9±6.5	32.5*	RB4	15.8±4.5	13.3±3.3	15.9*
LB4	17.1±6.3	11.8±3.8	31.0*	RB5	21.5±6.6	17.3±6.5	19.3*
LB5	17.0±5.2	11.4±3.6	33.0*	RLL	40.2±10.8	38.5±15.2	4.1
LLB6	80.2±20.3	67.8±22.6	15.5*	RLL7	61.7±19.3	49.8±15.7	19.2*
LLB	49.2±14.9	40.0±7.7	18.8*	RB6	27.6±9.1	31.5±20.8	−14.1
LB6	31.3±8.6	26.6±8.6	14.9 (P = 0.08)	RB7	23.2±19.7	15.2±4.8	34.5 (P = 0.06)
LB8	25.1±10.1	21.8±5.0	13.2	RB8	19.6±5.4	18.0±5.8	8.4
LB9	21.6±6.1	20.4±14.2	5.7	RB9	20.3±6.3	15.5±4.3	23.4*
LB10	24.8±5.8	20.9±4.5	15.8*	RB10	24.8±7.9	18.4±5.0	25.6*

Values are means ± SD. LMB, left main bronchus; LUL, left upper lobe; LB, left bronchus; LLB, left lower lobe; RMB, right main bronchus; RUL, right upper lobe; RB, right bronchus; BRONINT, intermediate bronchus; RLL, right lower lobe. *Significant difference between men and women ($P < 0.05$).
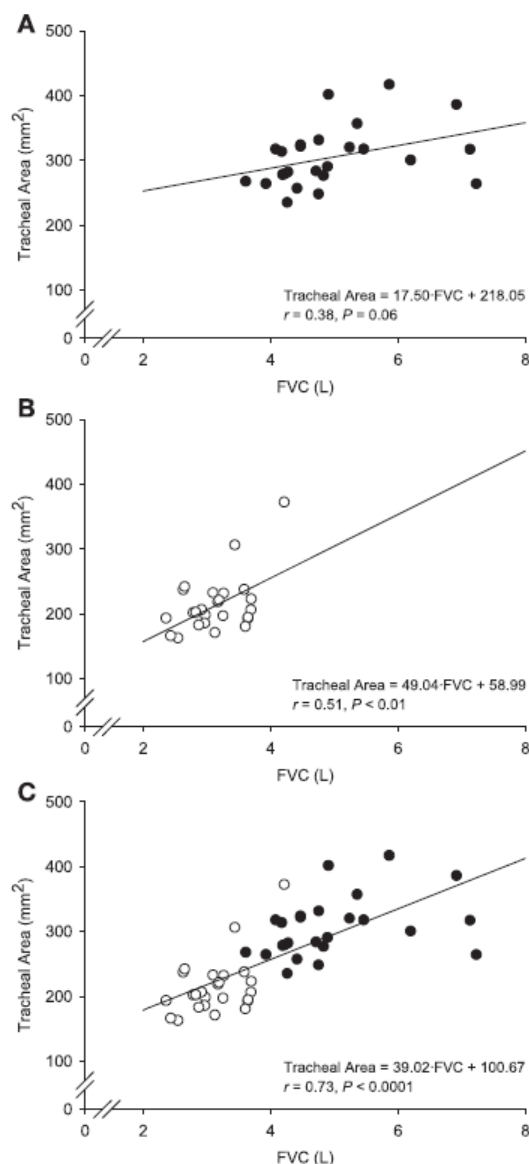
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Fig. 3. Tracheal area vs. forced vital capacity (FVC) in men (*A*), women (*B*), and with all subjects pooled together (*C*). Men are shown in solid circles and women in open circles.

women. Indeed these have all been reported in women and may negatively influence exercise capacity. This argument is supported by three separate sets of evidence. First, ratings of breathlessness and exercise intolerance are more pronounced in women with pulmonary disease (10, 22). This also appears to be the case in healthy older women (60–80 yr) (28). It is well known that ratings of breathlessness are multifactorial but may be related to mechanical constraints or a high work of breathing, among others. Second, there is now sufficient reason to suggest that healthy young women develop significant expiratory flow limitation during dynamic whole body exercise because of their smaller lungs and lower maximal expiratory flow rates, and therefore they may have a higher propensity for developing expiratory flow limitation compared with men (16, 25). Last, with increasing exercise intensity the work of breathing in women significantly increases out of proportion to men (15, 16). A high work of breathing is associated with significant neural and cardiovascular consequences (17, 30, 31) that can limit the ability to perform dynamic exercise (1, 29). Our observations were obtained in ex-smokers undergoing CT scans for clinical diagnostic purposes, and no measures of exercise capacity were made. As such, we are cognizant that the generalizability of our findings is narrow. However, our anatomic observations support the growing list of physiological evidence that points to a female pulmonary system that may be at mechanical disadvantage compared with their male counterparts during exercise.

To this point we have emphasized the differences in airway structure between men and women. It is important to note that many of the airways were indeed similar between men and women matched for lung size (see Table 5). The largest and most central airways were smaller in women, but the more distal airways were not different between men and women. Why would the more distal airways be similar between men and women with the most proximal airways being smaller in women? Moreover, do these similarities in the more distal airways somehow compensate for the differences we observed in the larger airways? Our cross-sectional approach does not provide an explanation for this apparent dissociation, and our observations may be complicated by both genetic and exogenous factors such as prolonged exposure to tobacco smoke or neonatal passive exposure. It is possible that the measurements of the more distal airways have less precision due to the resolution of the CT scanner than do the measurements of the central airways. However, while this size-dependent measurement bias could explain the similarities in the more distally measured airways, we view this as an unlikely explanation (see below).

Methodological considerations. Several aspects of our methodology and subject characteristics should be considered when interpreting our findings. A limitation of this study is that it was not originally designed to study airways in normal subjects. Rather our study was part of a lung cancer-screening cohort. Since the subjects in this study were all middle-aged or older former smokers, we emphasize that caution should be given when comparing our results with those from other subject groups or healthy individuals. Also, extensive remodeling of the small peripheral airways is commonly seen in smokers, particularly those with chronic obstructive pulmonary disease (COPD) (20), and it is thought that there are sex difference in the extent of this remodeling (24). However, our

study was not to relate airway dimensions to lung function under physiological or pathological conditions, but our observations do merit brief comment. For example, how might smaller airway areas influence the integrated pulmonary response to dynamic exercise? We postulate that smaller airway areas may be related to increased sensations of breathlessness, expiratory flow limitation, and higher work of breathing in

Table 4. *Relationship between airway luminal cross-sectional area and forced vital capacity for subjects of varying body size*

Airway:	All Subjects	Men	Women	Airway:	All Subjects	Men	Women
Trachea	$r = 0.73^*$	0.38	0.51^*	RMB	$r = 0.76^*$	0.48^*	0.49^*
LMB	$r = 0.67^*$	0.30	0.59^*	RUL	$r = 0.64^*$	0.34	0.26
LUL	$r = 0.53^*$	0.43^*	0.44^*	RB1	$r = 0.23$	0.04	0.35
LB1 + 2	$r = 0.57^*$	0.33	0.54^*	RB2	$r = 0.42^*$	0.13	0.24
LB1	$r = 0.26$	0.08	0.05	RB3	$r = 0.51^*$	0.47^*	0.16
LB2	$r = 0.28$	−0.06	0.32	BRONINT	$r = 0.66^*$	0.27	0.42^*
LB3	$r = 0.37$	0.06	0.63^*	RB4 + 5	$r = 0.36^*$	0.22	0.52^*
LB4 + 5	$r = 0.59^*$	0.46^*	−0.29	RB4	$r = 0.50^*$	0.45^*	0.38
LB4	$r = 0.39^*$	0.06	0.03	RB5	$r = 0.50^*$	0.47^*	0.47^*
LB5	$r = 0.66^*$	0.65^*	−0.16	RLL	$r = 0.36^*$	0.44^*	0.73^*
LLB6	$r = 0.39^*$	0.31	0.32	RLL7	$r = 0.24$	−0.21	0.47^*
LLB	$r = 0.51^*$	0.33	0.57^*	RB6	$r = -0.02$	0.28	0.01
LB6	$r = 0.45^*$	0.42^*	0.48^*	RB7	$r = 0.45^*$	0.40	0.30
LB8	$r = 0.24$	0.25	−0.38	RB8	$r = 0.29^*$	0.26	0.34
LB9	$r = 0.24$	0.57^*	0.21	RB9	$r = 0.48^*$	0.33	0.17
LB10	$r = 0.46^*$	0.27	0.48^*	RB10	$r = 0.46^*$	0.18	0.34

Values are means ± SD. *Significant correlation ($P < 0.05$).

data focused on relatively "large, central" airways, not the small peripheral airways that cause the airflow limitation in COPD. Furthermore, our study population presented with normal spirometry measurements, and thus most of them were less likely to have COPD. Therefore, we think it is unlikely that the large differences in Ai between men and women observed in the present study can be wholly explained by airway remodeling induced by COPD, or by the sex differences in this airway remodeling. However, further investigations in healthy nonsmokers are needed in this area.

In smokers, luminal area measurements can be influenced by the degree of emphysema, and the effect of tobacco on lung parenchyma and airways could contribute to the difference observed between men and women. In this study we found no difference in the degree of emphysema between groups (see Tables 1 and 2). Moreover, recent findings show that at all levels of disease severity, current and former male smokers with COPD have more extensive CT emphysema than women (6, 11) although this remains controversial. As an explanation for our findings, it possible that the female lungs in the present study had lower recoil and required less distending pressure to

reach TLC, and therefore a less (negative) pressure acting on the airways outside of the lung. This would result in less expansion and smaller cross-sectional area. Our experimental design does not provide a direct avenue of discounting this as a possibility.

Last, it is possible that the measurements we have obtained have been influenced by artifacts within both the CT image acquisition/reconstruction technique and the measurement technique. However, studies that have compared airways measured using CT to the same measurements obtained using histology have consistently shown that while CT underestimates lumen area, this error is systematic and greatest in airways <3 mm in diameter. The airways measured in our study were all above this range (4–20 mm diameter), and therefore we stand by our primary conclusion that the difference in Ai between men and women is an anatomically based phenomenon.

Summary. We used computed tomography to measure airway luminal areas of the large and central airways in men and women using two approaches. We determined the relationship between lung size and airway size in men and women of

Table 5. *Airway luminal cross-sectional area (mm²) of subjects matched for lung size*

Airway:	Men	Women	%Difference	Airway:	Men	Women	%Difference
Trachea	296.1±44.2	238.3±58.1	19.5*	RMB	193.2±28.1	147.3±26.7	23.8*
LMB	124.7±22.7	102.1±27.8	18.1*	RUL	76.6±18.3	65.3±8.7	14.75*
LUL	79.4±18.1	71.4±14.2	10.1	RB1	24.9±11.3	29.2±18.4	−17.3
LB1 + 2	19.2±13.7	21.3±13.1	−10.9	RB2	24.9±6.6	21.5±6.7	13.65
LB1	14.2±5.8	13.6±3.5	4.2	RB3	21.4±12.3	18.7±13.2	12.6
LB2	14.4±5.2	13.4±5.3	6.9	BRONINT	115.6±14.0	86.2±12.0	25.4*
LB3	34.3±19.1	33.1±19.5	3.5	RB4 + 5	34.5±9.2	33.3±5.7	3.5
LB4 + 5	35.7±13.0	29.3±7.9	17.9	RB4	15.3±4.0	15.0±3.9	2.0
LB4	16.9±7.5	13.0±5.2	23.1	RB5	22.0±6.5	21.4±7.3	2.7
LB5	16.6±4.9	11.4±4.2	31.3*	RLL	36.2±9.7	35.0±13.1	3.3
LLB6	73.1±19.4	71.4±22.9	2.3	RLL7	59.7±18.6	57.8±13.0	3.2
LLB	44.7±9.4	46.0±12.2	−2.9	RB6	24.0±11.1	27.8±7.2	−15.8
LB6	29.8±8.7	29.9±9.8	−10.0	RB7	18.9±4.6	16.7±3.4	11.6
LB8	25.7±13.9	21.3±4.4	17.1	RB8	18.9±5.6	20.1±7.1	−6.4
LB9	18.1±5.8	17.7±4.1	2.2	RB9	18.2±5.7	17.1±2.6	6.0
LB10	24.9±5.3	22.9±4.4	8.0	RB10	21.8±7.5	21.1±5.9	3.2

Values are means ± SD. *Significant difference between men and women ($P < 0.05$).

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varying stature and found that the airway luminal areas of the larger and central airways of women are smaller compared with those of men. We removed the effect of biological sex by matching men and women for lung size and found that women continue to have smaller airway luminal areas of the large airways below the tracheal carina. There are previous reports of significant male-female differences in tracheal size that are not accounted for by lung size. However, there are no studies that have determined if these differences exist beyond the trachea. This is the first study to address this question, and our findings provide an important advancement in our understanding of sex-based differences in airway geometry. We conclude that the larger and central airways in females are significantly smaller than those of males even after controlling for lung size.

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Respiratory mechanics during exercise in endurance-trained men and women

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The purpose of this study was to compare the mechanics of breathing including the measurement of expiratory flow limitation, end-expiratory lung volume, end-inspiratory lung volume, and the work of breathing in endurance-trained men ($n = 8$) and women ($n = 10$) during cycle exercise. Expiratory flow limitation was assessed by applying a negative expiratory pressure at the mouth. End-expiratory lung volume and end-inspiratory lung volume were determined by having subjects perform inspiratory capacity manoeuvres. Transpulmonary pressure, taken as the difference between oesophageal and airway opening pressure, was plotted against volume and integrated to determine the work of breathing. Expiratory flow limitation occurred in nine females (90%) and three males (43%) during the final stage of exercise. Females had a higher relative end-expiratory lung volume (42 ± 8 *versus* $35 \pm 5\%$ forced vital capacity (FVC)) and end-inspiratory lung volume (88 ± 5 *versus* $82 \pm 7\%$ FVC) compared to males at maximal exercise ($P < 0.05$). Women also had a higher work of breathing compared to men across a range of ventilations. On average, women had a work of breathing that was twice that of men at ventilations above $90 \, l \, min^{-1}$. These data suggest that expiratory flow limitation may be more common in females and that they experience greater relative increases in end-expiratory lung volume and end-inspiratory lung volume at maximal exercise compared to males. The higher work of breathing in women is probably attributed to their smaller lung volumes and smaller diameter airways. Collectively, these findings suggest that women utilize a greater majority of their ventilatory reserve compared to men and this is associated with a higher cost of breathing.

Expiratory flow limitation during exercise has been reported in both healthy men (Johnson *et al.* 1992) and women (McClaran *et al.* 1998). The presence of expiratory flow limitation may cause reflex inhibition of the hyperventilatory response and/or an alteration in operational lung volumes (Johnson *et al.* 1999). For example, with the onset of expiratory flow limitation, end-expiratory lung volume increases towards resting values. This dynamic hyperinflation permits increases in expiratory flow rates (Pellegrino *et al.* 1993*b*), but this comes at the expense of an increased elastic work of breathing because lung compliance is reduced as lung volume increases. Additionally, dynamic hyperinflation may hasten the fatigue of the respiratory muscles by requiring them to contract from a shorter length, which means that the muscular force required to ventilate the lungs is closer to the muscle's maximal capacity for force generation (Roussos *et al.* 1979).

