RESPIRATORY MECHANICS DURING EXERCISE IN ENDURANCE TRAINED MEN AND WOMEN

by

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ABSTRACT

A number of recent studies have shown that young healthy women may be susceptible to pulmonary system limitations during exercise including expiratory flow limitation (EFL). Several sex based differences in the anatomy and function of the pulmonary system have been reported as possible mechanisms. For example, women consistently have smaller lung volumes, smaller diameter airways, and a decreased diffusion surface area relative to height-matched men. These anatomical differences may also have implications in terms of breathing mechanics, particularly at maximal exercise. However, there have been no studies that have systematically compared the mechanics of breathing in men and women. Accordingly, the purpose of the present study was to provide a comprehensive assessment of breathing mechanics including the measurement of EFL, end expiratory lung volume (EELV), end inspiratory lung volume (EILV), and the work of breathing (W_b) in endurance trained men and women. It was hypothesized that women would develop EFL more frequently than men and that women would have greater relative increases in EELV and EILV at maximal exercise. It was also hypothesized that women would have a higher W_b across a range of ventilations. EFL was assessed by applying a negative expiratory pressure (NEP) at the mouth and comparing the resultant flow volume curve with that of the preceding control breath. If the NEP increased expiratory flow rate, the subject was considered non flow limited. Conversely, if application of the NEP did not illicit an increase in expiratory flow, the subject was considered flow limited. Operational lung volumes (i.e., EELV and EILV) were determined at various stages of exercise by having subjects perform inspiratory capacity manoeuvres. Flow, volume, oesophageal and airway opening pressure were continuously monitored throughout exercise. Trans-pulmonary pressure (P_{tp}) was taken as the difference between oesophageal and airway opening pressure which was then plotted against volume. The integral of the P_{tp}-volume loop was multiplied by breathing frequency to determine the W_b. A total of 18 endurance trained male (n=8) and female (n=10) athletes volunteered to participate in this study. Males had a higher absolute (mean ± SD; 5.30 ± 0.7 vs. 3.8 ± 0.4 $L \cdot min^{-1}$) and relative (69.5 ± 7.8 vs. 59.8 ± 4.8 $mL \cdot kg^{-1} \cdot min^{-1}$) $\dot{V}O_{2MAX}$ and a higher maximal minute ventilation (161 ± 25 vs. 120 ± 18 $L \cdot min^{-1}$) compared to females ($P<0.01$). Due to an abnormal response to the NEP in one male subject, EFL data was obtained in 7 of the 8 males. EFL occurred in 9 females (90%) and 4 males (57%) during the final stage of exercise. However, 8 (6F, 2M) of these subjects were later able to overcome EFL during the final stage of exercise through an alteration in breathing pattern. Females had a higher relative EELV (42±8 vs. 35±5 %FVC) and EILV (88±5 vs. 82±7 %FVC) compared to males at maximal exercise ($P<0.05$). Women also had a higher W_b compared to men across a range

of ventilations. On average, women had a W_b that was twice that of men at ventilations above 90 $L \cdot min^{-1}$. This data suggests that EFL may be more common in females and that they experience greater relative increases in EELV and EILV at maximal exercise compared to males. The higher W_b in women is likely attributed to their smaller lung volumes and smaller diameter airways. Collectively, these findings suggest that women utilize the majority of their ventilatory reserve compared to men but the associated cost may have physiological and performance based implications.

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INTRODUCTION

In general, the functional capacity of the healthy human pulmonary system including the lung, chest wall, and neural control systems, exceeds the demands placed upon it during heavy exercise. This is an impressive feat considering the major challenges the pulmonary system must face during exercise. First, exercise causes an increase in muscle metabolism which substantially reduces the oxygenation of mixed venous blood being delivered to the lungs. This means that alveolar to arterial gas exchange must be greatly increased. Second, there is a fourfold increase in cardiac output which means that the red blood cells will have considerably less time for the complete equilibration of O_2 and CO_2 between the alveoli and pulmonary capillaries. Third, since the lung receives all of the cardiac output, it has to maintain low vascular pressures and protect against the release of plasma water into the alveoli. Fourth, since gas transport is dependent on both blood flow and blood O_2 and CO_2 content, it is essential that the cardiovascular and pulmonary systems be matched to the increased metabolic demands, and that the substantial gas transport needs of both respiratory and locomotor muscles be considered. Finally, the healthy pulmonary system faces the major challenge of regulating alveolar partial pressure of oxygen (PAO_2) and carbon dioxide ($PACO_2$) through a considerable increase in alveolar ventilation ($\dot{V}A$) which can often exceed resting levels by more than 20 times. The capacity of the respiratory musculature for force development is adequate to meet this task, but it is essential that the physiological cost of ventilation ($\dot{V}E$) not be excessive.

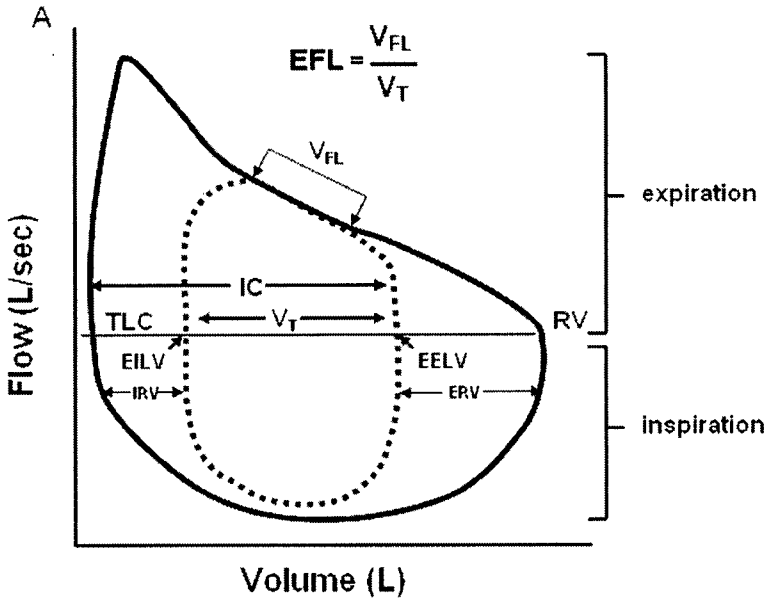
The ability for the healthy pulmonary system to meet these challenges is quite remarkable and for this reason, the lung and chest wall have traditionally been considered "overbuilt" for exercise and were not believed to impose a limitation to oxygen transport. However, there are some exceptions where the pulmonary system assumes a more critical role as a rate limiting factor to oxygen transport (29). Thus, it is now understood that the pulmonary system that was once thought to be "overbuilt" for exercise may in fact pose a limitation in some healthy individuals. Three examples of pulmonary system limitations include; (i) expiratory flow limitation (EFL) (61), (ii) exercise-induced arterial hypoxaemia (EIAH) (30), and (iii) exercise-induced diaphragmatic fatigue (57). Although these

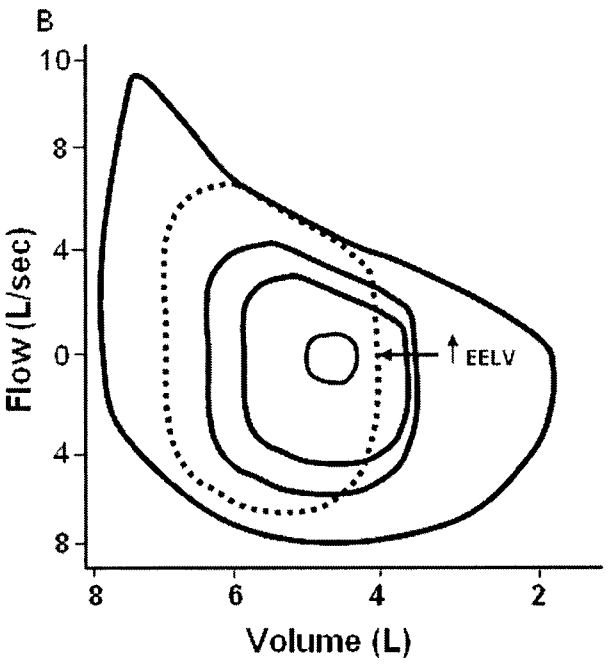
limitations are all interrelated, the present study is specifically concerned with EFL and the physiological cost of exercise hyperpnoea.

Generally, the airways are capable of meeting the ventilatory demands of intense and even maximal exercise, since ventilations up to 120-130 L/min are within the limits of the normal maximum flow volume loop (MFVL) (61). However, in some cases, the increase in $\dot{V}E$ that occurs during progressive exercise may cause a portion of the tidal breathing loop to intersect the MFVL. This intersection is known as EFL and is specifically defined as the percent of the tidal volume that meets or exceeds the expiratory boundary of the MFVL (figure 1A) (59, 60). The presence of EFL may cause reflex inhibition of the hyperventilatory response and/or an alteration in operational lung volumes (63). For example, with the onset of EFL, end expiratory lung volume (EELV) may increase (61, 62) back towards resting values resulting in a relative dynamic hyperinflation of the lungs (20) (figure 1B). Dynamic hyperinflation permits increases in expiratory flow rates (96), but results in a number of corresponding physiological consequences. For example, there will be an increased elastic work because lung compliance is reduced as lung volume increases. The reduction in inspiratory muscle length may substantially increase the work and O_2 cost of breathing, thus decreasing inspiratory muscle endurance time (116). 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 (104).

The higher metabolic demands of endurance athletes often requires a $\dot{V}E$ that is significantly higher than that of their untrained counterparts (63). This increased demand on the pulmonary system occurs despite the fact that the ventilatory capacity of the lungs and chest wall are generally the same between trained and untrained individuals (99). Additional research has shown that neither fitness or training has an effect on pulmonary function (61, 80, 88). One exception to this is in swimmers who generally have larger lungs compared with the normal population (5, 6, 23). The substantial ventilatory requirement of endurance athletes may result in EFL during exercise (38, 61) although this is not a universal finding (86). Trained female athletes may be particularly susceptible to developing EFL due

Figure 1. A) Schematic representation of the various lung volumes and determination of expiratory flow limitation (EFL) during maximal exercise. The solid line represents and maximal resting flow volume loop while the dotted line represents the maximal exercise tidal flow volume loop. The percent of the tidal breath (V_{FL}) that expiratory air flows meet or exceed the maximal expiratory flows are used as an estimate as to the degree of EFL. IC = inspiratory capacity; V_T = tidal volume; EILV = end inspiratory lung volume; EELV = end expiratory lung volume; IRV = inspiratory reserve volume; ERV= expiratory reserve volume; TLC = total lung capacity; RV = residual volume.
B) Schematic representation of the leftward (increase) shift in EELV with the onset of EFL at maximal ventilation (dotted line). The tidal flow volume loops (solid lines) represent rest, mild, and moderate levels of ventilation.





to their inherent anatomical differences. For example, it has been well documented that women tend to have lower peak expiratory flow rates and smaller lung volumes relative to age and height-matched men (7, 81, 83, 114). In addition, women typically have smaller diameter airways (83) and diffusion surface areas (114) compared to men. The smaller diameter airways, lower peak flow rates, and smaller lung volumes will result in a smaller MFVL and thus a smaller capacity to generate flow and volume during exercise. Therefore, for a given degree of $\dot{V}E$ during exercise, women may be more likely to experience EFL relative to men.

Currently, there are no studies that have directly compared men and women to determine which sex has a greater tendency to develop EFL. Additionally, those studies that have assessed EFL in women have utilized the traditional technique of superimposing tidal breaths within the MFVL (81, 119) (figure 1B). Although this is a widely used technique, it may lead to a false detection or overestimation of EFL for two primary reasons. Firstly, the thoracic gas compression artefact of the MFVL may underestimate the true capacity for flow generation (54). Secondly, the differences in the volume and time history that precede tidal expiration and forced expiratory manoeuvres may lead to a false detection of EFL (86). An alternative method to determine EFL is to apply a negative expiratory pressure (NEP) at the mouth, where the flow volume curve during the ensuing expiration is compared with the preceding control breath (86, 117). Unlike the traditional technique, the NEP technique does not require forced expiratory efforts or the correction for gas compression and the volume history of the control expiration and the subsequent expiration with the NEP is the same. Using the NEP technique, Mota et al. (86) only found EFL in one out of nine competitive male cyclists which is lower than previous reports in males where EFL was assessed using more traditional methods (38, 61). There are no studies currently available using the NEP technique in women. Given the results of Mota et al. (86) in men, it is conceivable that previous studies in women have also overestimated the degree of EFL.

Presumably, the higher incidence and degree of EFL in trained female athletes would likely result in a higher work of breathing (W_b) compared to males. There are a number of reasons in support of this theory as described by McClaran et al. (81). First, the smaller vital capacity in women would result in a smaller maximal exercise tidal volume such that the

greater dependence on breathing frequency (65) would augment dead space $\dot{V}_E$. Therefore, to attain similar levels of $\dot{V}_A$ as men, women would need an even higher $\dot{V}_E$. Second, the higher EELV due to EFL would reduce the initial length of the inspiratory muscles. Johnson et al. (61) found that the hyperinflation caused by the increases in EFL had a significant effect on the fraction of total inspiratory muscle capacity utilized and also caused a drop in dynamic compliance at an EILV/TLC of 86%. As pointed out by McClaran et al. (81), the highly fit women with an EILV/TLC of 90% during heavy exercise, would likely have an equal or greater fall in dynamic compliance, and therefore an increased elastic load on the inspiratory muscles over a greater portion of the tidal breath. Unfortunately, there is no direct evidence to support the theory of a higher W_b in women compared to men. The current understanding of pulmonary mechanics in athletes is based strictly on data obtained from the male population. Accordingly, the goal of the present study was to provide a comprehensive assessment of pulmonary mechanics during exercise in both sexes. Specifically, this study aimed at determining; (1) which sex has a greater tendency to develop EFL during exercise; (2) how EELV and EILV are regulated throughout exercise and (3) if there are sex differences in the mechanical W_b during exercise. Endurance trained athletes will be used as the model to answer these questions rather than sedentary individuals. The high metabolic and thus ventilatory requirements of athletes make this population more likely to develop EFL which will enable us to determine the physiological costs of breathing at the extreme end of the fitness continuum.

HYPOTHESES

1. Females will have a higher incidence of EFL compared to males.
2. Females will have greater relative increases in EELV and EILV at maximal exercise compared to males.
3. Females will have a higher W_b across a range of ventilations throughout exercise.

METHODS

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. All data was obtained at the Health and Integrative Physiology Laboratory at the University of British Columbia campus.

SUBJECTS

A total of 8 males and 10 females provided informed written consent to participate in this study. Since progesterone combined with oestrogen raises both $\dot{V}_A$ and chemosensitivity via central (13) and peripheral (113) receptor-mediated mechanisms, testing of females was limited to the early follicular phase when progesterone levels are reported to be lowest (71). Women were tested between days 3 and 8 of their menstrual cycle as determined via a self-reported menstrual history questionnaire (Appendix C). All subjects were highly trained, competitive 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.

GENERAL PROTOCOL

DAY 1 (familiarization day): All subjects completed consent forms, physical activity readiness questionnaires, and medical history questionnaires (Appendix C). Women completed an additional menstrual history questionnaire (Appendix C). Subjects then underwent basic anthropometric measures followed by pulmonary function testing. All subjects then sat quietly for 10 minutes while resting breathing and metabolic measures were collected. After a 5 minute self-selected warm up, subjects underwent an incremental cycle test to exhaustion to determine $\dot{V}O_2MAX$. Throughout the exercise test, subjects became familiar with performing inspiratory capacity (IC) manoeuvres. Following a brief recovery, subjects then performed 5 minutes of steady state exercise in order to become familiar with the NEP test.

DAY 2 (experimental day): Days 1 and 2 were separated by a minimum of 48 hours. Subjects returned to the laboratory where they were given a detailed description of the experimental procedures. Following the insertion of a balloon tipped catheter into the oesophagus, subjects sat comfortably on a chair while resting measures were obtained for 10 minutes. Resting data was collected for 10 minutes to ensure stable baseline levels for ventilatory and metabolic parameters. Upon completion of resting measures, subjects then performed a 5 minute self-selected warm up followed by a progressive cycle exercise test to exhaustion.

MEASUREMENTS

Pulmonary Function

Forced vital capacity (FVC), forced expiratory volume in 1second ($FEV_{1.0}$), $FEV_{1.0}$/FVC, peak expiratory flow (PEF), and maximum voluntary ventilation (MVV) were determined using a portable spirometer (Spirolab II, Medical International Research, Vancouver, BC). Measurements were obtained with subjects seated, utilizing standard protocols as detailed by the American Thoracic Society standards of care and expressed using prediction equations (1). 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 (IC) manoeuvres from functional residual capacity (FRC) until reproducible measurements were obtained.

Exercise Protocol

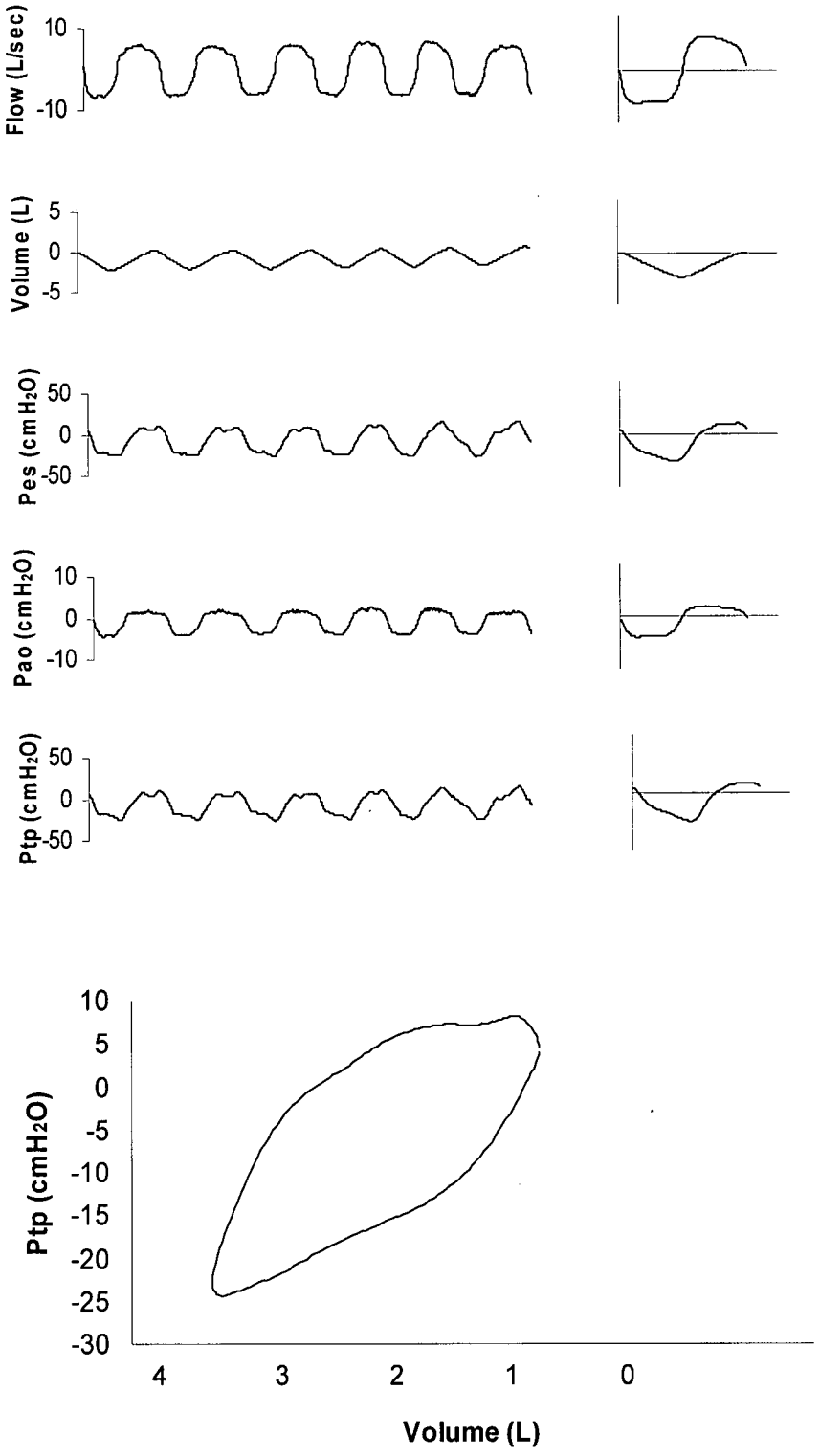
The exercise protocol for Day 1 and Day 2 were performed on an electronically braked cycle ergometer (Excalibur Sport, Lode, Gronigen, Netherlands). Males and females started the test at 200W and 100W respectively, with the workload increasing in a stepwise fashion by 30 W every 3 minutes until volitional exhaustion. This exercise protocol was used so that men and women would exercise for the same duration. The test was terminated when subjects could no longer maintain a cadence of 60 rpm. 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). 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. Heart rate was obtained from a commercially available heart rate monitor (Polar Electro, Kempele, Finland) and recorded every 30 sec. All metabolic data during the exercise test were recorded continuously at 200 Hz (PowerLab/16SP model ML 795, ADI, Colorado Springs, CO) and stored on a computer for subsequent analysis (Chart v5.3, ADInstruments, Colorado Springs, CO).

Work of Breathing

Volume was obtained by numeric integration of flow which was measured using a heated pneumotach (model 3813, Hans Rudolph, Kansas City, MO). Airway opening pressure (P_{ao}) was obtained via a port located in the mouthpiece and transferred by polyethylene tubing to a differential piezoelectric pressure transuducer (± 100 cmH_2O; Raytech Instruments; Vancouver, BC, Canada). Oesophageal (P_{es}) pressure was measured using a balloon-tipped catheter (no. 47-9005, Ackrad Laboratory, Cranford, NJ) attached to piezoelectric pressure transducer (± 100 cmH_2O; Raytech Instruments Inc). Both transducers were calibrated across a range of pressures using a digital manometer (2021P, Digitron, Torquay England). Viscous lidocaine (2%) 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 (85). 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 mL of air was injected in order to partially inflate the balloon and catheter as per manufacture specifications. Validity of the balloon pressure was checked by having the subjects blow against an occluded airway (12). If trans-pulmonary pressure (P_{tp}) remained constant while P_{ao} increased, placement was considered appropriate. Trans-pulmonary pressure was calculated as the difference between P_{es} and P_{ao}. The W_b was obtained by ensemble averaging several breaths and then using a customized software program (LabVIEW software V6.1, National Instruments) to integrate the averaged P_{tp}-tidal volume loop (92) (figure 2). The W_b multiplied by breathing frequency represents the amount of work done per minute by the respiratory system.