The substantial ventilatory requirement of male endurance athletes may result in expiratory flow limitation during strenuous exercise (Grimby *et al.* 1971; Johnson *et al.* 1992) although this is not necessarily a universal finding (Mota *et al.* 1999). Studies aimed at understanding the physiology of respiration during exercise have traditionally used male rather than female subjects. Only recently has there been an appreciable understanding that the respiratory responses to exercise in women may be different from that of men. Trained females may be particularly susceptible to developing expiratory flow limitation by virtue of their smaller diameter airways, smaller lung volumes and lower peak expiratory flow rates relative to age- and height-matched men (Mead, 1980; Thurlbeck, 1982; ATS, 1991; McClaran *et al.* 1998). These anatomically based differences ultimately result in a smaller maximum flow–volume loop which may cause women to experience expiratory flow limitation at a lower level of minute ventilation ($\dot{V}_E$) and oxygen consumption ($\dot{V}_{O_2}$) relative to men (McClaran *et al.* 1998; Hopkins & Harms, 2004). Increased susceptibility to expiratory flow limitation would presumably cause an increased level of

dynamic hyperinflation in women. Furthermore, it would be expected, based on mechanical grounds, that women would have a higher work of breathing for any level of absolute $\dot{V}_E$ due to their smaller lung volumes and airways as it is well established that these factors increase pulmonary flow resistance (Briscoe & Dubois, 1958). However, there is no published evidence to show that trained women have a higher work of breathing during exercise compared to men.

The limited number of studies that have attempted to assess expiratory flow limitation in exercising women have utilized the technique of superimposing tidal breaths within the maximum flow–volume loop (McClaran *et al.* 1998; Walls *et al.* 2002). Although this is a widely used method, it may lead to a false detection or overestimation of expiratory flow limitation for two primary reasons. First, the thoracic gas compression artefact of the maximum flow–volume loop may underestimate the true capacity for flow generation (Ingram & Schilder, 1966). Second, the differences in the volume and time history that precede tidal expiration and forced expiratory manoeuvres may lead to a false detection of expiratory flow limitation (D'Angelo *et al.* 1993; Mota *et al.* 1999). An alternative method to determine expiratory flow limitation is to apply a negative expiratory pressure at the mouth and compare the flow–volume curve of the ensuing expiration with the preceding control breath (Valta *et al.* 1994; Mota *et al.* 1999). Unlike the traditional method, the negative expiratory pressure technique does not require forced expiratory efforts or the correction for gas compression. Furthermore, the volume history of the control expiration and the subsequent expiration with the negative expiratory pressure is the same. To our knowledge, the negative expiratory pressure technique, which avoids these caveats, has not been used to assess expiratory flow limitation in female athletes.

Based on the above brief summary, we hypothesized that female endurance athletes with smaller lungs would be at an increased risk of developing expiratory flow limitation at maximal exercise. We also hypothesized that women would have a higher relative increase in operational lung volumes and a higher absolute work of breathing during progressive exercise compared to male endurance athletes.

Methods

Subjects

All experimental procedures and protocols were approved by the Clinical Screening Committee for Research and Other Studies Involving Human Subjects of the University of British Columbia, conforming to the *Declaration of Helsinki*. Each subject provided written informed consent prior to participating in this study. All subjects ($n = 8$ male; $n = 10$ female) were highly trained endurance athletes

(1 runner, 1 rower, 3 triathletes, 13 cyclists) and were excluded on the basis of any previous history of asthma, smoking, or cardiopulmonary disease. Progesterone combined with oestrogen raises both alveolar ventilation and chemosensitivity (Bayliss & Millhorn, 1992; Tatsumi *et al.* 1997). As such, testing of females was limited to the early follicular phase (days 3–8) (Lebrun *et al.* 1995) as determined via a self-reported menstrual history questionnaire. The use of oral contraceptives did not result in exclusion from this investigation.

General protocol

On Day 1 subjects underwent general pulmonary function testing and then sat quietly for 10 min while resting cardiorespiratory and metabolic measures were collected. After a 5 min self-selected warm-up, subjects underwent an incremental cycle test to exhaustion to determine maximal oxygen consumption ($\dot{V}_{O_2max}$). Throughout the exercise test, subjects became familiar with performing inspiratory capacity manoeuvres. Following a brief recovery, subjects then performed 5 min of steady-state submaximal exercise (~70% of maximum work) in order to become familiar with the negative expiratory pressure test. On Day 2, a balloon-tipped catheter was inserted into the oesophagus followed by 10 min of baseline measures. Subjects then performed the identical exercise protocol to that of Day 1. Experimental days were separated by a minimum of 48 h.

Pulmonary function

Forced vital capacity (FVC), forced expiratory volume in 1 s ($FEV_{1.0}$), $FEV_{1.0}/FVC$, and peak expiratory flow were determined using a portable spirometer (Spirolab II, Medical International Research, Vancouver, BC, Canada). Measurements were obtained with subjects seated, utilizing standard protocols and expressed as percentage predicted (ATS, 2002). Subjects with an $FEV_{1.0}/FVC < 80\%$ of predicted were excluded from the investigation. Upon completion of these tests, subjects were instructed to perform several inspiratory capacity manoeuvres from functional residual capacity for familiarization purposes.

Exercise protocol

Exercise was performed on an electronically braked cycle ergometer (Excalibur Sport, Lode, Gronigen, The Netherlands). Males and females started the test at 200 W and 100 W, respectively, with the workload increasing in a stepwise fashion by 30 W every 3 min until volitional exhaustion. This protocol was designed such that men and women would exercise for the same duration. Three minute stages were chosen in order to have steady-state

$\dot{V}_E$ such that expiratory flow limitation and changes in lung volumes could be determined. To determine $\dot{V}_{O_2max}$ on Day 1, subjects wore a nose clip and breathed through a mouthpiece connected to a non-rebreathing valve (model 2700B, Hans-Rudolph, Kansas City, MO, USA). Mixed expired gases were measured using calibrated CO_2 and O_2 analysers (Model CD-3A and Model S-3-A/I, respectively, Applied Electrochemistry, Pittsburgh, PA, USA). Inspiratory flow was measured using a calibrated pneumotachograph (model 3813, Hans Rudolph). The flow signal was integrated to obtain volume, breath timing and frequency, and to determine $\dot{V}_E$. Heart rate was obtained from a telemetric heart rate monitor (Polar Electro, Kempele, Finland) every 30 s. All ventilatory and expired gas data during the exercise test were recorded continuously at 200 Hz (PowerLab/16SP model ML 795, ADI, Colorado Springs, CO, USA) and stored on a computer for subsequent analysis (Chart v5.3, ADInstruments, Colorado Springs, CO, USA).

Expiratory flow limitation

Expiratory flow limitation was determined using the negative expiratory pressure technique as described by Mota *et al.* (1999). Briefly, the negative expiratory pressure technique involves the connection of a Venturi device (207A, model, Raytech Instruments, Vancouver, BC, Canada) to a tank of compressed gas capable of generating a range of negative pressures. A control box operated by the experimenter was used to activate and deactivate the negative expiratory pressure when it received a signal from the pneumotachograph. After expiration was initiated, the system took ~50 ms to reach the desired negative pressure which was set at approximately $-10\ cmH_2O$ (Mota *et al.* 1999). The valve remained opened for the entire expiration and was closed immediately at the onset of the next inspiration. The Venturi device was placed at the distal end of the pneumotachograph, which recorded the tidal flow–volume loops. Volume was obtained by numerical integration of the flow signal. The flow signal was corrected for any offset, using the assumption that inspired and expired volumes of the control breaths preceding the test breaths are the same (Koulouris *et al.* 1995). A minimum of three negative expiratory pressure tests were performed during the final workload as subjects were approaching volitional exhaustion. Expiratory flow limitation was considered present when there was an overlap between the negative expiratory pressure breath and preceding control expiration (Valta *et al.* 1994; Mota *et al.* 1999). The negative expiratory pressure technique has been validated in mechanically ventilated patients with concomitant determination of isovolume flow–pressure relationships (Valta *et al.* 1994) and in resting subjects with chronic obstructive pulmonary disease (Koulouris *et al.* 1995). The negative

expiratory pressure technique has been shown to be a simple and reliable method for determining expiratory flow limitation during exercise (Koulouris *et al.* 1997*a*; Mota *et al.* 1999).

Operational lung volumes

Changes in end-expiratory lung volume and end-inspiratory lung volume were evaluated from measurements of inspiratory capacity at rest, at the end of each exercise stage and immediately prior to cessation of exercise. End-expiratory lung volume was calculated by subtracting the resting FVC from the inspiratory capacity with the assumption that total lung capacity remains constant throughout exercise (Johnson *et al.* 1999). End-inspiratory lung volume was calculated by adding end-expiratory lung volume to the tidal volume. After a full explanation and demonstration to each subject, satisfactory technique and reproducibility of the inspiratory capacity manoeuvres ($\pm \sim 5\%$) were obtained during the familiarization day at rest and also during exercise. Subjects were given additional practice during the warm-up and rest period of Day 2. Prior to performing an inspiratory capacity manoeuvre, subjects were given a verbal prompt for the manoeuvre and encouragement throughout the manoeuvre (O'Donnell *et al.* 1998). Inspiratory capacity trials were considered acceptable when peak inspiratory oesophageal pressure matched those obtained at rest (Younes & Kivinen, 1984; Babb *et al.* 1991; Johnson *et al.* 1995). If subjects failed to achieve the pre-exercise target pressures, they were required to repeat the inspiratory capacity manoeuvre. When analysing the inspiratory capacity data, six breaths were selected prior to the inspiratory capacity manoeuvre in order to monitor changes in pneumotachograph drift and any alterations in breathing pattern immediately prior to the manoeuvre.

Work of breathing

Measurement of oesophageal pressure was obtained using a balloon-tipped catheter (no. 47-9005, Ackrad Laboratory, Cranford, NJ, USA) attached to piezoelectric pressure transducer ($\pm 100\ cmH_2O$; Raytech Instruments Vancouver, BC, Canada). Airway opening pressure was obtained via a port located in the mouthpiece and transferred by polyethylene tubing to a differential piezoelectric pressure transducer ($\pm 100\ cmH_2O$; Raytech Instruments, Vancouver, BC, Canada). Transducers were calibrated using a digital manometer (2021P, Digitron, Torquay UK). Viscous lidocaine (lignocaine) hydrochloride (2%) (Xylocaine, AstraZeneca Canada Inc. Mississauga, Ontario, Canada) was applied in the nasal and pharyngeal passages to minimize discomfort. The catheter was inserted through the nose and positioned

~45 cm down from the nostril (Milic-Emili *et al.* 1964). After the balloon was inserted, all the air was evacuated by pulling back on a syringe plunger until the plunger returned to a non-vacuum position. One millilitre of air was injected in order to partially inflate the balloon and catheter according to the manufacturer's specifications. Validity of the balloon pressure was checked by having the subjects blow against an occluded airway (Baydur *et al.* 1982). If trans-pulmonary pressure remained constant while airway opening pressure increased, placement was considered appropriate. Trans-pulmonary pressure was calculated as the difference between oesophageal pressure and airway opening pressure. The work of breathing was obtained by ensemble averaging several breaths and then using a customized software program (LabVIEW software V6.1, National Instruments) to integrate the averaged trans-pulmonary pressure–tidal volume loop (Otis, 1964). The work of breathing was then multiplied by breathing frequency to represent the amount of work done per minute by the respiratory system. The method for analysing the work of breathing was similar to previous studies where the work of breathing was compared between two groups (Milic-Emili *et al.* 1962; Cibella *et al.* 1999). The individual raw work of breathing data was plotted against a range of ventilations obtained during exercise. To facilitate comparison between men and women, curves corresponding to each subject were calculated assuming the following relationship between the work of breathing (W_b) and $\dot{V}_E$:

$$W_b = a\dot{V}_E^3 + b\dot{V}_E^2 \qquad (1)$$

This equation is based on the original work of Rohrer (1915) and is described in the classic work of Otis *et al.* (1950). The term $b\dot{V}_E^2$ represents the mechanical work done in overcoming the viscous resistance offered by the lung tissues to deformation and by the respiratory tract to the laminar flow of air. The term $a\dot{V}_E^3$ represents the work done in overcoming the resistance to turbulent flow. A value for constants a and b was then determined for each individual subject. All flow and pressure signals were amplified, filtered (low-pass) at 50 Hz, and digitized at 200 Hz by a 16-bit analog-to-digital converter (200B, Direc Physiologic Recording System; Raytech Instruments) using the data acquisition software program (Direc/Win version 2.21, Raytech Instruments Inc.) and stored for subsequent analysis.

Statistical analysis

Repeated measures ANOVA (Statistica 6.1, Stat Soft Inc., Tulsa, OK, USA) was used to examine differences in end-expiratory lung volume and end-inspiratory lung volume across different workloads. If significant F-ratios were detected, Tukey's *post hoc* test was applied to determine where the differences occurred.

Table 1. Subject characteristics

	Men (n = 8)	Women (n = 10)
Age (years)	25.9 ± 4.9	24.7 ± 2.8
Height (cm)	183.9 ± 6.6	$168.6 \pm 4.7^*$
Mass (kg)	76.6 ± 9.8	$63.3 \pm 4.2^*$
BMI (kg m^{-2})	22.6 ± 1.8	22.3 ± 0.9
BSA (m^2)	1.99 ± 0.15	$1.72 \pm 0.08^*$
FVC (l)	5.6 ± 1.0	$4.5 \pm 0.5^*$
FVC (%predicted)	105 ± 14	108 ± 13
FEV$_{1.0}$ (l)	5.1 ± 1.0	$3.8 \pm 0.5^*$
FEV$_{1.0}$ (%predicted)	106 ± 18	107 ± 13
FEV$_{1.0}$/FVC (%)	85.3 ± 4.2	84.5 ± 5.3
FEV$_{1.0}$/FVC (%predicted)	101 ± 5	98 ± 6
PEF (l s^{-1})	12.6 ± 1.4	$8.0 \pm 1.1^*$
PEF (%predicted)	124 ± 11	116 ± 18

Values are means $\pm$ s.d. Definitions of abbreviations: BMI, body mass index; BSA, body surface area; FVC, forced vital capacity; FEV$_{1.0}$, forced expiratory volume in 1 s; FEV$_{1.0}$/FVC, forced expiratory volume in 1 s/forced vital capacity; PEF, peak expiratory flow. *Significantly different from men ($P < 0.001$).

Mean comparisons between groups were performed using unpaired *t* tests with Bonferroni corrections where appropriate. Pearson product moment correlations were used to determine linear relationships between selected dependent variables. The level of significance was set at $P < 0.05$ for all statistical comparisons.

Results

Subject characteristics

Twenty-one endurance-trained subjects volunteered to participate in this study but two (1 male, 1 female) were excluded on the basis of an FEV$_{1.0}$/FVC $< 80\%$ of predicted and another was excluded on the basis of an irregular menstrual cycle. Subject characteristics are shown in Table 1. Women were significantly smaller in terms of height, mass and body surface area ($P < 0.001$). Women had a significantly smaller FVC, FEV$_{1.0}$ and peak expiratory flow compared to men ($P < 0.001$). All subjects were within or greater than predicted values for all pulmonary function variables and there were no significant differences between men and women ($P > 0.05$).

Exercise data

Table 2 shows metabolic, ventilatory and performance characteristics at maximal exercise on Day 1. Men had a significantly higher absolute and relative $\dot{V}_{O_2,max}$ compared to women ($P < 0.01$). Men had a significantly higher V_T ($P < 0.05$) and $\dot{V}_E$ ($P < 0.001$) compared to women at maximal exercise. Men achieved a higher power output at maximal exercise ($P < 0.001$). By design, duration of exercise tests were the same between men and women.

Table 2. Peak exercise values on Day 1

	Men (n = 8)	Women (n = 10)
$\dot{V}_{O_2}$ (ml kg^{-1} min^{-1})	69.5 ± 7.8	$59.8 \pm 4.8^*$
$\dot{V}_{O_2}$ (l min^{-1})	5.3 ± 0.7	$3.8 \pm 0.4^*$
$\dot{V}_{CO_2}$ (l min^{-1})	5.7 ± 0.6	$4.1 \pm 0.5^*$
RER	1.08 ± 0.07	1.07 ± 0.04
F_b (breaths min^{-1})	59 ± 9	58 ± 5
V_T (l)	3.1 ± 0.4	$2.3 \pm 0.3^*$
$\dot{V}_E$ (l min^{-1})	161 ± 25	$120 \pm 18^*$
$\dot{V}_E/\dot{V}_{O_2}$	30.6 ± 4.9	31.6 ± 3.0
$\dot{V}_E/\dot{V}_{CO_2}$	28.2 ± 3.0	29.6 ± 3.0
$\dot{V}_E/BSA$ (%)	81 ± 9	$69 \pm 9^*$
T_i (s)	0.47 ± 0.04	0.47 ± 0.07
T_e (s)	0.51 ± 0.07	0.52 ± 0.11
T_{tot} (s)	0.98 ± 0.09	0.99 ± 0.17
T_i/T_{tot}	0.48 ± 0.03	0.48 ± 0.03
T_e/T_{tot}	0.52 ± 0.03	0.52 ± 0.03
HR (beats min^{-1})	189 ± 8	190 ± 11
Exercise duration (s)	1273 ± 189	1229 ± 163
Peak power (W)	376 ± 30	$265 \pm 26^*$

*Significantly different from men ($P < 0.05$). Values are means $\pm$ s.d. Definitions of abbreviations: $\dot{V}_{O_2}$, oxygen consumption; $\dot{V}_{CO_2}$, carbon dioxide production; RER, respiratory exchange ratio; F_b, breathing frequency; V_T, tidal volume; $\dot{V}_E$, minute ventilation; $\dot{V}_E/\dot{V}_{O_2}$, ventilatory equivalent for oxygen; $\dot{V}_E/\dot{V}_{CO_2}$, ventilatory equivalent for carbon dioxide; $\dot{V}_E/BSA$, minute ventilation/body surface area; HR, heart rate; T_i, inspiratory time; T_e, expiratory time; T_{tot}, total time ($T_i + T_e$).

Expiratory flow limitation

Figure 1A and B shows individual flow–volume curves at maximal exercise in women and men, respectively. Subjects were considered flow limited if part of the negative expiratory pressure breath overlapped the preceding control breath. One male subject (subject 3) was excluded in the analysis of expiratory flow limitation because the negative expiratory pressure caused a sustained decrease in expiratory flow using previously defined criteria (Valta et al. 1994; Tantucci et al. 1999). Expiratory flow limitation was shown to occur in 3 of the 7 male subjects and 9 of the 10 female subjects during the final stage of exercise. Subject 3 (Fig. 1A) was the only female that did not develop expiratory flow limitation. Of all female subjects she also had the largest lungs (134% predicted FVC).

Overcoming expiratory flow limitation

Since multiple negative expiratory pressure tests were obtained throughout the final exercise stage, it could be determined if subjects could become flow limited early in a given exercise stage and later in the same stage, become non-flow limited. Several subjects who demonstrated

expiratory flow limitation (6 female, 1 male) during the initial portion of the final stage of exercise were later able to overcome the expiratory flow limitation. Figure 2 is representative of a single female subject (subject 9) who experienced expiratory flow limitation early during the final exercise stage but was later able to avoid the expiratory flow limitation through an alteration in her breathing pattern. As expected, in those subjects who demonstrated expiratory flow limitation and then did not later in the same exercise stage, there was no appreciable decline in $\dot{V}_E$ as the exercise progressed, but V_T decreased slightly and was coupled with an increase in F_b.

Operational lung volumes

Figure 3 shows changes in end-expiratory lung volume and end-inspiratory lung volume expressed as percentage of FVC at rest and during exercise in men and women. One female subject was not included in the analysis of lung volumes due to her inability to correctly perform inspiratory capacity manoeuvres. Mean values for end-expiratory lung volume decreased from rest in men and women with the onset of exercise. End-expiratory lung volume remained significantly below resting end-expiratory lung volume in men throughout all exercise intensities whereas females increased end-expiratory lung volume at 89% and 100% of maximum exercise workload (W_{max}), such that end-expiratory lung volume was no longer significantly different from rest. At maximum exercise, women had a significantly higher relative end-expiratory lung volume compared to men (42 ± 8 versus $35 \pm 5\%$FVC, $P < 0.05$). Mean end-inspiratory lung volume in men continued to increase from rest and throughout exercise but plateaued between 90 and 100% of W_{max} whereas mean end-inspiratory lung volume rose throughout all exercise intensities in women. At maximal exercise, relative end-inspiratory lung volume was significantly higher in women compared to men (88 ± 5 versus $82 \pm 7\%$FVC, $P < 0.05$).

Work of breathing

Figure 4 shows the individual raw traces relating the mechanical work of breathing and $\dot{V}_E$. The curves are of a continually increasing slope meaning that the mechanical work of breathing per any additional unit of air ventilated ($dW_b/d\dot{V}_E$) increases progressively with increasing $\dot{V}_E$. Without exception, the curve for each subject fits eqn (1) where the mean $r^2 = 0.99$. Mean values for constants a and b are shown in Table 3. Constant a was significantly higher in women compared to men ($P < 0.05$) but no differences were detected for constant b ($P > 0.05$). A mean curve for men and women was constructed based on the average values for each constant (Fig. 5). The curves

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have been extrapolated to $200 \, l \, min^{-1}$ for theoretical and visual purposes only. Constant b did not correlate with any variables in this study. However, constant a was significantly correlated with a number of variables as

shown in Table 4. The majority of significant correlations were only demonstrated when men and women were pooled into a single group. No variables were significantly correlated with constant a in men. However, $FEV_{1.0}$ and

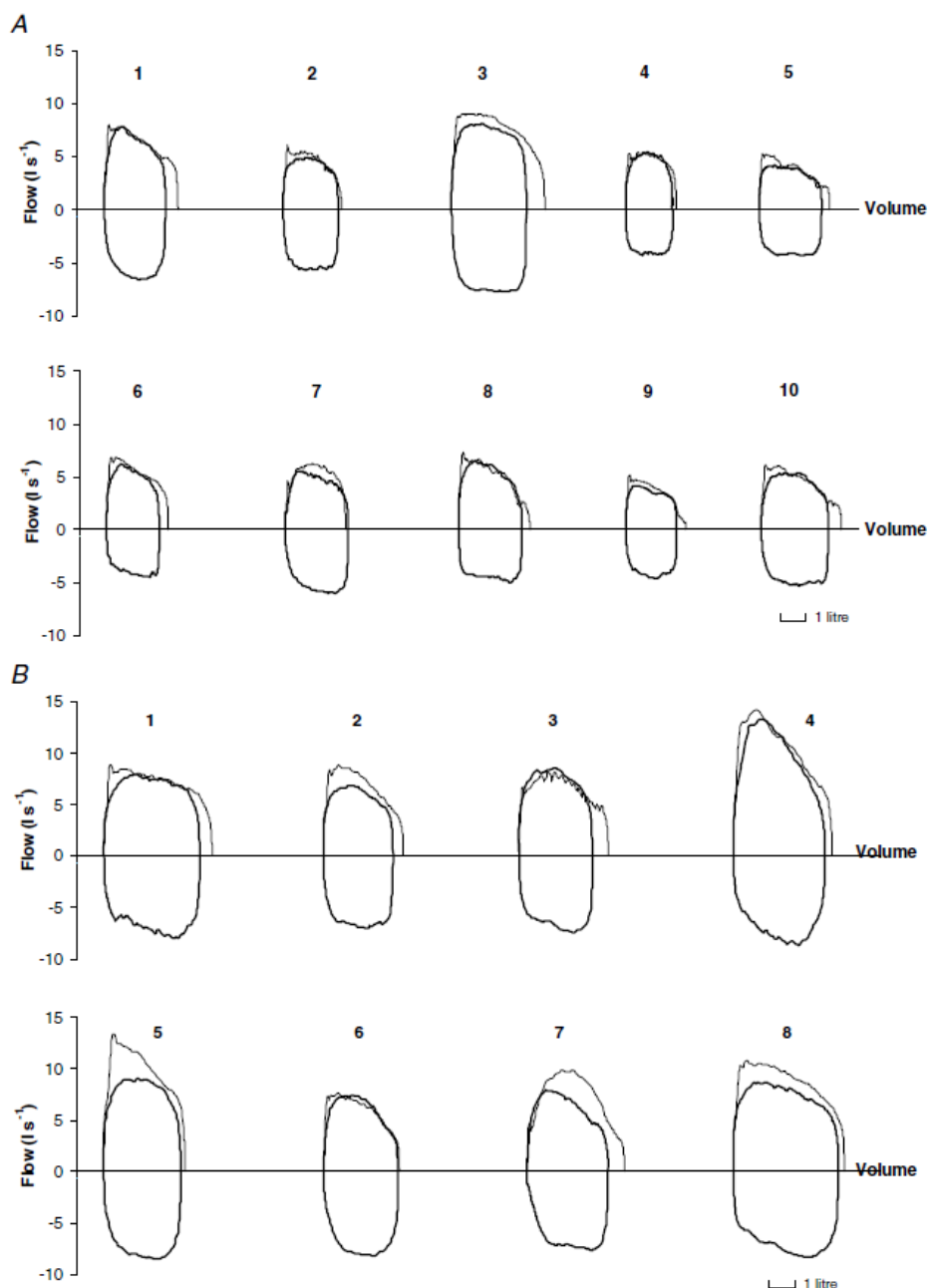**Figure 1**
Individual tidal flow–volume loops during the final stage of exercise in women (A; $n = 10$) and men (B; $n = 8$).
Dark lines represent the control breath and thin lines represent the negative expiratory pressure breath.

peak expiratory flow were correlated with constant *a* in women. There was also a trend for $FEV_{1.0}/FVC$ to be related to constant *a* in women ($r = -0.62$; $P = 0.08$). Figure 6 shows the work of breathing plotted against relative values of $\dot{V}_{O_2max}$. The work of breathing was not different between men and women at 75, 90 and 100% of $\dot{V}_{O_2max}$.

Discussion

This study was designed to systematically compare the mechanics of breathing in male and female endurance athletes during progressive exercise to exhaustion. Ours is the first investigation to utilize the negative expiratory pressure technique to assess expiratory flow limitation and is the only study which has measured the mechanical work of breathing in a group of female endurance athletes during exercise. The principle findings of this study are threefold. First, female endurance athletes experience expiratory flow limitation more frequently and at a lower level of $\dot{V}_E$ compared to male endurance athletes. Second, females experience greater dynamic hyperinflation during heavy exercise. Third, the total mechanical work of breathing is higher in females compared to males during progressive exercise. Our findings point to a female pulmonary system which is at a mechanical disadvantage compared to their male counterparts during exercise.

Expiratory flow limitation

We found that expiratory flow limitation occurs in both endurance-trained men and women at maximal exercise. However, expiratory flow limitation occurred more frequently in trained women even though maximal $\dot{V}_E$ was considerably lower than in the men. Nearly all women experienced expiratory flow limitation (90%) at maximal exercise, whereas expiratory flow limitation only occurred in 43% of males. This difference is probably attributable to the inherent differences in the structural and functional characteristics of the male and female pulmonary systems (Mead, 1980; Thurlbeck, 1982; ATS, 1991; McClaran *et al.* 1998). The women in this study had significantly smaller lungs and lower peak expiratory flow rates compared to men. The findings in the present study extend those of McClaran *et al.* (1998) and Guenette *et al.* (2004) that suggest women are more likely to utilize a greater percentage of their ventilatory reserve during exercise compared to men.

Endurance-trained humans are a unique model to understand respiratory mechanics during exercise because they have extremely high metabolic and ventilatory demands even though their pulmonary function (i.e. lung volumes and flow rates) is not markedly different from that of sedentary individuals (Reuschlein *et al.* 1968). The high ventilatory requirement could predispose them to expiratory flow limitation. However, the few investigations that have assessed expiratory flow limitation in endurance athletes have reported conflicting results with no studies making direct comparisons between men and women. Mota *et al.* (1999) suggest that expiratory flow limitation is rare in male endurance athletes whereas Johnson *et al.* (1992) have shown the opposite. The discrepancy may be due, in part, to methodological considerations associated with the assessment of expiratory flow limitation. As mentioned, the traditional method of assessing expiratory flow limitation involves the placement of tidal flow–volume loops within a maximum flow–volume loop and positioned according to end-expiratory lung volume. Koulouris (2002) has summarized a number of methodological concerns with this method. For example,
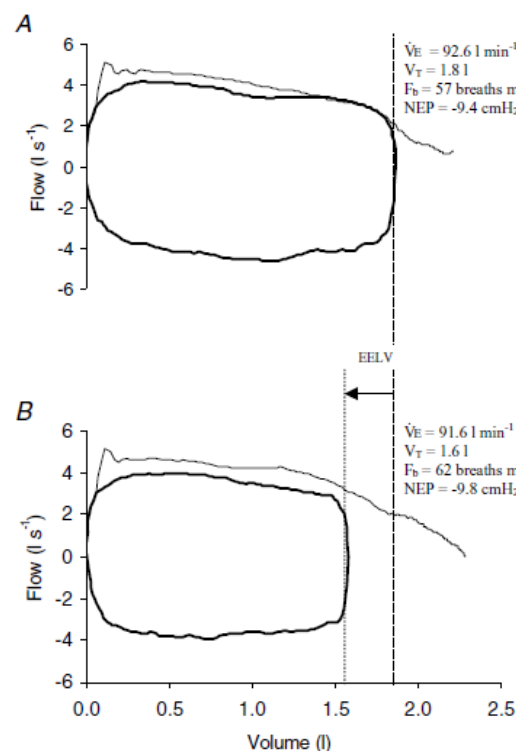
Figure 2
A, representative flow–volume loops of a single female subject (subject 9) experiencing expiratory flow limitation during the final stage of exercise as evidenced by the overlap between the control breath (dark line) and the negative expiratory pressure breath (thin line). *B*, flow–volume loops collected later during the final stage of exercise in the same subject. She is no longer flow limited because the negative expiratory pressure increased expiratory flow for the entire duration of the expiration. $\dot{V}_E$, minute ventilation; V_T, tidal volume; F_b, breathing frequency; NEP, negative expiratory pressure; $\Delta EELV$, change in end-expiratory lung volume.

the thoracic gas compression artefact of the maximum flow–volume loop may lead to a false detection or overestimation of expiratory flow limitation (Ingram & Schilder, 1966). In addition, differences in the volume and time history that precede tidal expiration and forced expiratory manoeuvres may result in a false detection of expiratory flow limitation (D'Angelo *et al.* 1993, 1994; Koulouris *et al.* 1997b). Since the volume and time history of a spontaneous tidal breath is different from that of an FVC manoeuvre, it is possible that the traditional method may be problematic, even when correction has been made for thoracic gas compression (Koulouris, 2002). The negative expiratory pressure technique is advantageous in that it does not require the correction for gas compression and the time history is the same between the control and negative expiratory pressure breaths. Using the negative expiratory pressure method, Mota *et al.* (1999) demonstrated that only 1 out of 9 male cyclists developed expiratory flow limitation at maximal exercise which is considerably lower than that previously reported in men (Johnson *et al.* 1992). Given these findings, it

remains possible that expiratory flow limitation may have previously been overestimated in women during exercise.