Figure 2. Determination of the work of breathing. Flow was continually monitored and integrated to get volume. P_{tp} was determined by subtracting the P_{es} from P_{ao}. The curves to the right of the raw traces are averages. The single volume trace was determined by integrating the averaged flow signal and then applying a correction to offset any drift. The averaged P_{tp} is plotted against the volume to provide a representative P_{tp} -volume loop which is then shifted according to EELV. This loop was then integrated and the corresponding area is multiplied by the breathing frequency in order to determine the total mechanical W_b.

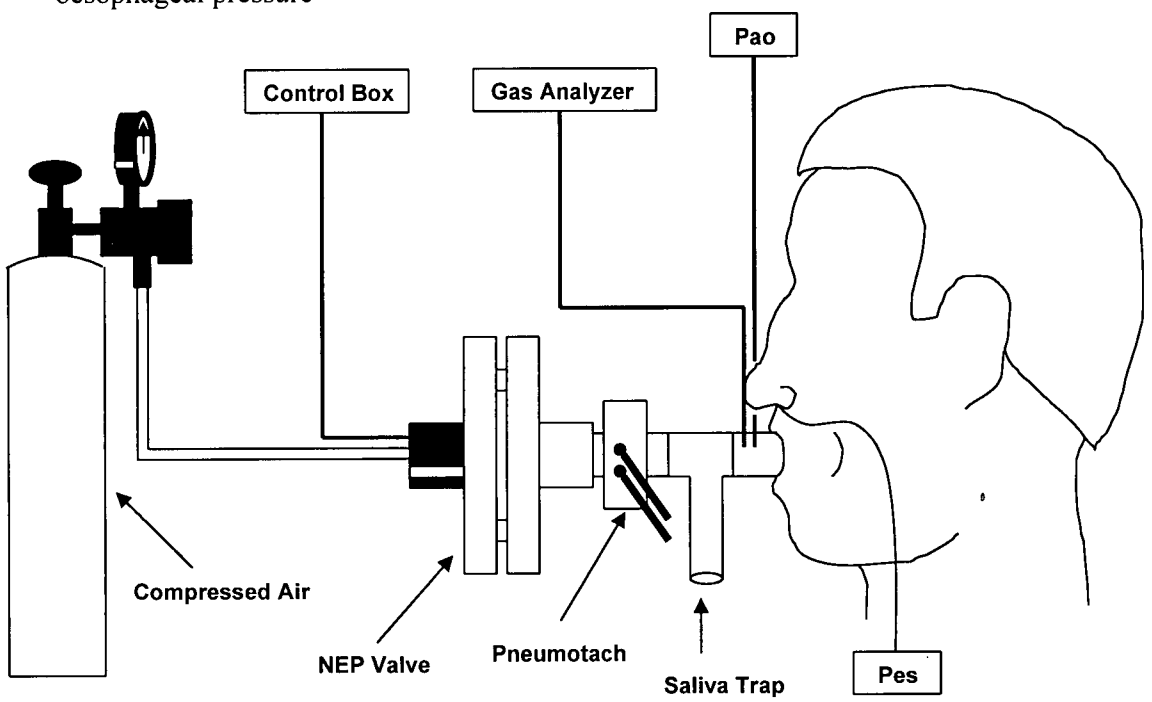


All flow and pressure signals were amplified, filtered (low-pass) at 50 Hz, and digitized at 200 Hz by a 16-bit analog-to-digital converter (200B, Direc Physiologic Recording System; Raytech Instruments) using the Direc/Win data acquisition software program (version 2.21, Raytech Instruments Inc.). The digitized data was stored on the computer hard drive for subsequent analysis.

Determination of Expiratory Flow Limitation

Expiratory flow limitation was determined using the NEP technique and was nearly identical to that used in a similar athletic group by Mota et al. (86). The NEP technique involved the connection of a Venturi device (207A, 4" NEP Valve, Raytech Instruments Inc.) to a tank of compressed gas capable of generating a range of negative pressures. A control box was used to activate and deactivate the NEP when it received a signal from the pneumotach. After expiration was initiated, the system took ~50 ms to reach the desired negative pressure which was set at approximately -10 cmH_2O (118). 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 (figure 3). The pneumotach was calibrated before each

Figure 3. Schematic diagram of equipment setup. P_{ao} = pressure at airway opening, P_{es} = oesophageal pressure



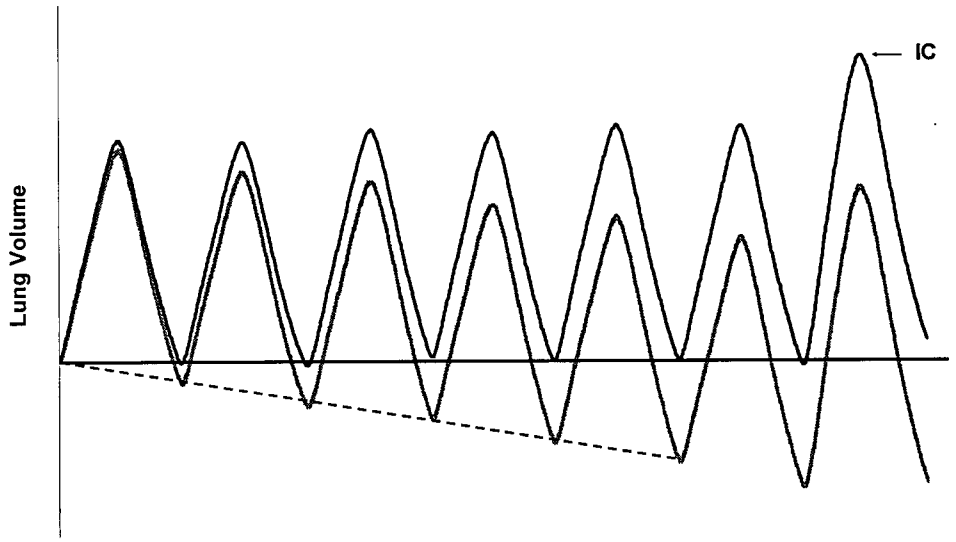
test using a 3L calibration syringe over a range of flow rates to ensure linearity. Volume was obtained by numerical integration of the flow signal. The flow signal was corrected for any offset, using the assumption that inspired and expired volumes of the control breaths preceding the test breaths are the same (69, 97). The NEP test was applied during the final 30 seconds of each 3 minute exercise stage. However, 3 NEP tests were performed during the final workload as subjects were approaching volitional exhaustion. Expiratory flow limitation was considered present when part of the tidal expiratory FV curve with the NEP was superimposed on that of the preceding expiration (86, 117). The percentage of EFL was determined by calculating the percent of the tidal volume encompassed by flow limitation (69, 86, 117).

Determination of EELV and EILV

Changes in EELV and EILV were evaluated from measurements of IC at rest, at the end of each exercise stage and immediately prior to cessation of exercise. EELV was calculated by subtracting the resting TLC from the IC with the assumption that TLC remains constant throughout exercise (63). EILV was calculated by adding EELV to the tidal volume. After a full explanation and demonstration to each subject, satisfactory technique and reproducibility of the ICs 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 IC, subjects were given a few breaths warning before the manoeuvre, a prompt for the manoeuvre (i.e., "at the end of a normal breath out, take a big powerful breath all the way in"), and then verbal encouragement throughout the manoeuvre (89). Subjects were also instructed to generate as much pressure as possible and to ensure that their lungs were full and then provide an additional effort to fill up the lungs even more (34). Acceptable IC trials required that peak inspiratory P_{es} match those obtained at rest. If the maximal inspiratory pressure obtained during exercise is similar to that obtained repeatedly at full inflation (TLC) at rest, one can be confident that TLC was reached during the manoeuvres (8, 62, 122). If subjects failed to achieve the pre-exercise target pressures, they were required to repeat the IC manoeuvre. When analyzing the IC data, 6 breaths were

selected prior to the IC in order to monitor changes in pneumotach drift and any alterations in breathing pattern immediately prior to the IC (figure 4).

Figure 4. Schematic trace of 6 breaths prior to an IC manoeuvre. The grey line represents the original data that is non-drift corrected. The dotted line is the slope drawn from the start of the first inspiration to the start of the second last inspiration. This slope was used to calculate the drift. The solid black line represents the drift corrected volume signal. The IC is always taken as the distance from the x-axis to the peak of the drift corrected IC breath. The x-axis represents functional residual capacity. By correcting to the second last inspiration, any alterations in breathing immediately prior to the IC can be taken into account when determining the IC.



Data Analysis

Pearson product moment correlations were used to determine linear relationships between selected dependent variables. Repeated measures ANOVA were used to examine differences in EELV, EILV and W_b across different workloads. Descriptive comparisons between groups and all post hoc analyses for main effects were performed using *t*-tests with Bonferroni corrections when appropriate. The level of significance was set at $P<0.05$ for all statistical comparisons. Analyses were performed using a computer software program (Statistica 6.1, Stat Soft Inc., Tulsa, OK).

The method for analyzing the W_b was similar to previous studies where the W_b was compared between two groups (22, 84). The individual raw W_b data was plotted against a range of ventilations. To facilitate comparison between men and women, curves corresponding to each subject were calculated assuming the following relationship between the W_b and $\dot{V}E$ as deduced by Otis et al. (93):

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

A value for constant 'a' and 'b' was then determined for each individual subject.

RESULTS

Subject Characteristics and Resting Pulmonary Function

Twenty one subjects volunteered to participate in this study but two 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. Physical characteristics of men and women are shown in table 1. Women were significantly smaller in terms of height, weight, and body surface area ($P<0.001$). Table 2 shows resting pulmonary function and percent predicted values for each variable in men and women. Women had a significantly smaller FVC, $FEV_{1.0}$, PEF and MVV compared to men ($P<0.001$). All subjects were within or greater than predicted values for all pulmonary function variables. There were no significant difference in predicted values between men and women. Table 3 shows resting ventilatory data obtained as an average over the last minute of the 10 minute rest period. There was no difference in resting V_T, F_b, and $\dot{V}_E$ between men and women. However, women had a slightly lower resting $P_{ET}CO_2$ compared to men ($P<0.05$).

Table 1. Descriptive and anthropometric data. Values are means ± SD. Definitions of abbreviations: BMI = body mass index; BSA = body surface area. * Significantly different from men ($P<0.001$).