McClaran *et al.* (1998) measured expiratory flow limitation in a group of fit ($\dot{V}_{O_2max} = 62.9$ ml kg^{-1} min^{-1}) and less fit ($\dot{V}_{O_2max} = 48.1$ ml kg^{-1} min^{-1}) women and found evidence of expiratory flow limitation in 12 of the 14 (86%) fit women and 4 of the 15 (27%) less fit women. Using a similar technique, Walls *et al.* (2002) found expiratory flow limitation in 7 out of 8 (88%) recreationally active women ($\dot{V}_{O_2max} = 46.8$ ml kg^{-1} min^{-1}). Both studies examining expiratory flow limitation in women have utilized the traditional approach, and given the findings of Mota *et al.* (1999) in men with the negative expiratory pressure technique, it is possible that they overestimated expiratory flow limitation in women. However, the results from the present study are in excellent agreement with previous expiratory flow limitation studies in women, despite the fact that we used a technique that is less likely to incorrectly characterize subjects as being flow limited.
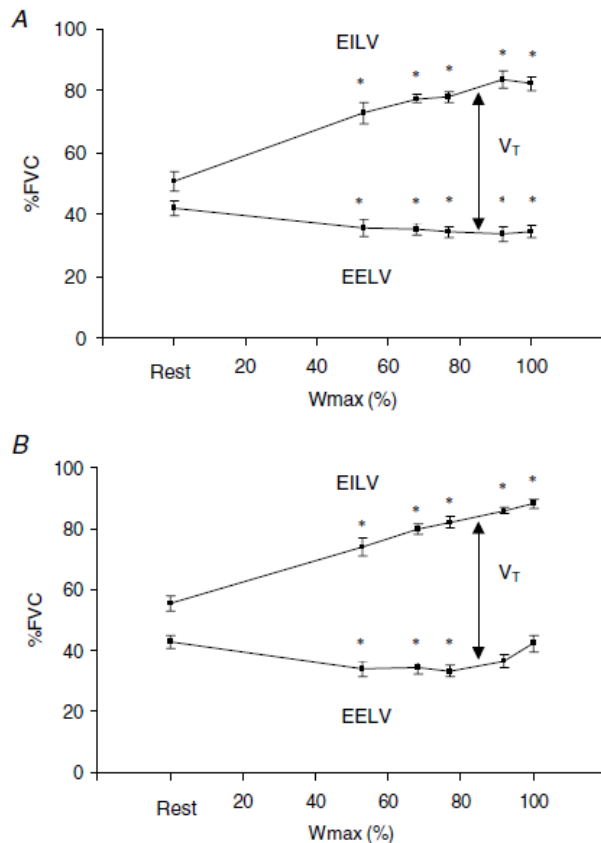

Figure 3
Subdivision of lung volumes, expressed as percentage forced vital capacity (FVC) at rest and during progressive exercise to maximal workload (W_{max}) in men (*A*) and women (*B*). EILV, end-inspiratory lung volume; EELV, end-expiratory lung volume; V_T, tidal volume. Values are means ± s.e.m. *Significantly different from rest ($P < 0.05$).

Table 3. Mean values of constants *a* and *b* from eqn (1)

	Men (n = 8)	Women (n = 9)
a (J min^{-1})/(l min^{-1})^3 ($\times$ 10^{-5})	6.4 ± 4.1 (2.5–14.0)	19.0 ± 14.4* (7.0–54.8)
b (J min^{-1})/(l min^{-1})^2 ($\times$ 10^{-3})	6.8 ± 3.2 (2.0–10.3)	6.4 ± 7.4 (−5.2–14.9)

Constant *a* represents the work done in overcoming the resistance to turbulent flow. Constant *b* represents the work done in overcoming viscous resistance (see text). Values are means ± s.d. with ranges in parentheses. *Significantly different from men ($P < 0.05$).

To this point, we have presented our findings as suggesting that athletes either do or do not develop expiratory flow limitation at maximal exercise. Indeed, this is a commonly used representation of expiratory flow limitation during exercise (McClaran *et al.* 1998; Mota *et al.* 1999; Walls *et al.* 2002). However, we favour the concept that expiratory flow limitation is not an all-or-none phenomenon but rather it is a physiological situation where compensatory 'strategies' are available. For example, seven (6 female and 1 male) subjects in this study developed expiratory flow limitation during the final workload but were later assessed as being non-flow-limited according to the negative expiratory pressure test. Figure 2 illustrates a typical example of this where there was a general trend for subjects to slightly decrease V_T and increase F_b such that total $\dot{V}_E$ did not change. This tachypnoeic breathing pattern probably occurred because increasing V_T would have been too difficult due to the expiratory flow limitation and the increasing elastic load on inspiration. When the tidal flow–volume loops are aligned, it can be seen that end-expiratory lung volume shifts, resulting in the avoidance of expiratory flow limitation. Importantly, it should be noted that the magnitude of the negative expiratory pressure was identical in both tests. It should also be noted that there may have been a bronchodilatory response which enabled some subjects to overcome expiratory flow limitation. Our finding that some subjects exhibit expiratory flow limitation and then do not, could partially explain the discrepancy between the work of Mota *et al.* (1999) and Johnson *et al.* (1992).
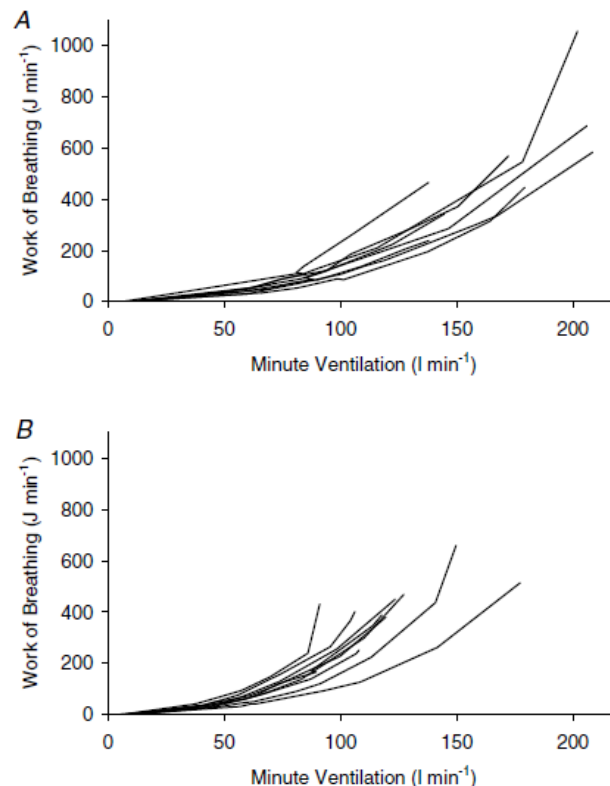

Figure 4
Individual responses of the work of breathing *versus* minute ventilation in men (*A*) and women (*B*).

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Operational lung volumes

It is well known that end-expiratory lung volume decreases during exercise in men (Lind & Hesser, 1984; Sharratt *et al.* 1987; Henke *et al.* 1988*b*; Johnson *et al.* 1992; Mota *et al.* 1999) and this was also observed in the present study. Henke *et al.* (1988*a*) suggest that mechanical feedback from the lung and chest wall cause active expiration and reductions in functional residual capacity. Decreasing end-expiratory lung volume may then optimize diaphragm length and in turn lower or minimize the inspiratory work of breathing. Inspiratory work of breathing is reduced by recovering some of the work done by the expiratory muscles during the previous expiration (Collett & Engel, 1986; Road *et al.* 1986; Henke *et al.* 1991; Johnson *et al.* 1991). Although men and women demonstrated similar changes in end-expiratory lung volume throughout the majority of submaximal exercise, there were significant differences as subjects approached maximal exercise (Fig. 3*B*). The women in this study increased end-expiratory lung volume such that it was no longer significantly below resting values. In fact, when normalized to FVC, the women in this study had a significantly higher end-expiratory lung volume compared to men at maximal exercise. This is in contrast to a recent study which found no differences in operational lung volumes between men and women when normalized to FVC (Vogiatzis *et al.* 2005). The difference between these studies is probably a function of subject fitness levels. Vogiatzis *et al.* (2005) used untrained subjects with women only achieving a maximal $\dot{V}_E$ of 69 l min^{-1} whereas subjects in the present study ventilated at a much higher rate (120 l min^{-1}). These untrained women did not increase end-expiratory lung volume at maximal exercise because their ventilatory load was probably insufficient to elicit expiratory flow limitation. Therefore, we would
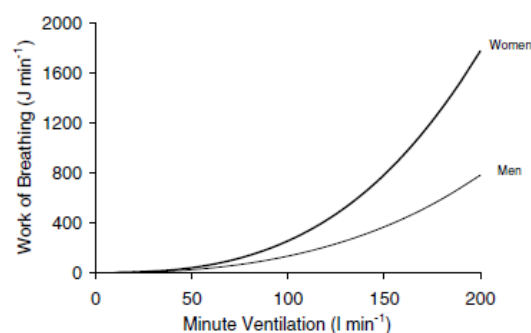
Table 4. Correlation analysis of spirometry data with constant *a*

Variable *vs.* constant *a*	All ($n = 17$)	Men ($n = 8$)	Women ($n = 9$)
FVC	−0.54*	NS	NS
FEV$_{1.0}$	−0.59*	NS	−0.69*
FEV$_{1.0}$/FVC	−0.49*	NS	−0.62
PEF	−0.68*	NS	−0.76*

Definitions of abbreviations: FVC, forced vital capacity; FEV$_{1.0}$, forced expiratory volume in 1 s; FEV$_{1.0}$/FVC, forced expiratory volume in 1 s/forced vital capacity; PEF, peak expiratory flow. NS, non-significant correlation ($P > 0.05$). *Statistically significant correlation ($P < 0.05$).

argue that our results at submaximal exercise are in good agreement with the findings of Vogiatzis *et al.* (2005) and the difference at maximal exercise between these studies is probably due to the presence or absence of expiratory flow limitation.

Increases in end-expiratory lung volume have previously been shown to increase in men that are capable of achieving very high levels of muscular work and the increases are often associated with the presence of expiratory flow limitation (Olafsson & Hyatt, 1969; Grimby *et al.* 1971; Johnson *et al.* 1992). Increasing end-expiratory lung volume permits utilization of the higher flow rates available at larger lung volumes. Pellegrino *et al.* (1993*a*) investigated the effect of expiratory flow limitation on end-expiratory lung volume by imposing an expiratory threshold load in male subjects. They concluded that the increase in end-expiratory lung volume during exercise is associated with expiratory flow limitation and that compression

Figure 5
Mean curves relating the work of breathing to minute ventilation in men (thin line) and women (thick line). Both curves are based on mean values of constants *a* and *b* from eqn (1). Each curve has been extrapolated to 200 l min^{-1}.
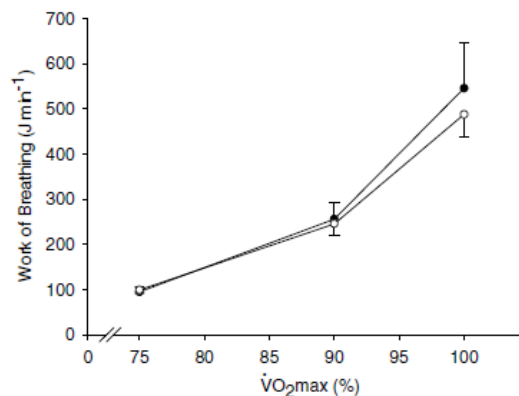

Figure 6
Work of breathing at various percentages of maximal oxygen consumption ($\dot{V}_{O_2max}$) in men (●) and women (○). Values are means ± S.E.M.

of airways downstream from the flow-limited segment may elicit a reflex mechanism that influences breathing pattern by prematurely terminating expiration. McClaran *et al.* (1998) also examined the effect of expiratory flow limitation on end-expiratory lung volume in a group of women by having subjects breath a helium inspirate (79% He, 21% O_2) in order to increase the size of the maximum flow–volume loop and thus eliminate expiratory flow limitation. Reducing expiratory flow limitation caused subjects to maintain a lower end-expiratory lung volume. Furthermore, the lower end-expiratory lung volume occurred only when HeO_2 caused a significant reduction of expiratory flow limitation. These findings are consistent with ours and those of Pellegrino *et al.* (1993*a*) implying an interrelationship between expiratory flow limitation and the increase in end-expiratory lung volume associated with heavy exercise.

This study has also shown that relative end-inspiratory lung volume is significantly higher at maximal exercise in women compared to men. Given the higher end-expiratory lung volume in women, it is not surprising then that end-inspiratory lung volume is also higher as this occurs in order to preserve the exercise V_T. The end-inspiratory lung volumes in these women are approaching levels that may be considered a marker of ventilatory constraint and an index of increased ventilatory work (Belman *et al.* 1996). The higher end-inspiratory lung volume would likely increase the elastic load on the inspiratory muscles over a greater portion of the tidal breath relative to men.

Work of breathing

The relationship between work of breathing and $\dot{V}_E$ during exercise has been examined in several studies (Milic-Emili *et al.* 1962; Coast *et al.* 1993; Cibella *et al.* 1999). However, all of the above-mentioned studies were exclusively conducted with male subjects. Since women generally have smaller vital capacities compared to height-matched men, it would be expected that women would have an increased elastic work of breathing. It would also be expected that the dimensions of the airways would be smaller or fewer in number. When coupled with a smaller vital capacity, the resistance to flow would consequently be higher (Otis, 1954). As expected, the men were able to achieve a higher $\dot{V}_E$ and V_T compared to women at maximal exercise. Figure 5 shows the mean relationship between the work of breathing and $\dot{V}_E$ in men and women. It can be seen that the work of breathing is essentially the same at rest and during very low levels of exercise $\dot{V}_E$ (i.e. $< 60\,l\,min^{-1}$). However, as $\dot{V}_E$ increases beyond this point, the work of breathing in women significantly increases out of proportion to men. In fact, the work of breathing in women is approximately twice that of men at ventilations beyond $90\,l\,min^{-1}$. Thus,

the physiological cost of moving a given amount of air in and out of the lungs is substantially higher in women. The present results are in good agreement with Topin *et al.* (2003) who suggest a higher O_2 cost of breathing in women.

The method of analysis in the present study is similar to those used by other researchers that have compared the work of breathing between two groups (Milic-Emili *et al.* 1962; Cibella *et al.* 1999). Constant *a* was significantly higher in women which may be an indication that the higher work of breathing in women is associated with the additional work needed to overcome the resistance to turbulent flow. This may explain why the magnitude of the difference between men and women increased out of proportion with increasing levels of $\dot{V}_E$. Based on mechanical grounds, it would be expected that subjects with larger lung volumes would have lower pulmonary resistance (Briscoe & Dubois, 1958) and thus a lower work of breathing for a given level of $\dot{V}_E$. The women in this study had significantly smaller lungs compared to the men and this may be one of many major reasons for their higher work of breathing. We observed inter-individual variability for constants *a* and *b* which may be related to the differences in lung volumes (see Table 3 for range). Consistent with the concept of differences in lung volumes was the observation that when men and women were pooled together, there was a significant, albeit modest, correlation between lung volume and constant *a* (Table 4). Moreover, the female with the largest lungs (subject 3) had the lowest work of breathing relative to other women and she did not demonstrate expiratory flow limitation.

Although we did not measure airway diameter, the increased resistive work in women is probably dependent on their inherently smaller diameter airways (Mead, 1980). It was noted that constant *a* was significantly correlated with $FEV_{1.0}$ and peak expiratory flow in women and also when all subjects were pooled together. Since the shape of the maximum flow–volume loop is determined, in part, by the diameter of the airways, it seems likely that the relatively strong and significant negative correlations between constant *a* and the $FEV_{1.0}$ and peak expiratory flow is an indication that airway diameter is partially responsible for the higher work of breathing in women. Although differences in lung volume and airway diameter are explanations for the present findings we make this claim cautiously as it is based on correlative rather than causative evidence.