	Men (n=8)	Women (n=10)
Age (years)	25.9 ± 4.9	24.7 ± 2.8
Height (cm)	183.9 ± 6.6	168.6 ± 4.7*
Weight (kg)	76.6 ± 9.8	63.3 ± 4.2*
BMI ($kg \cdot m^2$)	22.6 ± 1.8	22.3 ± 0.9
BSA (m^2)	1.99 ± 0.15	1.72 ± 0.08*

Table 2. Pulmonary function data. Values are means ± SD. Definitions of abbreviations: FVC = forced vital capacity; $FEV_{1.0}$ = forced expiratory volume in 1 second; $FEV_{1.0}$/FVC = forced expiratory volume in 1 second / forced vital capacity; PEF = peak expiratory flow; MVV = maximum voluntary ventilation. * Significantly different from men ($P<0.001$).

	Men (n=8)	Women (n=10)
FVC (L)	5.6 ± 1.0	4.5 ± 0.5*
FVC (%predicted)	105 ± 14	108 ± 13
$FEV_{1.0}$ (L)	5.1 ± 1.0	3.8 ± 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 ($L \cdot sec^{-1}$)	12.6 ± 1.4	8.0 ± 1.1*
PEF (%predicted)	124 ± 11	116 ± 18
MVV ($L \cdot min^{-1}$)	208 ± 12	153 ± 20*
MVV (%predicted)	138 ± 17	132 ± 19

Table 3. Resting ventilatory data. Values collected during the final minute of the 10 minute seated resting period. Values are means ± SD. Definitions of abbreviations: $P_{ET}CO_2$ = partial pressure of end tidal CO_2; V_T = tidal volume; F_b = breathing frequency; $\dot{V}_E$ = minute ventilation. * Significantly different from men ($P<0.05$).

	Men (n=8)	Women (n=10)
$P_{ET}CO_2$ (mmHg)	43.4 ± 1.9	40.7 ± 2.1*
V_T (L)	0.79 ± 0.15	0.77 ± 0.21
F_b ($breaths \cdot min^{-1}$)	16.7 ± 1.9	15.0 ± 3.2
$\dot{V}_E$ ($L \cdot min^{-1}$)	11.6 ± 1.5	10.1 ± 2.1

Exercise Data

Table 4 shows metabolic, ventilatory, and performance characteristics at maximal exercise on day 1. Men had a significantly higher absolute and relative $\dot{V}O_2MAX$ compared to women ($P<0.01$) but there were no differences in predicted $\dot{V}O_2MAX$ (37). Men had a significantly higher V_T ($P<0.05$) and $\dot{V}_E$ ($P<0.001$) compared to women. Men achieved a higher power output at maximal exercise ($P<0.001$) but the duration of the exercise test was the same.

Table 4. Peak exercise values during the incremental cycle exercise test on Day 1. Values are means ± SD. 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; $\dot{V}_E$/MVV = minute ventilation / maximum voluntary ventilation; $\dot{V}_E$/BSA = minute ventilation / body surface area; HR = heart rate.
* Significantly different from men ($P<0.05$)

	Men (n=8)	Women (n=10)
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	69.5 ± 7.8	59.8 ± 4.8*
$\dot{V}O_2$ (% predicted)	120 ± 11	116 ± 9
$\dot{V}O_2$ ($L \cdot min^{-1}$)	5.3 ± 0.7	3.8 ± 0.4*
$\dot{V}CO_2$ ($L \cdot min^{-1}$)	5.7 ± 0.6	4.1 ± 0.5*
RER	1.08 ± 0.07	1.07 ± 0.04
F_b ($breaths \cdot min^{-1}$)	59 ± 9	58 ± 5
V_T (L)	3.1 ± 0.4	2.3 ± 0.3*
$\dot{V}_E$ ($L \cdot min^{-1}$)	161 ± 25	120 ± 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 ± 9*
HR (bpm)	189 ± 8	190 ± 11
Exercise Duration (sec)	1273 ± 189	1229 ± 163
Peak Power (W)	376 ± 30	265 ± 26*

Expiratory Flow Limitation

Figure 5 and 6 show individual flow volume curves at maximal exercise in men and women respectively. Subjects were considered flow limited if part of the NEP breath overlapped the preceding control breath. One male subject (subject 3) was excluded in the analysis of EFL because the NEP caused a sustained decrease in expiratory flow. If the NEP elicits a sustained increase in upper airway resistance, such that the flow with NEP remains below the control flow until the end of expiration, the NEP test is no longer valid for the assessment of EFL (112, 117). Expiratory flow limitation was shown to occur in 4 of the 7 male subjects (57%) and 9 of the 10 female subjects (90%) during the final stage of exercise.

Since 3 NEP tests were obtained throughout the final exercise stage, it could be determined if subjects could become flow limited on a given breath and later become non flow limited on a different breath (i.e., overcome EFL by adopting a different breathing "strategy"). Several subjects that experienced EFL (6 women, 2 men) during the initial portion of the final stage of exercise were later able to overcome the EFL. Figure 7 is a representative figure of a single female subject (subject 9) that experienced EFL early during the final exercise stage but was later able to avoid the EFL through an alteration in her breathing pattern. As expected, there was no appreciable decline in $\dot{V}E$ as the exercise progressed, but rather a slight alteration in V_T and F_b, such that V_T would decrease slightly and F_b would increase. It can be seen that there was a shift in EELV that occurred in order to avoid EFL.

Figure 5. Individual tidal flow volume loops from 8 male athletes during the final stage of exercise. Dark lines represent the control breath and thin lines represent the breath with the NEP.

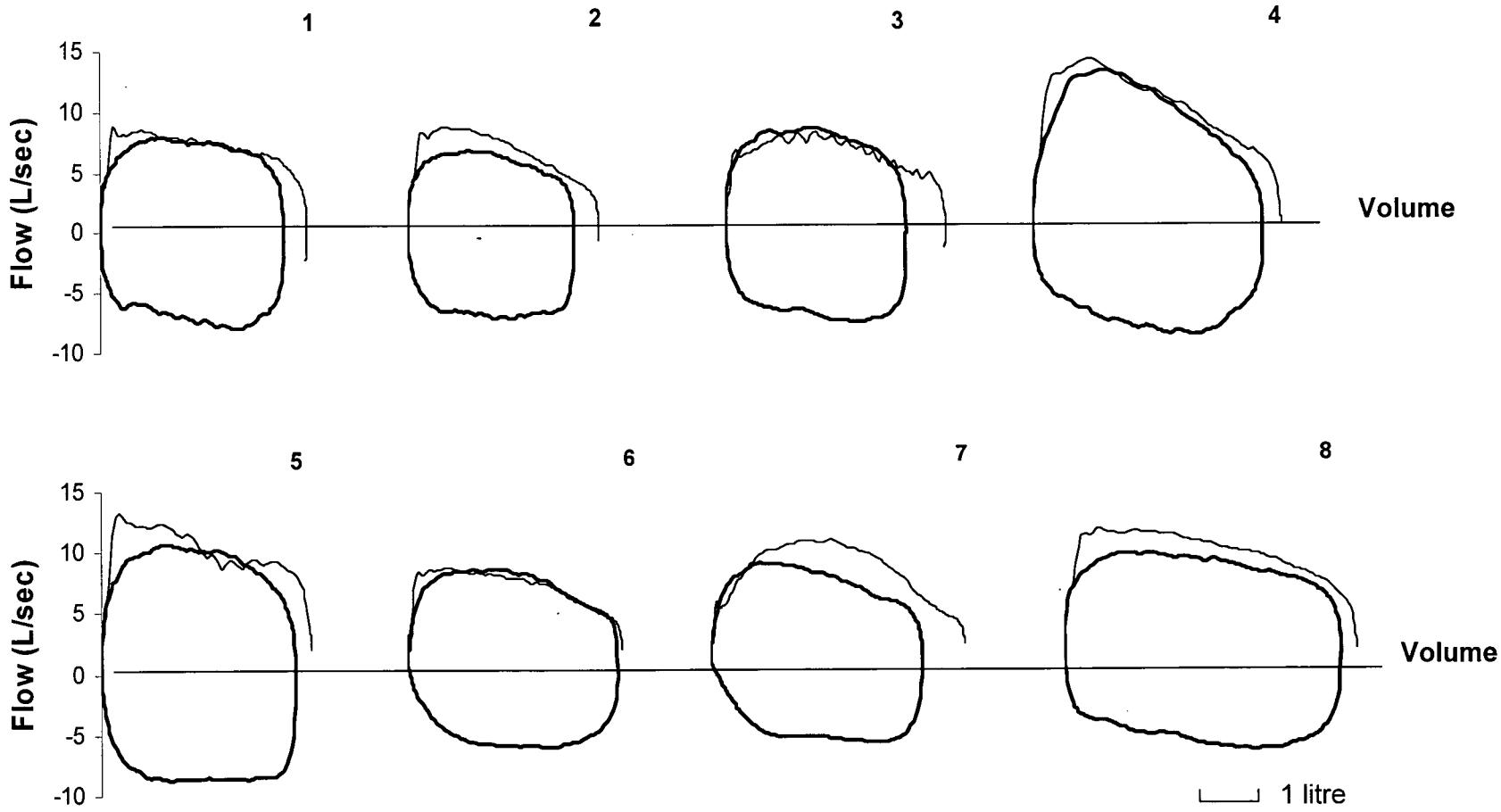


Figure 6. Individual tidal flow volume loops from 10 female athletes during the final stage of exercise. Dark lines represent the control breath and thin lines represent the breath with the NEP.

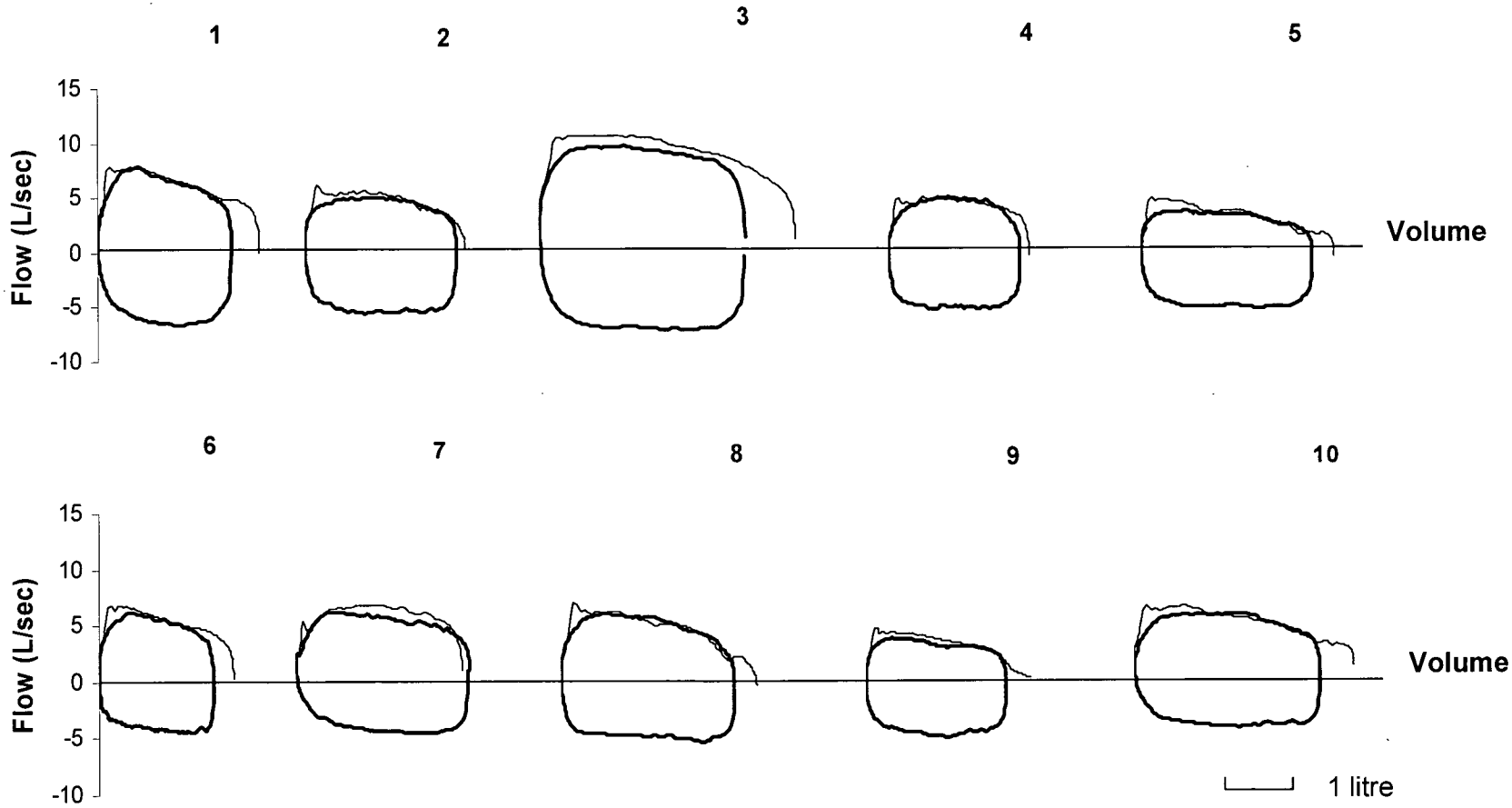
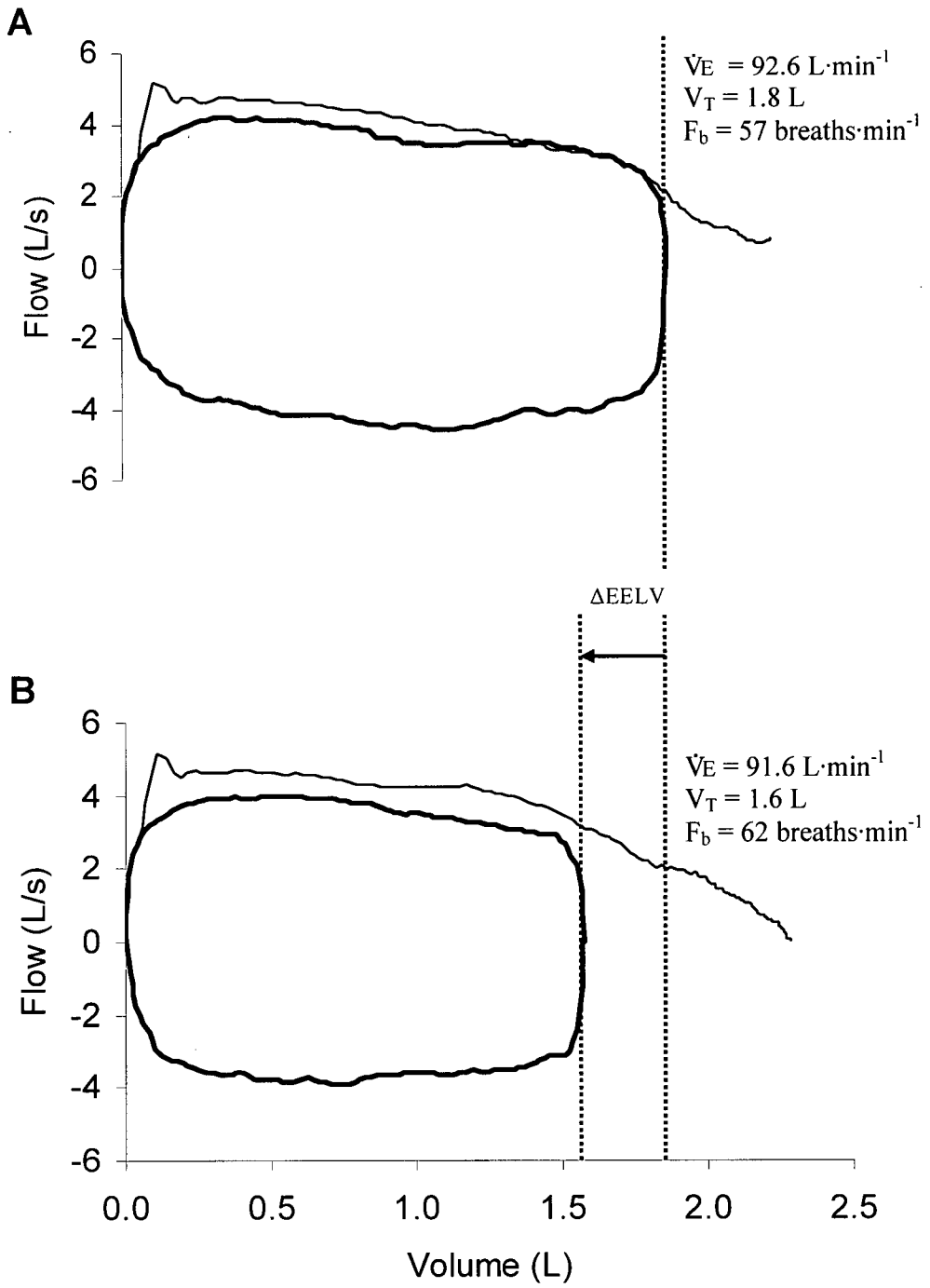


Figure 7. A) Representative flow volume loops of a single female subject (subject 9) experiencing EFL during the final stage of exercise as evidenced by the overlap between the control breath (dark line) and the NEP breath (thin line). B) Flow volume loops collected later during the final stage of exercise in the same subject. She is no longer flow limited because the NEP increased expiratory flow for the entire duration of the expiration. $\dot{V}_E$ = minute ventilation; V_T = tidal volume; F_b = breathing frequency; ΔEELV = change in end expiratory lung volume.



EELV and EILV

Figure 8 shows changes in EELV and EILV expressed as percentage of FVC at rest and during exercise in men (A) and women (B). Table 5 shows EELV and EILV expressed in liters and percentage of FVC in men and women. One female subject was discarded for the analysis of lung volumes due to her inability to correctly perform IC manoeuvres. Mean values for EELV decreased from rest in men and women with the onset of exercise. EELV remained significantly below resting EELV in men throughout all exercise intensities whereas females increased EELV at 89% and 100% of maximum exercise workload (W_{max}), such that EELV was no longer significantly different from rest. At maximum exercise, women had a significantly higher relative EELV compared to men (42±8 vs. 35±5 %FVC, $P<0.05$). Mean EILV in men continued to increase from rest and throughout exercise but hit a plateau between 90 and 100% of W_{max} whereas mean EILV rose throughout all exercise intensities in women. At maximal exercise, relative EILV was significantly higher in women compared to men (88±5 vs. 82±7 %FVC, $P<0.05$).

Figure 8. Subdivision of lung volumes, expressed as % forced vital capacity (FVC) at rest and during progressive exercise to maximal exercise (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 ± SE. * Significantly different from rest ($P<0.05$).

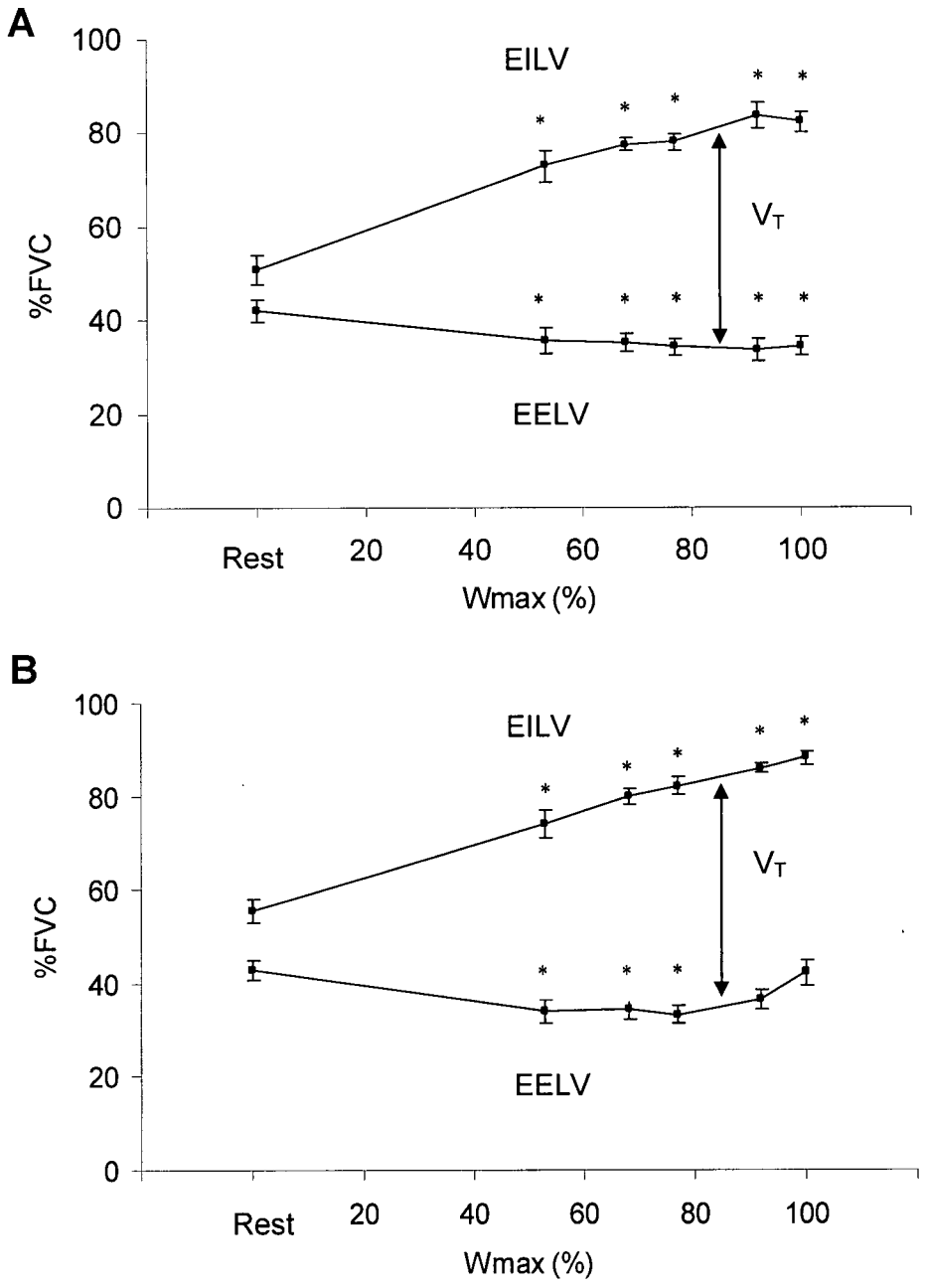


Table 5. Changes in operational lung volumes at rest and different levels of maximal exercise (W_{max}). Values are means ± SD. Definitions of abbreviations: EELV = end expiratory lung volume; EILV = end inspiratory lung volume; FVC = forced vital capacity. * Significantly different from rest; † Significantly different from men ($P<0.05$). Comparisons between men and women were made on relative EELV and EILV (i.e., %FVC).