In this study we have shown that women are more likely to have expiratory flow limitation, hyperinflation and increased work of breathing. When expressed at the same level of absolute minute ventilation women have higher work of breathing (Fig. 5). When expressed at a relative intensity men and women appear to have a similar work of breathing (Fig. 6). We interpret our collective findings to mean that at a given workload expiratory flow limitation is more common in women because their ventilations are

higher and equally or more importantly their maximum flow–volume envelopes are smaller. Comparing the work of breathing at equal relative intensities brings the work of breathing data together because the ventilations are substantially different between men and women at a given absolute work rate. Men and women have a similar work of breathing at 100% $\dot{V}_{O_2max}$ and yet women have a minute ventilation that is substantially lower ($\sim$25%) than their male counterparts. Therefore, a similar work of breathing at 100% $\dot{V}_{O_2max}$ between men and women suggests that the physiological cost of breathing is higher in women.

Critique of methods

The negative expiratory pressure technique has been validated in mechanically ventilated patients (Valta *et al.* 1994) and in resting patients with chronic obstructive pulmonary disease (Koulouris *et al.* 1995). It has been used during exercise in competitive cyclists (Mota *et al.* 1999) and in healthy untrained individuals and patients with chronic obstructive pulmonary disease (Koulouris *et al.* 1997*a*). Koulouris *et al.* (1997*a*) suggest that the negative expiratory pressure technique is more reliable for assessing expiratory flow limitation during exercise compared to conventional methods. However, some have questioned the validity of this technique during exercise because the negative expiratory pressure may induce reflex inhibition of expiratory muscle activity which leads to a decrease in expiratory driving pressure (de Bisschop *et al.* 2006). These authors suggest that expiratory flow limitation is dependent on the magnitude of the negative pressure. For example, they found evidence of expiratory flow limitation when a 5 hPa ($\sim$5.1 cmH$_2$O) negative pressure was used but not when the negative pressure was increased to 9 hPa ($\sim$9.2 cmH$_2$O). We used a negative pressure of approximately -10 cmH$_2$O which should be sufficient to avoid any false detection of expiratory flow limitation (Mota *et al.* 1999). Figure 2 illustrates that a subject can be flow limited or not flow limited irrespective of the negative pressure used.

The measurement of end-expiratory lung volume was determined using inspiratory capacity manoeuvres. End-expiratory lung volume may be altered by performing inspiratory capacity manoeuvres which could happen as subjects prepare to perform the manoeuvre or transiently after the manoeuvre is performed (Johnson *et al.* 1999). To account for any alterations in end-expiratory lung volume, several breaths were monitored prior to the inspiratory capacity manoeuvre so that the necessary corrections could be made. Subjects were also given extensive practice during the familiarization day and also during the experimental testing day. Since inspiratory capacity manoeuvres are dependent on subject motivation, it is possible that subjects will not fully inspire to total lung capacity when

performing the manoeuvre. To account for this potential limitation, continuous measurements of oesophageal pressure were made so that maximal inspiratory pressure during the inspiratory capacity manoeuvres could be monitored. If the maximal inspiratory pressure obtained during exercise was similar to that obtained repeatedly at total lung capacity at rest, there can be confidence that total lung capacity was reached during the manoeuvres (Younes & Kivinen, 1984; Babb *et al.* 1991; Johnson *et al.* 1995). If subjects failed to achieve the pre-exercise target pressures, they were required to repeat the inspiratory capacity manoeuvre during exercise. Therefore, it is unlikely that the inspiratory capacity was underestimated at any point during exercise.

In estimating the forces involved in breathing there are inherent assumptions that merit discussion. Our estimation of the work of breathing does not take into account the flow-resistive work done on tissues of the thorax and abdomen. Although this is considered to make up a relatively small portion of the total work of breathing, particularly during increasing levels of $\dot{V}_E$ (Agostoni, 1961), we acknowledge that our work of breathing values may in fact underestimate the total mechanical work of breathing. Within the calculation of the work of breathing, several factors have previously been considered negligible. As Otis *et al.* (1950) described, the respiratory system is almost continuously accelerating and decelerating and the work to overcome inertia probably contributes to the overall work of breathing. The kinetic energy imparted to the air may also be a relevant factor. However, the often used equation to determine the work of breathing (see eqn (1)) does not include these potential influences as they have been typically considered minor. It is possible that with high levels of ventilation during exercise these typically neglected influences may be of greater importance. To our knowledge quantifiable estimates of inertia and kinetic energy are not available. However, given the magnitude of difference we observed between men and women for the work of breathing it is unlikely that our overall conclusion and interpretation would be different by including these additional influences.

Conclusions

The results from this study indicate that the pulmonary system of endurance-trained females may be at a disadvantage compared to their male counterparts during intense exercise. We demonstrated that female athletes tend to develop expiratory flow limitation more frequently than male athletes. It was also observed that women have higher relative increases in end-expiratory lung volume and end-inspiratory lung volume at maximal exercise. Finally, women tend to have a higher work of breathing across a wide range of ventilations during exercise compared to men. Many of the differences

observed in this study are due to the smaller lungs and presumably the smaller diameter airways in women. Several studies have reported that women may be susceptible to pulmonary system limitations during exercise including exercise-induced arterial hypoxaemia (Harms *et al.* 1998; Richards *et al.* 2004) and expiratory flow limitation (McClaran *et al.* 1998). These studies, in combination with the present findings, suggest that the female pulmonary system may be at a disadvantage compared to their male counterparts during intense exercise. However, our understanding of female pulmonary responses to exercise remains incomplete. In order to fully address questions of sex-based differences future studies must consider the importance of matching subjects for age, body and lung size, and aerobic capacity.

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Sex differences in the resistive and elastic work of breathing during exercise in endurance-trained athletes

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Guenette JA, Querido JS, Eves ND, Chua R, Sheel AW. Sex differences in the resistive and elastic work of breathing during exercise in endurance-trained athletes. *Am J Physiol Regul Integr Comp Physiol* 297: R166–R175, 2009. First published May 6, 2009; doi:10.1152/ajpregu.00078.2009.—It is not known whether the high total work of breathing (WOB) in exercising women is higher due to differences in the resistive or elastic WOB. Accordingly, the purpose of this study was to determine which factors contribute to the higher total WOB during exercise in women. We performed a comprehensive analysis of previous data from 16 endurance-trained subjects (8 men and 8 women) that underwent a progressive cycle exercise test to exhaustion. Esophageal pressure, lung volumes, and ventilatory parameters were continuously monitored throughout exercise. Modified Campbell diagrams were used to partition the esophageal-pressure volume data into inspiratory and expiratory resistive and elastic components at 50, 75, 100 l/min and maximal ventilations and also at three standardized submaximal work rates (3.0, 3.5, and 4.0 W/kg). The total WOB was also compared between sexes at relative submaximal ventilations (25, 50, and 75% of maximal ventilation). The inspiratory resistive WOB at 50, 75, and 100 l/min was 67, 89, and 109% higher in women, respectively ($P < 0.05$). The expiratory resistive WOB was 131% higher in women at 75 l/min ($P < 0.05$) with no differences at 50 or 100 l/min. There were no significant sex differences in the inspiratory or expiratory elastic WOB across any absolute minute ventilation. However, the total WOB was 120, 60, 50, and 45% higher in men at 25, 50, 75, and 100% of maximal exercise ventilation, respectively ($P < 0.05$). This was due in large part to their much higher tidal volumes and thus higher inspiratory elastic WOB. When standardized for a given work rate to body mass ratio, the total WOB was significantly higher in women at 3.5 W/kg (239 ± 31 vs. 173 ± 12 J/min, $P < 0.05$) and 4 W/kg (387 ± 53 vs. 243 ± 36 J/min, $P < 0.05$), and this was due exclusively to a significantly higher inspiratory and expiratory resistive WOB rather than differences in the elastic WOB. The higher total WOB in women at absolute ventilations and for a given work rate to body mass ratio is due to a substantially higher resistive WOB, and this is likely due to smaller female airways relative to males and a breathing pattern that favors higher breathing frequency.

respiratory mechanics; pulmonary ventilation; breathing pattern

A GROWING NUMBER OF INVESTIGATIONS aimed at characterizing the healthy female respiratory response to exercise have reported sex-based differences in pulmonary gas exchange (8, 18) and respiratory mechanics (7, 12). These studies show that young adult women free from respiratory disease may be more susceptible to pulmonary limitations during exercise, which is likely associated with women having smaller lungs and airways relative to size-matched males (13, 23).

There have been few attempts to systematically compare the work of breathing (WOB) between sexes (7, 9). We recently demonstrated that the WOB was higher in endurance-trained women at moderate to high levels of minute ventilation compared with trained males with no differences in the total WOB when comparisons were made at different percentages of maximal aerobic capacity ($\dot{V}_{O_{2max}}$) (7). On average, women had a WOB that was approximately twice as high as men at ventilatory rates above 50 l/min. However, the analysis used in this previous study was limited in that it did not provide specific information regarding the individual components that make up the total WOB. The WOB can be subdivided into the work of the respiratory muscles to overcome the elasticity of the lung during inspiration, the work required to overcome airflow resistance during inspiration, the work of the expiratory muscles to overcome the elastic outward recoil of the chest wall, and the work required to overcome airflow resistance during expiration. It is currently unknown which of these factors contribute to the higher total WOB in women compared with men.

On the basis of mechanical grounds, we hypothesized that the resistive WOB would be higher in women because of their inherently smaller diameter airways (11, 13). However, this hypothesis may be an oversimplification because it is unknown whether women adopt a unique breathing pattern to minimize one WOB component at the expense of another. To this end, we reanalyzed data from our previous investigation (7) by partitioning the respiratory pressure-volume data into four distinct WOB components across a range of ventilations and also at 3 different body mass-corrected work rates achieved by all subjects. The WOB was compared at different mass-corrected work rates to determine whether the WOB is higher for a given level of external muscular work. Furthermore, we examined sex differences in breathing pattern to determine the effect of tidal volume and breathing frequency on the WOB.

METHODS

Subjects. Sixteen endurance trained athletes (8 men and 8 women) volunteered to participate in this study. Endurance-trained athletes were used instead of untrained individuals because they are capable of generating higher levels of minute ventilation compared with their untrained counterparts. This permits physiological comparisons across a wider range of values. Moreover, the mechanical work of breathing appears to be independent of fitness level (14). The subjects gave informed written consent and all experimental procedures received institutional ethical approval and conformed to the Declaration of Helsinki. All subjects were healthy nonsmokers and did not have any previous history of cardiopulmonary disease. Subjects with a forced expired volume in 1 s ($FEV_{1.0}$) to forced vital capacity (FVC) ratio of <80% of predicted were excluded from the investigation.

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Experimental overview. Subjects participated in two testing sessions separated by a minimum of 48 h. All women were tested during the early follicular phase (*days 3* to *8*) of the menstrual cycle as determined via a self-reported menstrual history questionnaire. On the first day, subjects performed general spirometry to assess lung function and an incremental cycle test to exhaustion to determine $\dot{V}_{O_{2max}}$. They also received extensive practice on how to perform inspiratory capacity maneuvers at rest and during exercise. The second day served as the primary testing day, which included 10 min of seated quiet breathing followed by an incremental cycle test to exhaustion using the identical exercise protocol as used on *day 1*.

Pulmonary function. FVC, $FEV_{1.0}$, $FEV_{1.0}/FVC$, and peak expiratory flow were obtained using routine spirometry according to standardized procedures and expressed using prediction equations (1).

Maximal cycle exercise. Subjects performed an incremental test to exhaustion on a cycle ergometer using a step protocol. Men and women began cycling at 200 W and 100 W, respectively, with the work rate increasing by 30 W every 3 min. Ventilatory and mixed expired metabolic parameters were assessed using a customized metabolic cart consisting of a calibrated pneumotachograph (model 3813; Hans Rudolph, Kansas City, MO) and calibrated CO_2 and O_2 analyzers (Models CD-3A and Model S-3-A/I, respectively; Applied Electrochemistry, Pittsburgh, PA).

Flow, volume, and pressure. Inspiratory and expiratory flow was measured using a heated and calibrated pneumotachograph (model 3813, Hans Rudolph, Kansas City, MO) attached to a mouthpiece. Inspiratory and expiratory volume was obtained through numerical integration of the flow signal. Esophageal pressure (P_{eso}) was obtained by placing a 10-cm-long latex balloon (no. 47–9005; Ackrad Laboratory, Cranford, NJ) ~45 cm down from the nostril (15) after application of a local anesthetic. All air was removed from the balloon by having subjects perform a Valsalva maneuver. The balloon was then inflated with 1 ml of air as per manufacturer specifications. P_{eso} was measured using a calibrated piezoelectric pressure transducer (±100 cmH_2O; Raytech Instruments, Vancouver, BC, Canada).

End expiratory lung volume. End-expiratory lung volume (EELV) was determined by having subjects perform inspiratory capacity (IC) maneuvers at rest and during exercise as previously described (7). Two to three IC maneuvers were obtained near the middle and end of each 3-min exercise bout and additional IC maneuvers were performed immediately prior to exhaustion. EELV was calculated as the difference between FVC and the IC volume. FVC was used to calculate EELV rather than total lung capacity because it was not possible to measure residual lung volume in our subjects. FVC maneuvers were performed before and immediately after exercise with the largest FVC value being used for the analysis.

Work of breathing. The muscular WOB was determined using modified Campbell diagrams as described by Roussos and Campbell (19) and using the technique of Yan et al. (25). Flow, volume, and pressures from several breaths (~5–20) corresponding to ~50, 75, and 100 l/min and 25, 50, 75, and 100% of maximal ventilation were selected for each subject and ensemble averaged using a customized software program (Bibo, LabVIEW software V6.1; National Instruments, Austin, TX). The same procedure was performed to compare the WOB at 3.0, 3.5, and 4.0 W/kg. These ventilatory rates and workloads were selected because nearly all male and female subjects were successfully able to reach these values. Additional Campbell diagrams were also generated for each stage of exercise to determine the relationship between average inspiratory flow and the inspiratory resistive WOB. An example of the modified Campbell diagram in a representative male subject at ~75% of maximal minute ventilation (i.e., 115 l/min) is shown in Fig. 1. A line was drawn connecting the points of zero flow (i.e., EELV and EILV) representing the dynamic compliance of the lung. The compliance of the chest wall was derived using previously published data (3) based on both age and sex as done by others (2, 4, 21, 25). The chest wall compliance line was positioned through functional residual capacity (EELV at rest) and extended to
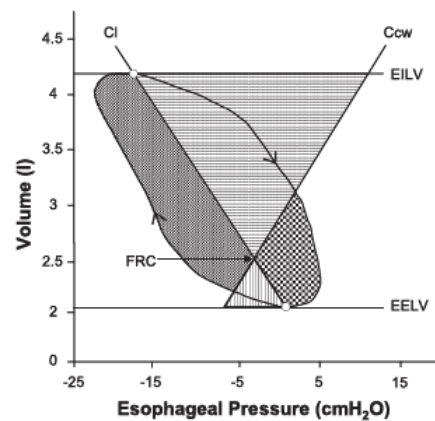

Fig. 1. Example of a modified Campbell diagram obtained from a male subject during exercise at ~75% of maximum ventilation (115 l/min). Oblique hatching represents the inspiratory resistive work of breathing. Horizontal hatching represents the inspiratory elastic work of breathing. Stippling represents the expiratory resistive work of breathing. Vertical hatching represents the expiratory elastic work of breathing. FRC, functional residual capacity; EELV, end-expiratory lung volume; EILV, end-inspiratory lung volume; Cl, dynamic lung compliance; Ccw, chest wall compliance. Upward arrow represents inspiration and downward arrow represents expiration. Small open circles represent zero flow points.

EELV and EILV. The muscular WOB was then partitioned into four separate components. The area inside of the P_{eso}-loop to the left of the lung compliance line (oblique hatching) represents the work needed to overcome airflow resistance during inspiration (i.e., inspiratory resistive work). The area enclosed by the lung compliance and chest wall compliance lines (horizontal hatching) represents the work needed to overcome lung elasticity (i.e., inspiratory elastic work). The area to the right of the chest wall compliance line (stippling) represents the active muscular work needed to overcome airflow resistance during expiration (i.e., expiratory resistive work). Lastly, the area between the lung and chest wall compliance lines below functional residual capacity (vertical hatching) represents the work needed to overcome the outward elastic recoil of the chest wall to maintain EELV below functional residual capacity (i.e., expiratory elastic work). The sum of all four areas shown in Fig. 1 represents the total WOB. All WOB values were multiplied by breathing frequency, representing a unit of power (i.e., J/min). However, as conventionally used, we will refer to this throughout the manuscript as the WOB rather than the power of breathing.