Men (n=8)	%W_{max}					
	Rest	53%	68%	77%	92%	100%
EELV (L)	2.72 ± 0.63	2.30 ± 0.72*	2.28 ± 0.53*	2.23 ± 0.52*	2.19 ± 0.58*	2.24 ± 0.59*
EELV (%FVC)	42.15 ± 6.56	35.54 ± 7.92	35.32 ± 5.74*	34.39 ± 5.09*	33.84 ± 6.94*	34.48 ± 5.36*
EILV (L)	3.27 ± 0.77	4.70 ± 0.99*	5.00± 0.88*	5.04 ± 0.91*	5.39 ± 0.94*	5.33 ± 1.06*
EILV (%FVC)	50.80 ± 8.60	72.85 ± 9.53*	77.42 ± 3.91*	77.99 ± 5.47*	83.75 ± 8.15*	82.39 ± 6.50*
Women (n=9)	%W_{max}					
	Rest	54%	67%	78%	89%	100%
EELV (L)	1.97 ± 0.46	1.57 ± 0.32*	1.59 ± 0.28*	1.55 ± 0.23*	1.70 ± 0.36	1.94 ± 0.34
EELV (%FVC)	42.90 ± 6.71	34.73 ± 7.44*	35.02 ± 6.01*	34.26 ± 5.21*	37.17 ± 6.38	42.23 ± 8.06†
EILV (L)	2.53 ± 0.63	3.38 ± 0.33*	3.62 ± 0.38*	3.73 ± 0.38*	3.94 ± 0.45*	4.07 ± 0.46*
EILV (%FVC)	54.84 ± 7.24	74.57 ± 9.01*	79.39 ± 5.50*	81.71 ± 5.35*	86.19 ± 3.27*	88.12 ± 4.59*†

Work of Breathing

Figure 9 shows the individual raw traces relating the mechanical W_b and $\dot{V}E$ in men (A) and women (B). The curves are of a continually increasing slope meaning that the mechanical W_b per any additional unit of air ventilated ($dW_b / d\dot{V}E$) increases progressively with increasing $\dot{V}E$. In all cases the data points closely fit equation 1 (r^2=0.99) (see methods). An individual value for constant 'a' and 'b' was determined for each subject. The mean value for each constant is shown in table 6. Constant 'a' was significantly higher in women compared to men (P<0.05). A mean curve for men and women was constructed based on the average values for each constant (figure 10). Figure 10 for men and women have been extrapolated to 200 L/min for theoretical and visual purposes only. Figure 11 shows a comparison of the W_b across differing levels of $\dot{V}E$. Women had a higher W_b at almost all levels of $\dot{V}E$ (i.e., 60, 90, 120, 150 L/min, P<0.05).

Table 6. Mean values of constant 'a' and 'b' from equation 1. Values are means ± SD.
* Significantly different from men (P<0.05).

	Men (n=8)	Women (n=9)
a $(J/min)/(L/min)^3$ $(x\ 10^{-5})$	6.4 ± 4.1	19.0 ± 14.4*
b $(J/min)/(L/min)^2$ $(x\ 10^{-3})$	6.8 ± 3.2	6.4 ± 7.4

Figure 9. Individual responses of the work of breathing vs. minute ventilation in men (A) and women (B).

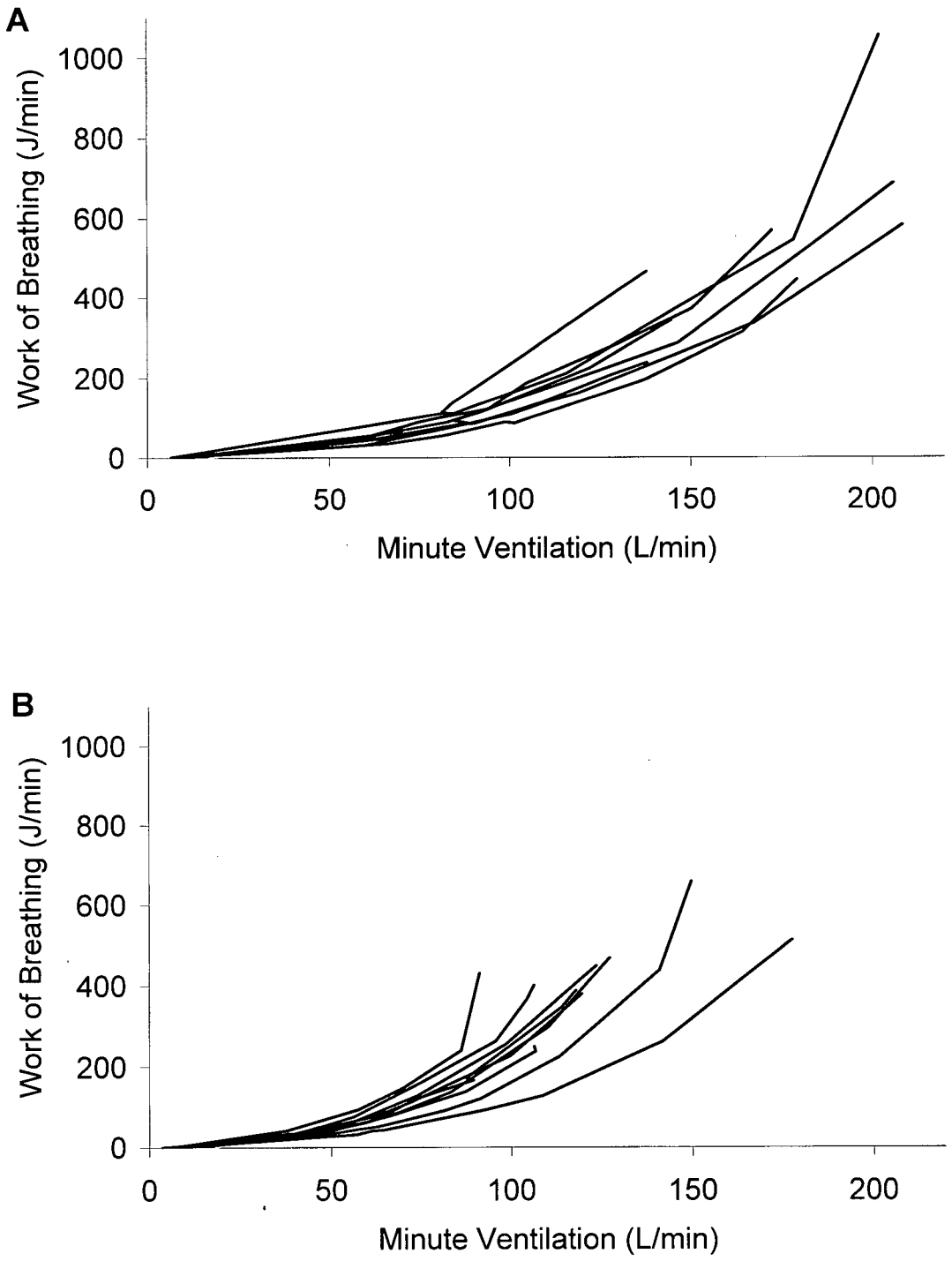


Figure 10. Mean curve relating the work of breathing vs. minute ventilation in men (thin line) and women (thick line). Both curves are based on mean values of constant 'a' and 'b' from equation 1. Each curve has been extrapolated to 200 L/min. See text for calculation of curves.

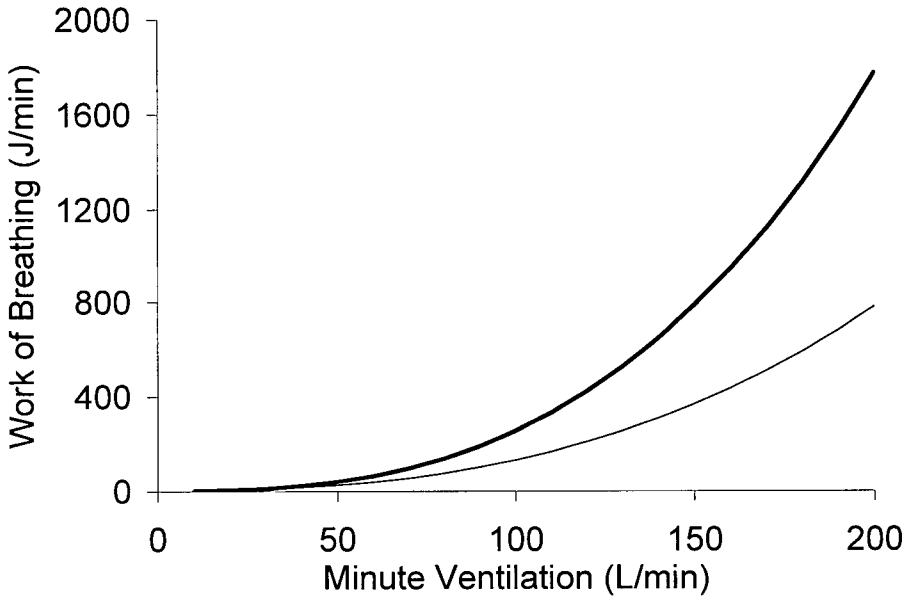
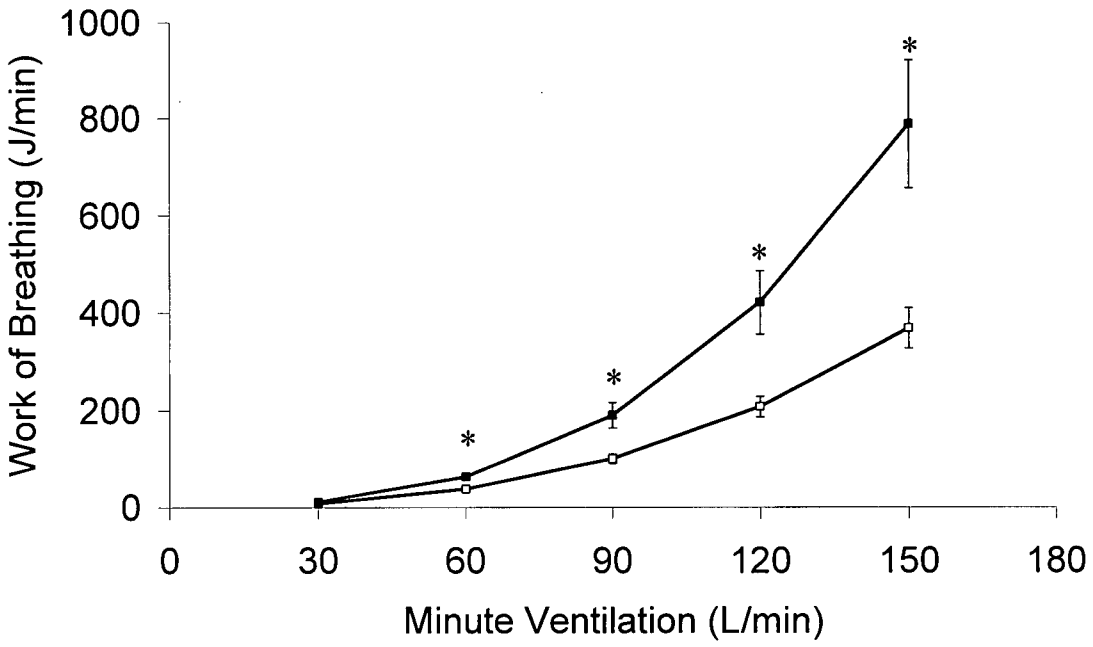


Figure 11. Work of breathing vs. minute ventilation in men and women at specific ventilations. ■ = average value for women; □ = average value for men; bars = SE. * Significantly different from men ($P<0.05$).

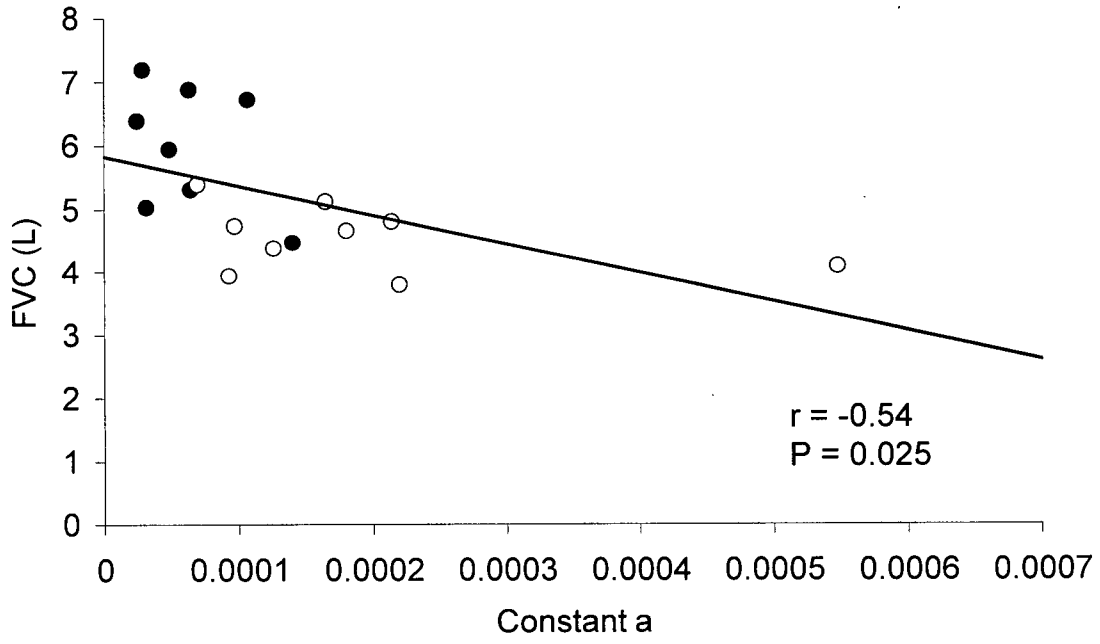


Constant 'b' did not correlate with any variables in this study. However, constant 'a' was significantly correlated with a number of variables as shown in table 7. Constant 'a' was significantly related to all spirometric variables and a number of variables at maximal exercise. The majority of significant 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 PEF were correlated with constant 'a' in women. There was also a trend for $FEV_{1.0}$/FVC and MVV to be related to constant 'a' in women (P=0.08 and 0.07 respectively). Figure 12 shows the relationship between FVC and constant 'a' in all subjects. There appeared to be one female outlier which slightly weakened each correlation. She had a significantly higher value for constant 'a' compared to all other subjects.

Table 7. Correlation analysis of spirometry and maximal exercise data with constant 'a'. Definitions of abbreviations: FVC = forced vital capacity; $FEV_{1.0}$ = forced expiratory volume in 1 second; $FEV_{1.0}/FVC$ = forced expiratory volume in 1 second / forced vital capacity; PEF = peak expiratory flow; MVV = maximum voluntary ventilation; F_b = breathing frequency; V_T = tidal volume; $\dot{V}_E$ = minute ventilation; $\dot{V}O_2$ = oxygen consumption; $\dot{V}CO_2$ = carbon dioxide production; $\dot{V}_E/\dot{V}O_2$ = ventilatory equivalent for oxygen; $\dot{V}_E/\dot{V}CO_2$ = ventilatory equivalent for carbon dioxide; $\dot{V}_E$/MVV = minute ventilation / maximum voluntary ventilation; $\dot{V}_E$/BSA = minute ventilation / body surface area. NS; non-significant correlation ($P>0.05$); * Statistically significant correlation ($P<0.05$).

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*
MVV	-0.71*	NS	-0.62
F_b	NS	NS	NS
V_T	NS	NS	NS
$\dot{V}_E$	-0.55*	NS	NS
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	-0.57*	NS	NS
$\dot{V}O_2$ ($L \cdot min^{-1}$)	-0.62*	NS	NS
$\dot{V}CO_2$	-0.58*	NS	NS
$\dot{V}_E/\dot{V}O_2$	NS	NS	NS
$\dot{V}_E/\dot{V}CO_2$	NS	NS	NS
$\dot{V}_E$/MVV	NS	NS	NS
$\dot{V}_E$/BSA	-0.55*	NS	NS

Figure 12. Relationship between forced vital capacity (FVC) and constant 'a.' Male subjects are denoted by solid black circles and women are denoted by open circles.



DISCUSSION

This is the first study to systematically compare the mechanics of breathing in male and female endurance athletes. More specifically, this study is the first to utilize the NEP technique to assess EFL and the first to measure the mechanical W_b in a group of female athletes during exercise. The principle findings in this study are 3 fold. (1) Female endurance athletes experience EFL more frequently and at a lower level of $\dot{V}E$ compared to male endurance athletes; (2) Females experience greater relative rises in EELV and EILV at maximal exercise compared to males; (3) The total mechanical W_b is higher in females compared to males during exercise.

Expiratory Flow Limitation

The present study has demonstrated that EFL occurs in both endurance trained men and women at maximal exercise. However, EFL appears to occur more frequently in trained women even though maximal $\dot{V}E$ was considerably lower than the men. Nearly all women experienced EFL (90%) at maximal exercise, whereas EFL occurred in only about half of the men (57%). The findings in the present study suggest that EFL is more common in endurance trained women. This is most likely due to the inherent differences in the structural and functional characteristics of the male and female pulmonary systems. The women in this study had significantly smaller lungs, lower peak flow rates and thus a smaller MFVL compared to men. It would be expected then that women would experience EFL at a lower or comparable level of $\dot{V}E$ than men. The findings in the present study extend those of McClaran et al. (81) that suggest women are more likely to utilize a greater percentage of their ventilatory reserve during exercise. These authors based their comparison on previously published data in men. A recent study by Guenette et al. (39) also found that highly trained women utilize a greater portion of their ventilatory reserve (i.e., $\dot{V}E/MVV$) at maximal exercise compared to highly trained men.

Endurance athletes are a unique population because they have excessively high metabolic and ventilatory demands even though their pulmonary function (i.e., lung volumes and flow rates) is not appreciably different from that of sedentary individuals (99). The high ventilatory requirement of endurance athletes might make them particularly susceptible to

EFL. However, the few investigations that have assessed EFL in endurance athletes have reported conflicting results. Mota et al. (86) suggests that EFL is rare in competitive male cyclists and the discrepancy in the literature may be due in part, to methodological considerations associated with the assessment of EFL. For example, EFL has been assessed in endurance athletes using two primary methods. The first method is the most traditional and involves the placement of tidal flow volume loops within a MFVL according to EELV. The second method is the most direct and is based on a comparison of pressure volume loops that meet or exceed the P_{tp} necessary to generate maximal flow (Pcrit). Using this direct method, Grimbly (38) found that one male subject reached EFL while cycling whereas two did not. However, all three subjects demonstrated EFL when the traditional method was used. The most probable explanation for this inconsistency comes from the thoracic gas compression artefact (54). If gas compression is not corrected for when superimposing tidal breaths within the MFVL, then EFL could be falsely detected or overestimated. Further evidence for methodological discrepancies comes from the work of Johnson et al. (61). These authors measured EFL in eight endurance trained male runners and found EFL to occur in half of them using the direct approach. These authors also used the traditional method but they corrected the MFVL for the thoracic gas compression artefact. Despite this correction, there was still a discrepancy between the two methods because the tidal flow volume loops impinged on the pre-exercise MFVL even though the expiratory P_{tp} did not attain Pcrit. As pointed out by Mota et al. (86), if Johnson et al. (61) used the post exercise MFVL with the traditional method, all eight runners should have developed EFL. This discrepancy is likely attributable to differences in the volume and time history that precede tidal expiration and forced expiratory manoeuvres (26, 27, 68). Since the volume and time history of a spontaneous tidal breath is different from that of an FVC manoeuvre, it is axiomatic that the traditional method be problematic even when the thoracic gas compression has been corrected for. Given the above considerations, Mota et al. (86) utilized the NEP technique to assess EFL in a group of competitive male cyclists. The NEP 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 NEP breaths. These authors demonstrated that only 1 out of 9 male cyclists developed EFL at maximal exercise which is lower than previous reports in male

athletes (61). Given these findings, it remains possible that EFL may also have been overestimated in previous studies on women.

McClaran et al. (81) measured EFL 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 EFL in 12 of the 14 (86%) fit women and 4 of the 15 (27%) less fit women. These authors used the traditional approach of placing tidal breaths within the MFVL but they did not take into account the thoracic gas compression artefact. Interestingly, Walls et al. (119) corrected for gas compression and still found EFL in 7 out of 8 (88%) recreationally active women ($\dot{V}O_2MAX$ = 46.8 ml·kg^{-1}·min^{-1}). All studies looking at EFL in women have utilized some variation of the traditional method, and given the findings of Mota et al. (86) in men, it is possible that previous studies have over-detected EFL in women. Accordingly, it was the purpose of the present study to assess sex differences in EFL using a technique that is less likely to detect false positives.

The present study utilized the NEP technique to assess EFL whereas McClaran et al. (81) used the traditional approach without any correction for the thoracic gas compression artefact. Interestingly, both studies found that almost all of the trained subjects developed EFL with only a few exceptions. Although it still remains possible that McClaran et al. (81) may have overestimated the degree of EFL in their women, the high prevalence found in both studies suggest that EFL is extremely common in fit women.