Data processing. All raw data were recorded continuously at 200 Hz using a 16-channel data acquisition system (PowerLab/16SP model ML 795, AD Instruments, Colorado Springs, CO) and stored on a computer for subsequent analysis (Chart v5.3, AD Instruments).

Statistical analysis. Descriptive characteristics were compared between sexes using unpaired *t*-tests. Preplanned comparisons were used to compare men and women for the various WOB components and the ventilatory parameters at the target ventilations and work rates using unpaired *t*-tests. Linear regression analysis using Pearson correlations was performed to test for associations between specific WOB components and pulmonary function parameters. The α level was set a priori at 0.05 for all statistical comparisons. Values were presented throughout the manuscript as means ± SD with the exception of Figs. 2, 4, 5, 6, and 7, where values are reported as means ± SE.

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RESULTS

Subject characteristics. The subjects used in the present investigation completed a study that has been previously published (7). The present study has 8 males and 8 females, while our previous manuscript reported data on 8 males and 10 females. Two females were excluded from the present analysis because they did not have P_{eso} data ($n = 1$) or were unable to correctly perform IC maneuvers ($n = 1$). Males and females were not different for age (25.9 ± 4.9 vs. 24.9 ± 3.1 yr, respectively), but men were taller (183.9 ± 6.6 vs. 168.8 ± 4.0 cm, $P < 0.0001$), heavier (76.6 ± 9.8 vs. 64.3 ± 3.6 kg, $P < 0.01$) and had a higher Vo_{2max} (69.5 ± 7.8 vs. 59.2 ± 4.7 ml·kg^{-1}·min^{-1}, $P < 0.01$). Table 1 summarizes the pulmonary function data for the present study. As expected, women had smaller FVC, $FEV_{1.0}$, and peak expiratory flows compared with men. All subjects were within normal values for all pulmonary function measures except peak expiratory flows, which were typically $>120\%$ of predicted. There were no significant sex differences in percent predicted values for any pulmonary function parameters.

Work of breathing vs. absolute minute ventilation. Figure 2 shows the total WOB (*A*) and the constituent components of the WOB (*B–E*) at comparable absolute ventilations and at maximal ventilation in men and women. The total WOB was significantly higher in women at 75 and 100 l/min but not at 50 l/min. The total WOB at 50, 75, and 100 l/min was 23, 33, and 48% higher in women, respectively, but was 45% higher in men at maximal ventilations. The inspiratory resistive WOB was 67, 89, and 109% higher in women at 50, 75, and 100 l/min, respectively ($P < 0.05$), while the expiratory resistive WOB was only significantly higher in women at 75 l/min. The expiratory resistive WOB at 75 l/min was 131% higher in women. There was no significant difference in the elastic WOB during inspiration or expiration at any absolute ventilation. However, the inspiratory elastic WOB was 42% higher in men at maximal ventilations ($P = 0.05$). Figure 3 shows Campbell diagrams for an individual male and female subject matched for minute ventilation, tidal volume, and breathing frequency. This figure demonstrates the significantly higher pressures needed to maintain the same ventilatory loads resulting in a much higher inspiratory and expiratory resistive work, with little difference in elastic work.

Work of breathing vs. relative submaximal minute ventilation. The total WOB plotted against percentages of maximal minute ventilation is shown in Fig. 4. Men had a significantly higher total WOB for any given percentage of maximal ven-

tilation compared with women. The total WOB was 120, 60, and 50% higher in men at 25, 50, and 75% of maximal minute ventilations, respectively. While each component of the WOB was higher in men for a given percentage of minute ventilation, the largest (and statistically significant) differences were seen with the inspiratory elastic WOB (data not shown). The absolute ventilations in men vs. women corresponding to 25, 50, and 75% of maximal ventilations were 48.4 ± 6.5 vs. 32.8 ± 7.0 l/min, 90.3 ± 14.7 vs. 62.9 ± 12.2 l/min, and 134.9 ± 22.1 vs. 94.9 ± 18.4 l/min, respectively. Thus, the absolute ventilations were, on average, 31% higher in men when comparing sexes at the aforementioned relative minute ventilations. The tidal volumes were 75, 37, and 36% higher in men at 25, 50, and 75% of maximal minute ventilations ($P < 0.05$), respectively with little to no difference in breathing frequency.

Work of breathing vs. external muscular work. Figure 5 shows the total WOB (*A*), inspiratory resistive WOB (*B*), and expiratory resistive WOB (*C*) vs. work rate (corrected for body mass) in men and women. Figure 5A demonstrates that the total WOB was significantly higher in women at ~3.5 W/kg (239 ± 31 vs. 173 ± 12 J/min, $P < 0.05$) and 4.0 W/kg (387 ± 53 vs. 243 ± 36 J/min, $P < 0.05$). The inspiratory resistive WOB was higher in women at ~3.0 W/kg (56 ± 8 vs. 41 ± 4 J/min, $P = 0.05$), 3.5 W/kg (93 ± 15 vs. 56 ± 5 J/min, $P < 0.05$) and 4.0 W/kg (162 ± 24 vs. 81 ± 2 J/min, $P < 0.05$). The expiratory resistive WOB was higher in women at ~3.5 W/kg (44 ± 11 vs. 20 ± 3 J/min, $P < 0.05$) and 4.0 W/kg (67 ± 16 vs. 35 ± 8 J/min, $P = 0.05$). There were no significant differences in the inspiratory or expiratory elastic components at any work rate.

Breathing pattern. Figure 6 shows the breathing frequency (*A*) and tidal volume (*B*) response to exercise. It can be seen that men achieved a maximal minute ventilation that was considerably higher than women (180.2 ± 28.7 vs. 126.2 ± 24.2 l/min, respectively). Generally, women breathed with a significantly higher breathing frequency and lower tidal volume to achieve the same absolute minute ventilation as men. Breathing frequency was significantly different between sexes at 75 and 100 l/min while tidal volume was attenuated in women at all ventilations above 50 l/min. Figure 7 shows the effect of tidal volume (*A*) and breathing frequency (*B*) on the inspiratory elastic WOB at the four venitlatory points (i.e., 50, 75, 100 l/min and maximal ventilation). For any given tidal volume, the inspiratory elastic WOB is considerably higher in women. However, the inspiratory elastic WOB is lower in women for any given breathing frequency. Although these are physiologically significant observations, specific statistical procedures could not be performed on the data presented in Fig. 7.

Lung size vs. work of breathing. Figure 8 summarizes the relationships between different components of the WOB and FVC with all subjects pooled together. The WOB values shown in this figure are from a minute ventilation corresponding to 100 l/min. We chose to report data at 100 l/min for the regression analysis for three reasons. First, we wanted to report data at the highest range of absolute ventilations when the work and metabolic cost of breathing are highest. Second, this ventilation tended to show the largest sex-based difference in the total WOB and inspiratory resistive WOB. Finally, all subjects achieved 100 l/min of ventilation during exercise. FVC was used as a surrogate of total lung capacity (less

Table 1. *Pulmonary function data*

	Men ($n = 8$)	Women ($n = 8$)
FVC, liters	6.0 ± 0.3 (4.5–7.2)	4.5 ± 0.2 (3.8–5.4)*
FVC, %predicted	105 ± 5 (84–125)	108 ± 5 (92–134)
$FEV_{1.0}$, liters	5.1 ± 0.4 (3.5–6.4)	3.8 ± 0.2 (3.0–4.5)*
$FEV_{1.0}$, %predicted	106 ± 6 (80–133)	106 ± 5 (80–131)
PEF, l/s	12.6 ± 0.5 (10.4–14.8)	8.1 ± 0.4 (6.5–10.0)*
PEF, %predicted	124 ± 4 (109–138)	118 ± 7 (91–148)
$FEV_{1.0}/FVC$, %	85.3 ± 1.5 (78.3–88.6)	84.6 ± 2.1 (74.3–92.4)
FEV1.0/FVC, %predicted	101 ± 2 (92–106)	98 ± 2 (86–109)

FVC, forced vital capacity; $FEV_{1.0}$, forced expired volume in 1 s; PEF, peak expiratory flow. *Significantly different from men ($P < 0.01$). Ranges are presented in parentheses.
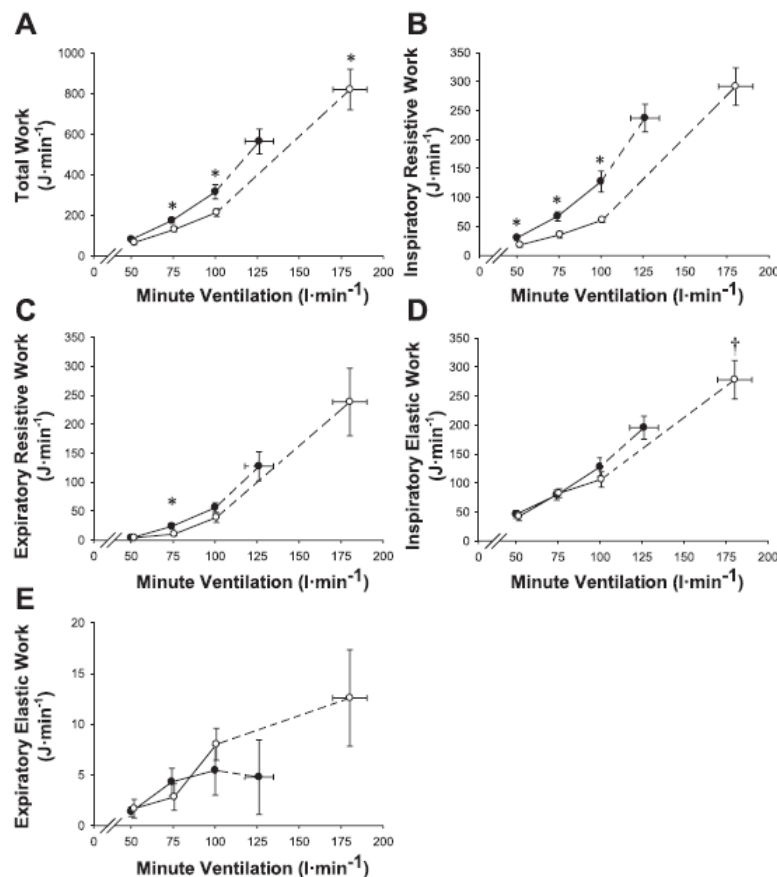
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Fig. 2. Total work of breathing (*A*), inspiratory resistive work (*B*), expiratory resistive work (*C*), inspiratory elastic work (*D*), and expiratory elastic work (*E*) vs. minute ventilation in men (○) and women (●). The last data point represents maximal minute ventilation. Values are mean ± SE. *Significantly different between groups ($P < 0.05$); †$P = 0.05$.
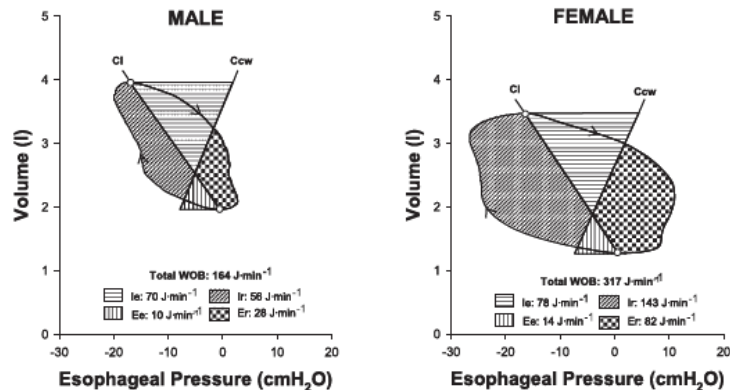
residual volume) to determine the relationship between lung size and the WOB. FVC was significantly and linearly related to the total WOB, the inspiratory resistive WOB, and the expiratory resistive WOB. When partitioned into individual groups, the correlation coefficients relating FVC to the total WOB, the inspiratory resistive WOB, and expiratory resistive WOB in women was 0.92 ($P = 0.001$), 0.78 ($P = 0.02$), and 0.73 ($P = 0.04$), whereas the men were 0.46 ($P = 0.25$), 0.27 ($P = 0.52$), and 0.75 ($P = 0.03$), respectively.

Flow vs. work of breathing. The inspiratory resistive WOB was plotted against the corresponding average inspiratory flow throughout all exercise intensities in each individual subject as shown in Fig. 9*A*. All of the raw data points from Fig. 9*A* were fitted with a second-order polynomial (mean r^2 for all subjects = 0.99 ± 0.01) to produce a mean curve for all men and women as shown in Fig. 9*B*. Fig. 9, *A* and *B* show that the inspiratory resistive WOB was higher in women for any given flow rate above ~2 l/s, and the magnitude of this difference increased disproportionally in women with increasing flow. Figure 9*B* also shows the response of an individual female subject with FVC and peak expiratory flows that were 134 and 148% above predicted values, respectively.

DISCUSSION

Our present understanding of the WOB during exercise is primarily based on studies conducted in males but a recent study by our group demonstrated significant differences in the total WOB between men and women for a given absolute minute ventilation (7). The present study adds to the previous literature by systematically measuring the elastic and resistive WOB in exercising women. The novel findings from this study are four-fold. First, the inspiratory resistive WOB was higher in women for any given absolute minute ventilation while the expiratory resistive WOB was higher in women at only 75 l/min. There were no sex differences in the inspiratory or expiratory elastic WOB across any absolute minute ventilation. However, the total WOB was actually higher in men when compared across relative percentages of maximal ventilations, due to their higher absolute tidal volumes and thus higher minute ventilations. Second, the total WOB and the inspiratory and expiratory resistive WOB were higher in women when performing the same relative external muscular work. Third, the WOB was inversely related to lung size and presumably airway size. Lastly, the inspiratory resistive WOB was consid-

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Fig. 3. Modified Campbell diagrams from an individual male and female subject matched approximately for absolute minute ventilation [100 vs. 101 l/min (STPD)] tidal volume (2.1 vs. 2.2 liters), breathing frequency (52 vs. 49 breaths/min), age (24 vs. 25 years), and mass (64.6 vs. 64.2 kg), respectively. The male was slightly taller than the female subject (181 vs. 167 cm, respectively). Oblique hatching represents the inspiratory resistive work of breathing (Ir). Horizontal hatching represents the inspiratory elastic work of breathing (Ie). Stippling represents the expiratory resistive work of breathing (Er). Vertical hatching represents the expiratory elastic work of breathing (Ee). Cl, dynamic lung compliance; Ccw, chest wall compliance. Upward arrow represents inspiration and downward arrow represents expiration. Small open circles represent zero flow points.

erably higher for a given level of inspiratory flow compared with men, demonstrating the importance of airway size in determining the mechanical cost of breathing. We interpret our findings to mean that the higher total WOB observed in exercising women at absolute ventilations is due to a higher resistive WOB, which can be attributed to relatively smaller lungs and airways.

Resistive work of breathing vs. minute ventilation. In our previous work (7), we plotted the total WOB against a range of ventilatory rates and fit the data points to the following equation as originally described by others (10, 17): $WOB = aV_E^3 + bV_E^2$. The term bV_E^2 describes the mechanical work done in overcoming the viscous resistance offered by the lung tissues to deformation and by the respiratory tract to the laminar flow of air. The term aV_E^3 represents the work done in overcoming the resistance to turbulent flow. We found that the constant a was significantly higher in women meaning that the higher total WOB in women is associated with the additional resistive work due to turbulent airflow. Although this is an instructive analysis, it does not permit a quantitative measure of the individual factors that make up the total WOB at specific time or physiological points. By using a more extensive approach, we have now partitioned the total WOB into its individual components at specific values of minute ventilation and at standardized work rates. During progressive exercise, we found that both inspiratory and expiratory resistive work were significantly higher in women over a range of ventilatory rates (Fig. 2, *B* and *C*). Interestingly, we observed significant differences in inspiratory resistive work at low levels of minute ventilation (50 l/min), and the magnitude of difference increased as ventilation increased up to 100 l/min. Figure 3 provides a compelling example of the high pressures that are needed in a female subject to achieve the same minute ventilation as a male subject. It is important to note that the male and female subject shown in Fig. 3 have been matched for both breathing frequency, tidal volume, age, and body mass. Despite the fact that both subjects are breathing at the same volume and rate, the inspiratory and expiratory resistive work components are considerably higher in the female subject.

Elastic work of breathing vs. minute ventilation. The elastic work required to increase and decrease the volume of the lung is related to the elastic forces that develop in the tissues of the lung and chest wall. Unique to this study, we found that there were no sex differences in the elastic WOB at any absolute ventilation. However, with further examination, it can be seen in Fig. 7A that the inspiratory elastic WOB is substantially higher in women for a given tidal volume. This can be attributed to the fact that women are breathing at a higher percentage of their total lung capacity for a given level of ventilation, which reduces the compliance of the lungs. Perhaps more important is the observation that women adopt a higher breathing frequency for a given minute ventilation, which acts to reduce the inspiratory elastic WOB (Fig. 7B). This type of breathing pattern comes at the expense of an increased resistive WOB. This is an important observation if one is to consider how breathing patterns are regulated in humans in an effort to minimize the total WOB. The "principle of minimum effort" was first used to describe that for a given alveolar ventilation, there is a breathing frequency that is optimal (17). This pattern is adopted because if the breathing frequency is too low, then large amounts of elastic work are required, whereas if the breathing frequency is too high, then respiratory muscle work is expended to ventilate dead space (i.e., wasted ventilation). During exercise the diaphragm generates most of the inspiratory driving force and appears to remain within the favorable part of its length-tension curve (6).
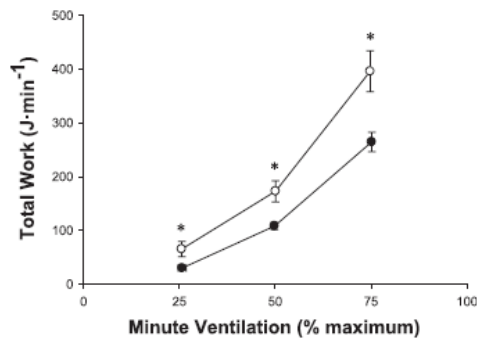

Fig. 4. Total work of breathing vs. relative percentages of maximal minute ventilation in men (○) and women (●). Values are expressed as means ± SE. *Significantly different between groups ($P < 0.05$).
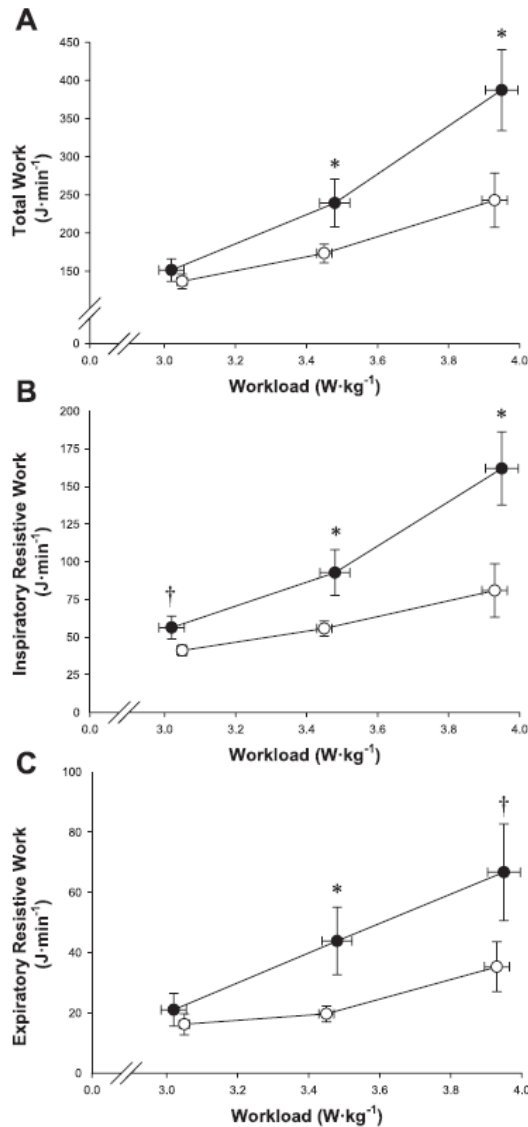
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Fig. 5. Total work (A), inspiratory resistive work (B), and expiratory resistive work of breathing (C) vs. work rate in men (○) and women (●). Values are expressed as means ± SE. *Significantly different between groups (P < 0.05); †P = 0.05.

parison above 50 l/min, which is consistent with our previous findings using a different analysis technique (7). In our previous work, we also compared the total WOB between men and women at different percentages of $\dot{V}o_{2max}$ and found that the total WOB was modestly higher in men at $\dot{V}o_{2max}$, but there was no statistically significant difference (7). However, in the present study, we have shown that the total WOB is significantly higher in men when compared at maximal ventilations (Fig. 2) and also at submaximal relative percentages of maximal ventilation (Fig. 4). We attribute this discrepant finding to the fact that the modified Campbell diagram technique takes into account the compliance of the chest wall, which allows us to calculate the additional part of the inspiratory elastic WOB, which extends beyond the area directly within the pressure-volume loop (see Fig. 1). Indeed, it can be seen in Fig. 2 that the only component to approach statistical significance at maximal ventilations was the inspiratory elastic WOB (P = 0.05). The primary driving force for the higher total WOB at submaximal relative ventilations was also the inspiratory elastic WOB (data not shown). This is due to the fact that for a given relative percentage of maximal ventilation, the tidal volume is considerably higher in men with little to no differ-
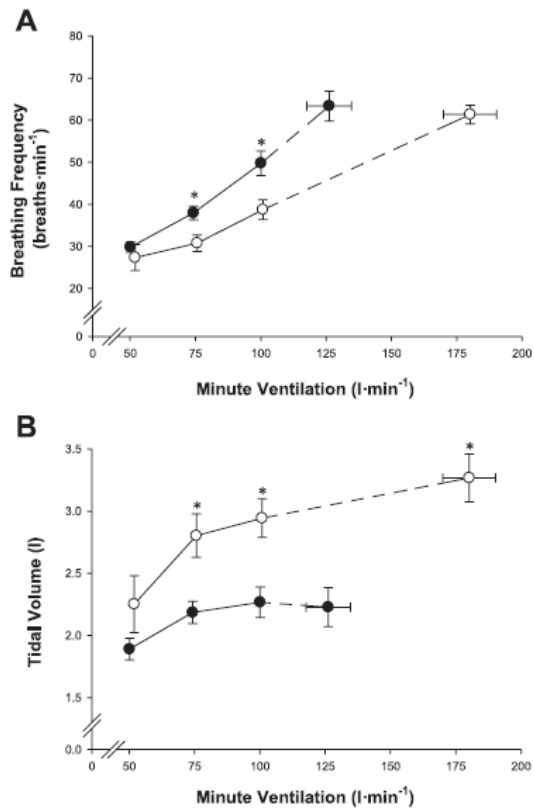

Fig. 6. Breathing frequency (A) and tidal volume (B) vs. minute ventilation in men (○) and women (●). The last data point represents maximal minute ventilation. Values are expressed as means ± SE. *Significantly different between groups (P < 0.05).

This suggests that under spontaneously breathing conditions, the diaphragm tension or O_2 cost is what is being minimized during exercise. On the basis of the present study, it appears that women use a higher breathing frequency to minimize the elastic WOB, which comes at the expense of a higher resistive WOB.

Total work of breathing vs. minute ventilation. The total WOB was higher in women at any absolute ventilation com-

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a physiologically relevant comparison because it minimizes the potential confounding effect of body size differences. Moreover, it allows us to compare the physiological cost of breathing betweens sexes for a given standardized external work load. Even when normalized for body mass, the total WOB is higher at 3.5 and 4.0 W/kg with the inspiratory and expiratory resistive WOB components accounting for the vast majority of this difference (Fig. 5). At 3.5 and 4.0 W/kg, the inspiratory resistive WOB was 67 and 100% higher, respectively in women, while the expiratory resistive WOB was 123 and 89% higher in women, respectively. It is important to note that these comparisons do not take into account lean body mass since
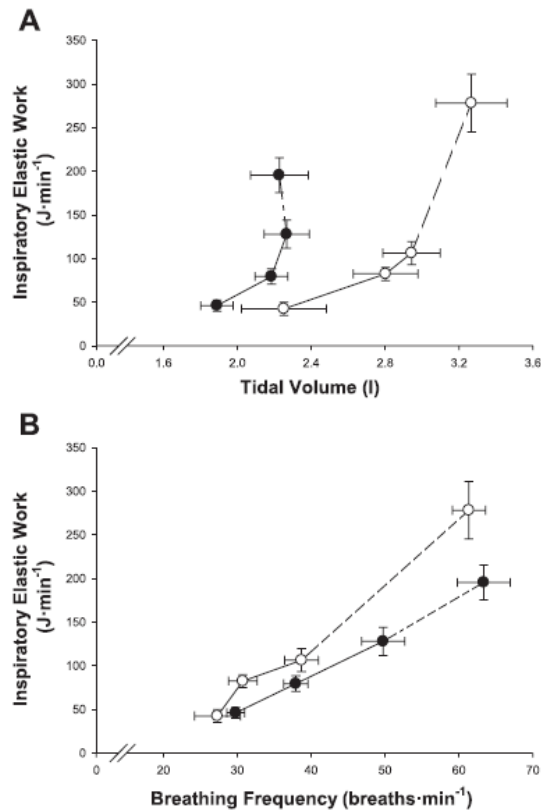

Fig. 7. Inspiratory elastic work of breathing vs. tidal volume (A) and breathing frequency (B) at the four ventilatory points (i.e., 50, 75, 100 l/min and maximal ventilation) in men (○) and women (●). Values are expressed as means ± SE.

ence in breathing frequency. This will substantially increase the inspiratory elastic WOB and thus the total WOB when comparisons are made at relative intensities. We have purposely limited the majority of our analysis and interpretation in this study and in our previous work (7) to absolute ventilations to determine whether the mechanical cost of moving a given amount of air in and out of the lungs is different between sexes. Examining the mechanics of breathing between sexes at relative ventilations is a difficult comparison because men are utilizing a much higher tidal volume and thus have higher minute ventilations than women. For example, the men in this study were breathing 54 l/min higher than women at maximal exercise. Despite the fact that maximal ventilations were 43% larger in our male subjects, it is interesting to note that there were no significant differences in the resistive WOB components. This lends further support to the finding that women have a substantially higher resistive WOB than men.

Work of breathing vs. external muscular work. Fig. 5 shows the WOB required to perform the same relative external work on the cycle ergometer. Rather than using absolute work rate (i.e., power) in Watts, we have normalized the work rate by expressing it in Watts per kilogram body mass, which provides
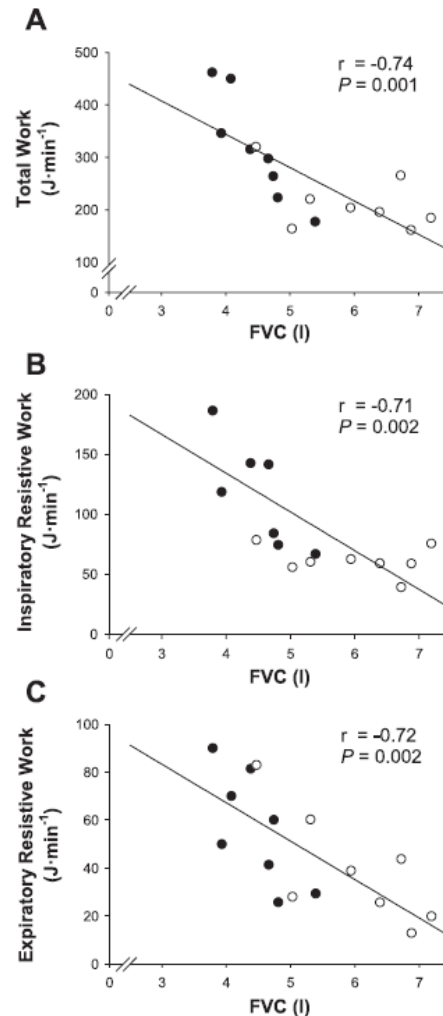
Fig. 8. Regression analysis of the total work (A), inspiratory resistive work (B), and expiratory resistive work (C) vs. forced vital capacity (FVC) in men (○) and women (●). Work of breathing values are obtained at a minute ventilation of 100 l/min.
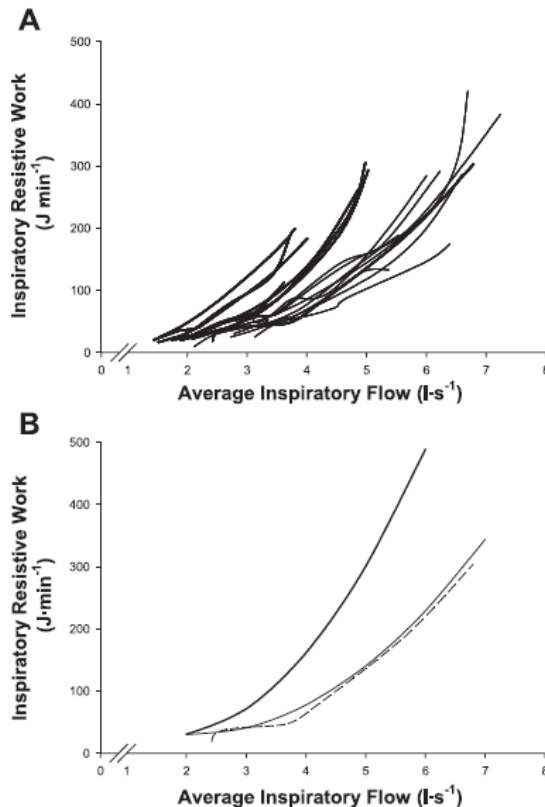
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Fig. 9. Inspiratory resistive work of breathing vs. average inspiratory flow in individual male (thin lines) and female (thick lines) subjects (*A*). *B*: mean inspiratory resistive work of breathing vs. average inspiratory flow in women (thick line) and men (thin line) and an individual female subject (dashed line) with larger than average FVC and peak expiratory flow values.

WOB. We do not have a direct measurement of airway size in our subjects but similar correlation coefficients were also observed when relating the resistive WOB components against peak expiratory flows, which may serve as a crude surrogate for airway size. We are cognizant of the limits of correlative evidence and therefore do not attempt to overstate these findings. However, in an effort to provide a more mechanistic understanding of the higher resistive WOB in women, particularly on inspiration, we performed additional analyses as shown in Fig. 9. In this analysis, Fig. 9*A* shows the inspiratory resistive WOB for a given level of inspiratory flow in individual subjects while Fig. 9*B* represents the group average. These data show that for a given level of flow, the resistive WOB is higher in women, and the magnitude of this difference increases disproportionally with increasing flow. Fig. 9*B* includes one female subject superimposed with the mean curves. This subject had unusually large lungs and peak expiratory flows (>130% predicted). In fact, her FVC and peak expiratory flows were relatively close to the group mean values for men. Interestingly, her inspiratory resistive WOB response for a given level of flow was nearly identical to the average curve for the male subjects. These observations in conjunction with correlative evidence points to an anatomical basis (i.e., smaller lungs and airways) for the WOB differences that we observed during exercise. Additional physiological and performance-based consequences of these anatomical differences in lung and airway size have been reviewed elsewhere (20).

Our sex-based comparisons were made between men and women of significantly different statures. It could be argued that our findings simply reflect size differences rather than a true male-female difference in lung and airway size. However, there is reason to suggest that our findings would be similar between men and women of comparable sizes (i.e., men and women matched for total lung capacity). We make this claim based on two lines of anatomical evidence. First, in healthy young men and women matched for total lung capacity, women have significantly smaller tracheal areas (2.79 vs. 1.99 cm^2) as assessed by acoustic reflectance (11). As such, the reduced female tracheal area would result in a higher WOB for a given level of minute ventilation. Air flow is determined, in part, by Poiseuille's law, and the factors governing it are internal diameter, length, gas viscosity, and airflow pressure, where the radius is raised to the fourth power. As such, even a small difference in airway radius is magnified and would have an effect on airflow resistance and the accompanying WOB. Second, the relationship between airway size (estimated from maximal expiratory flow/static recoil pressure at 50% vital capacity), and lung size (vital capacity) shows that adult men have airways that are 17% larger than those of women (13). This has been termed "dysanapsis" to reflect the dissociation between airway size and lung parenchymal size (5). Given the brief summary presented above, coupled with the findings of the present study, it appears that the higher resistive WOB seen in women is due to inherently smaller airways.

Methodological considerations. Our measures of the work done by the respiratory muscles do not take into account the distorting forces of the chest wall observed at high levels of minute ventilation. Volume displacement of the rib cage and abdomen can be independent of one another (19). Phrased differently, this means that all of the respiratory muscles do not necessarily shorten during inspiration nor do all of the muscles

body composition was not measured in these subjects. It has been suggested that sex differences are minimized or completely abolished in laboratory-based experiments when comparing sexes for a given power-to-lean body mass ratio (22). Although all subjects were lean endurance athletes of similar training status, it would be expected that the women would still have a higher percentage of body fat and therefore less muscle mass. Therefore, it is important to acknowledge that this interpretation has its limitations because the female participants are still working at a slightly higher percentage of their maximum output relative to their male counterparts. This will certainly account for some of the differences observed in our WOB values. However, we cannot directly assess the impact of this limitation in our study without a measurement of lean body mass and therefore do not attempt to overstate these findings.

Sex vs. size differences. We observed statistically significant associations between the resistive WOB at 100 l/min and FVC (see Fig. 8). As would be expected, women had lower FVC values than men, which were inversely related to a higher resistive WOB. Therefore, those with the smallest lungs and presumably the smallest airways had the highest resistive

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of expiration shorten during expiration. We recognize this as a critique of the modified Campbell approach and that our measure of the WOB may be underestimates. However, it is unlikely that this systematic underestimation applied to all subjects equally would have had any substantive effect on our overall conclusion that women have a higher WOB during exercise owing to a greater resistive WOB. This is supported by optoelectronic plethysmography measures, which suggest that men and women utilize muscles of the rib cage compartment and those of the abdomen to the same extent (24).

The compliance of the chest wall is required to determine the various WOB components using the modified Campbell diagram method. It is typically very difficult to reliably measure the compliance of the chest wall because naive subjects have a difficult time completely relaxing their respiratory muscles. Therefore, we based our chest wall compliance values on previously published data taking into account both age and sex (3), as done by others (2, 4, 21, 25). There are limitations with this approach that warrant discussion. For example, the chest wall compliance values we used were obtained in healthy volunteers with normal static lung volumes and $FEV_{1.0}$ values, and it is assumed that the values were measured in untrained individuals. Therefore, we are making the assumption that the compliance of the chest wall is similar between trained and untrained subjects. To our knowledge, there are no studies that have studied the effect of fitness on the compliance of the chest wall. Although there are inherent limitations in using "normative" data and applying it to elite athletes, we do not think that this had an effect on our main conclusions regarding sex differences in the WOB. We base this assumption on several factors. First, according to Estenne et al. (3), there are no sex differences in the compliance of the chest wall (at least in untrained individuals). Our men and women were of similar relative fitness levels, so it is reasonable to assume that this lack of a sex difference should persist along the fitness continuum, and any potential errors in our chest wall compliance values would likely be a systematic error across all subjects. Secondly, the vital capacity values in our men (6.0 ± 1.0 l) and women (4.5 ± 0.5 l) were nearly identical to the age-matched men (6.0 ± 0.9 l) and women (4.3 ± 0.4 l) from whom we derived are chest wall compliance values. Finally, we have calculated that even a ±10% change in the compliance of the chest wall would only affect the elastic components by approximately ±3–5%, the inspiratory resistive WOB by less than ±0.5%, and the expiratory resistive WOB by ±1.5–3%.

The elastic WOB measurements that we and others (21, 25) have made may be underestimations because the calculations are based on tidal volume measured at the mouth, which ignores gas compression (16). Gas compression is typically negligible in healthy individuals at rest or during exercise. However, under conditions where expiratory flow limitation is present, this could increase the magnitude of underestimation. We did observe expiratory flow limitation in many of our female and male subjects (7) during high levels of exercise, but we do not believe that this had a major influence on our findings or overall interpretation of the present study, particularly at submaximal workloads. Specifically, we observed no demonstrable difference between men and women in the elastic WOB at low levels of ventilation (50 l/min) when expiratory flow limitation is not present. This absence held true at higher levels of ventilation, suggesting that any underestimation was most likely consistent across all ventilatory rates. Thus, any underestimation due to gas compression likely had a negligible influence on our findings and conclusion that the elastic WOB is similar between men and women for a given level of ventilation. The current study design does not allow us to determine the direct role of expiratory flow limitation and the corresponding changes in operational lung volumes between sexes because flow limitation was typically observed at maximal to near-maximal intensities. As such, it would be difficult to isolate the effects of expiratory flow limitation on our WOB values in men and women because any potential differences would be masked by the fact that men have much higher tidal volumes and thus ventilations at maximal exercise. A novel study design would be required to assess sex-based differences in expiratory flow limitation and the direct corresponding effect on the WOB.

Perspectives and Significance

This study is the first to systematically assess the mechanisms of a higher WOB in women during dynamic exercise. Describing sex differences in breathing mechanics poses a significant challenge because of the inherent difficulties in comparing men and women due to known differences in body size and an incomplete understanding of the most appropriate allometric scaling factor to use. In the present study, we have made a number of unique physiological comparisons. First, comparing the WOB for a given level of ventilation allowed us to quantify the additional cost of breathing necessary to move a fixed amount of air through smaller lungs and airways in women. Second, comparing men and women at different percentages of maximal ventilations allowed us to determine the cost of breathing for a given relative intensity. Third, comparing sexes at a standardized size-corrected work rate enabled us to determine whether there are differences in the WOB for a given level of external muscular work. The data from this study suggest that the higher overall WOB in women during dynamic exercise is due to a substantially greater resistive work during inspiration and expiration with no differences in the elastic WOB. However, much of these differences are reversed when comparisons are made at relative intensities. We conclude that sex-based differences in lung and airway size result in the higher work and thus O_2 cost of breathing in women during exercise for a given absolute level of ventilation or exercise intensity.

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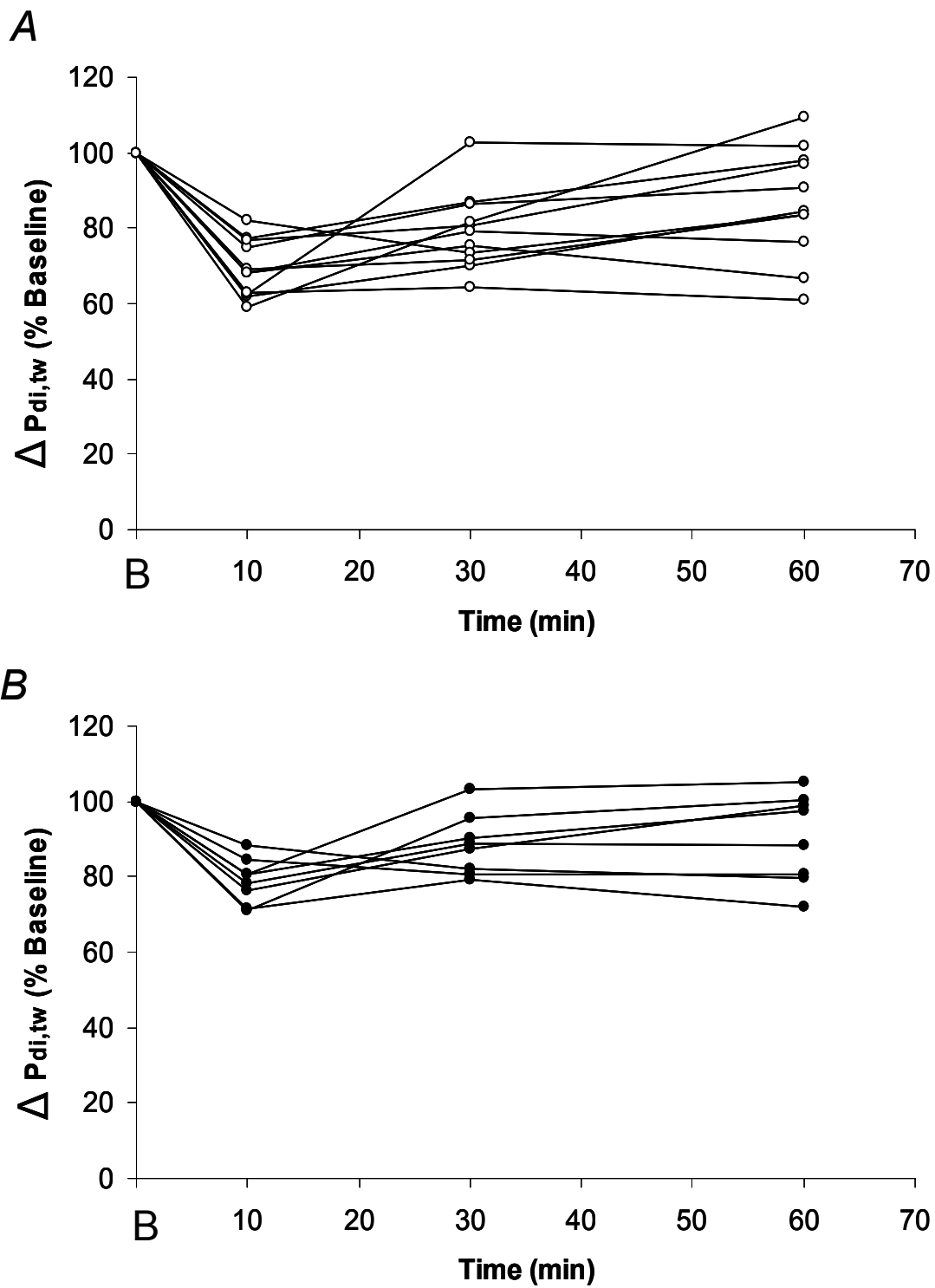# APPENDIX IV: Individual diaphragm fatigue response in men and women

Figure A.IV.1: Response of twitch trans-diaphragmatic pressure ($P_{di,tw}$) during recovery in the individual male (A) and female (B) subjects that developed diaphragm fatigue (defined as a drop in $P_{di,tw}$ of $\geq 15\%$). B, baseline.

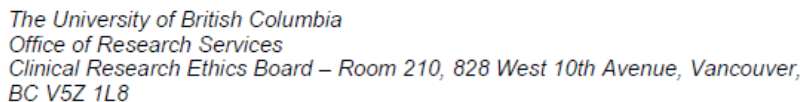# APPENDIX V: Certificates of ethical approval

The University of British Columbia
Office of Research Services,
Clinical Research Ethics Board – Room 210, 828 West 10ᵗʰ Avenue, Vancouver, BC V5Z 1L8

Certificate of Expedited Approval: Amendment
Clinical Research Ethics Board Official Notification

PRINCIPAL INVESTIGATOR	DEPARTMENT	NUMBER	
Sheel, W.	Human Kinetics		C05-0072

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT

UBC Campus

CO-INVESTIGATORS

Guenette, Jordan. ; Koehle, Michael. Human Kinetics; Lusina, Sarah, Human Kinetics; MacNutt, Meaghan, Forest Sciences; McKenzie, Donald, Human Kinetics; Road, Jeremy, Medicine; Witt, Jonathan, Human Kinetics

SPONSORING AGENCIES

Unfunded Research

TITLE :

Ventilatory Responsiveness and the Work of Breathing in Men and Women with Exercise Induced Arterial Hypoxaemia

APPROVAL DATE (yy/mm/dd)	TERM (YEARS)	AMENDMENT	AMENDMENT APPROVED
05-03-23	1	Protocol Amendment Version 2 dd 14 July 2005; Subject Consent Form Version 4 dd 14 July 2005; Addition of Co-Investigator; Change in title	**19 September 2005**

CERTIFICATION

In respect of clinical trials:

1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The amendment(s) for the above-named project has been reviewed by the Chair of the University of British Columbia Clinical Research Ethics Board and the accompanying documentation was found to be acceptable on ethical grounds for research involving human subjects.

The CREB approval period for this amendment expires on the one year anniversary date of the CREB approval for the entire study.

Approval of the Clinical Research Ethics Board by one of:
Dr. Gail Bellward, Chair
Dr. James McCormack, Associate Chair

ETHICS CERTIFICATE OF EXPEDITED APPROVAL: AMENDMENT

PRINCIPAL INVESTIGATOR:	DEPARTMENT:	UBC CREB NUMBER:
William Sheel	UBC/Education/Human Kinetics	H08-00671

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:	
Institution	Site
UBC	Vancouver (excludes UBC Hospital)
Other locations where the research will be conducted: N/A	

CO-INVESTIGATOR(S):
Donald C. McKenzie
Jeremy D. Road
Meaghan MacNutt
Jordan Querido
Simone Tomczak

SPONSORING AGENCIES:
N/A

PROJECT TITLE:
Respiratory Muscle Fatigue in Men and Women

REMINDER: The current UBC CREB approval for this study expires: March 9, 2010

AMENDMENT(S):			AMENDMENT APPROVAL DATE:
Document Name	Version	Date	March 9, 2009
Protocol:			
Protocol	3	March 1, 2009	

CERTIFICATION:

In respect of clinical trials:
1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The amendment(s) for the above-named project has been reviewed by the Chair of the University of British Columbia Clinical Research Ethics Board and the accompanying documentation was found to be acceptable on ethical grounds for research involving human subjects.

Approval of the Clinical Research Ethics Board by :

Dr. Stephen Hoption Cann,
Associate Chair

APPENDIX VI: Candidate's list of research publications

REFEREED JOURNAL PUBLICATIONS

1. **Guenette JA**, Dominelli PB, Reeve SS, Durkin CM,Eves ND and Sheel AW. Effect of thoracic gas compression and bronchodilation on the evaluation of expiratory flow limitation during exercise in healthy humans. *Respiratory Physiology and Neurobiology.* In Press.

2. Koehle MS, **Guenette JA** and Warburton DER. Oximetry, heart rate variability, and the diagnosis of mild to moderate acute mountain sickness. *European Journal of Emergency Medicine.* In Press.

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ABSTRACTS AND CONFERENCE PROCEEDINGS

1. **Guenette JA**, Vogiatzis I, Zakynthinos S, Boushel R, Wagner PD and Roussos C. Influence of respiratory muscle blood flow and hypoxemia on exercise induced diaphragmatic fatigue in humans. *Medicine and Science in Sports and Exercise.* 41: S55, 2009. Invited oral presentation (Featured Science Symposium), Seattle, Washington, USA.

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