Although more EFL was found in women, over half of the male subjects also experienced EFL. The experimental protocol was nearly identical to Mota et al. (86) but it appears that there is a discrepancy in the number of subjects experiencing EFL. Mota et al. (86) exclusively used competitive male cyclists whereas the present study used 6 male cyclists, 1 rower and 1 runner. However, the runner (subject 3) was excluded from the analysis of EFL (see results). Therefore, both studies had relatively homogenous and thus comparable populations. There are two possible explanations for the differences in the present data in men with those of Mota et al. (86). Firstly, the most likely explanation is that the men in this study achieved a higher level of $\dot{V}E$ at maximal exercise (161 vs. 147 L·min^{-1}). Secondly, there were slight differences in the timing of the NEP application between the two studies. Based on visual inspection of the individual flow volume curves, it appears that a

few subjects in the study by Mota et al. (86) had the NEP activated during the middle portion of the tidal breath. In the present study, the NEP was applied almost immediately (i.e., 50ms delay) at the onset of expiration. This ensures that EFL across the entire portion of the tidal breath will not be missed. Although unlikely, it is possible that Mota et al. (86) may have missed small amounts of EFL in some subjects because the NEP was not applied instantaneously. The spike artefact typically associated with the NEP may have masked any flow limitation. This argument is based on subject 4 and 5 (figure 5) in the present study. These subjects experienced small amounts of EFL earlier in the tidal expiration with no concomitant ventilatory constraint during the later portion of the tidal expiration. If the NEP had been applied after this point, these subjects would not have been considered non flow limited.

Up to this point, the present study and others (81, 86, 119) have determined that athletes either do or do not develop EFL at maximal exercise. However, it is possible that flow limited subjects can make the necessary adjustments to overcome any previously experienced ventilatory constraint. Thus, it is important to recognize that EFL is not an all or none phenomenon but rather a phenomenon that has compensatory strategies. For example, eight subjects in this study developed EFL during the final workload but were later assessed as being non flow limited according to the NEP test. Figure 7 illustrates a typical example of a subject that made ventilatory adjustments to overcome previously experienced EFL. The general trend was for subjects to slightly decrease V_T and increase F_b such that total $\dot{V}_E$ did not change. This tachypneic breathing pattern likely occurred because increasing V_T would have been too difficult due to the EFL and the increasing elastic load on inspiration. When the tidal flow volume loops are aligned, it can be seen that EELV increased causing a relative hyperinflation and thus the avoidance of EFL (figure 7). It should be noted that the magnitude of the NEP was the same in both tests. There are other compensatory strategies that may have occurred in order to avoid and or overcome EFL. For example, some subjects may have had a significant exercise-induced bronchodilation which would increase the MFVL. Previous studies have shown that bronchodilation is present for a short period of time immediately after exercise in normal subjects (120).

Operational Lung Volumes

Several studies have demonstrated that EELV decreases during exercise (49, 61, 73, 86, 107) while others have shown that it remains relatively constant (64, 111). Although this has previously been considered a contentious issue, the vast majority of studies suggest that EELV decreases throughout submaximal exercise. Those studies that demonstrated a decrease also showed considerable intersubject variability with some subjects decreasing EELV only at the highest exercise levels (107). However, in most subjects, the changes are progressive with increasing exercise intensity (49, 73, 107). The men in this study decreased EELV at the onset of exercise and maintained this pattern of breathing even at the highest workloads. The women followed a nearly identical pattern throughout submaximal exercise but began increasing EELV at the highest exercise intensities. It appears that men and women in this study regulate EELV in the same manner during submaximal exercise. Henke et al. (47) suggest that mechanical feedback from the lung and chest wall cause active expiration and reductions in functional residual capacity. Decreasing EELV may then be a strategy that optimizes diaphragm length and lowers the inspiratory W_b. Inspiratory W_b is reduced by recovering some of the work done by the expiratory muscles during the previous expiration (25, 48, 59, 102).

Although men and women demonstrated similar changes in EELV throughout the majority of submaximal exercise, there were differences at near maximal and maximal exercise. The women in this study started to increase EELV at 89% and 100% of W_{max} such that EELV was no longer significantly lower than resting EELV (figure 8). The relative EELV was also significantly higher in women compared to men at maximal exercise. The most probable explanation for these findings comes from the fact that nearly all women experienced EFL. Increases in EELV have previously been shown to increase in individuals that are capable of achieving very high levels of exercise and is often associated with the presence of EFL (38, 61, 90). Increasing EELV allows subjects to utilize the higher flow rates available at the larger lung volumes. Pellegrino et al. (95) investigated the effect of EFL on EELV by imposing an expiratory threshold load in male subjects. These authors concluded that the increase in EELV during exercise is associated with EFL and that compression of airways downstream from the flow-limited segment may elicit a reflex

mechanism that influences breathing pattern by prematurely terminating expiration. McClaran et al. (81) also examined the effect of EFL on EELV in a group of women by having subjects breath HeO_2 (79% He, 21% O_2) in order to increase the size of the MFVL and thus eliminate EFL. These authors found that reducing EFL caused their subjects to maintain a lower EELV. Furthermore, the lower EELV with the HeO_2 occurred only when HeO_2 caused a significant reduction of EFL. These findings are in good agreement with those of Pellegrino et al. (95) which suggest an interrelationship between EFL and the increase in EELV associated with heavy exercise. The present results are in excellent agreement with these studies. This study showed a clear change in breathing pattern that resulted in the ability for many subjects to overcome previously experienced EFL (figure 7). This is the first study to illustrate such a finding using the NEP technique during exercise. In this regard, the NEP test is advantageous in that EFL can be assessed on a breath by breath basis whereas the traditional approach is based on an average of several breaths.

It is important to note that a link between EFL and EELV is not a universal finding. Mota et al. (86) found that a group of competitive cyclists increased EELV at maximal exercise despite only one subject experiencing EFL. The pattern of change in EELV in Mota's study is nearly identical to what was found in the women of the present study. However, 90% of the women in this study actually developed EFL, all of which increased EELV back to near resting levels. The single female that was not flow limited did not increase EELV. This is in direct opposition to Mota's study because 9 of their 10 subjects were not flow limited yet their group still increased EELV. Mota et al. (86) attribute their findings to a reflexive mechanism which is triggered when an individual approaches EFL in an effort to avoid dynamic compression. These authors argue against the possibility of recording falsely low ICs but there are two methodological concerns that argue against this. Firstly, they did not measure P_{es} to ensure that subjects reached maximal inspiratory pressure during the IC manoeuvres. Secondly, subjects performed ICs immediately after the application of the NEP. The NEP tends to increase EELV which is clearly evident in nearly all the flow volume loops in Mota's and the present study (figure 5 and figure 6). This means that the subjects will be initiating the IC well above functional residual capacity which will result in an underestimation of IC and thus, overestimating EELV. To avoid this problem,

subjects in the present study performed the IC prior to each NEP test. The NEP was then applied after breathing patterns returned back to normal levels. The results from this study indicate that there is a link between EFL and the exercise induced increases in EELV. It is proposed that a reflexive inhibition of respiratory motor output occurs in response to EFL rather than a reflex that is triggered in order to avoid EFL as has been suggested by others (82, 86).

This study has also shown that relative EILV is significantly higher at maximal exercise in women compared to men. Given the higher EELV in women, it is not surprising then that EILV also be higher as this occurs in order to preserve the exercise VT. The EILV in these women are approaching levels that may be considered a marker of ventilatory constraint and an index of increased ventilatory work (15). The higher EILV 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 W_b and $\dot{V}E$ during exercise has been explored in several studies (4, 22, 24, 84). However, all data points have been exclusively obtained in men with only one exception (4). There was one female subject in this study (n=8) and she had the highest W_b compared to the group mean (544.1 J/min vs. 391.8 J/min) despite having a lower maximal $\dot{V}E$ relative to the group mean (139 L/min vs. 147 L/min). Furthermore, her O_2 cost of breathing was higher at 70% and 100% of $\dot{V}O_2MAX$ compared to the group mean. This is one of the only data points where the W_b has been measured in a woman. Given the smaller lung volumes and smaller diameter airways in women relative to height matched men, it seems intuitive to speculate that women should have a higher W_b. However, there is no data to support this hypothesis. Accordingly, the present study sought to test this hypothesis by measuring the W_b in a group or endurance trained athletes of both sexes.

The men and women tested in this study were of similar athletic calibre and both had a $\dot{V}O_2MAX$ within the same predicted value. As expected, the men were able to achieve a higher maximal $\dot{V}E$ and VT compared to women. Figures 10 and 11 show the mean relationship between the W_b and $\dot{V}E$ in men and women. It can be seen that the W_b is

essentially the same at rest and during very low levels of exercise $\dot{V}E$ (i.e., <60 $L \cdot min^{-1}$). However, as $\dot{V}E$ increases beyond this point, the W_b in women begins to significantly increase out of proportion to men. In fact, the W_b in women is approximately twice that of men at ventilations beyond 90 L/min. 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. (115) that suggest a higher O_2 cost of breathing in women. These authors found that the O_2 consumption of the respiratory muscles was significantly higher in women compared to age-matched men.

The method of analysis in the present study is similar to those used by other researchers that have compared the W_b between two groups (22, 84). A curve relating W_b and $\dot{V}E$ was generated according to equation 1 (see methods). The term $b\dot{V}E^2$ represents the mechanical work done in overcoming the viscous resistance offered by 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 (79). Constant 'a' was shown to be significantly higher in women which may be an indication that the higher W_b 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 $\dot{V}E$. The increasing flow rates associated with the rise in $\dot{V}E$ likely caused a shift from predominantly laminar to turbulent flow. The increase in turbulent flow would likely cause an increase in the resistive W_b in women.

Based on mechanical grounds, it would be expected that subjects with larger lung volumes would have lower pulmonary resistance (18) and thus a lower W_b 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 W_b. There was a high degree of variability in constants 'a' and 'b' which may be related to the differences in lung volumes. In fact, when men and women were pooled together, there was a significant, albeit modest, correlation between lung volume and constant 'a' (figure 12).

Although airway diameter was not measured, the increased resistive work in women is also dependent on their inherently smaller diameter airways (83). It was noted that constant 'a' was significantly correlated to $FEV_{1.0}$ and PEF in women and also when all

subjects were pooled together (table 7). Since the shape of the MFVL 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 PEF is an indication that airway diameter is partially responsible for the present results. Although lung volume and airway diameter are explanations for the present findings, caution should be taken because these mechanisms are based on indirect and correlative data.

The higher W_b in women is also attributable to the smaller VT that occurs during exercise. A lower VT means that women would have to rely on a higher F_b which increases the amount of dead space $\dot{V}E$ and therefore an even higher $\dot{V}E$ would be necessary to obtain a similar $\dot{V}A$ as men (81). The women in this study will also have a higher W_b at maximal exercise because of the high prevalence of EFL and the corresponding effect on EELV and EILV. It is important to note that further partitioning of the P_{tp} -volume loops of each subject would likely shed more light on the specific contributions of each W_b component to the results in this study. However, the present study was primarily interested in measuring the total mechanical W_b and determining if it was higher in women. The specific components of work that contribute to the total mechanical W_b is beyond the scope of this study.

The high W_b in women is going to have a number of physiological and performance based implications. The high degree of EFL and its associated increases in EELV and EILV might make women prone to diaphragmatic fatigue, particularly at very high exercise intensities. There seems to be an association between the W_b, diaphragm fatigue, and exercise performance in men, which is based on the reflexive effects of respiratory muscle fatigue which increases sympathetic vasoconstrictor outflow and reduces limb blood flow during prolonged exercise (31). Harms et al. (46) hypothesized that a high W_b will influence locomotor muscle performance and thus whole body endurance performance. To test this hypothesis, cyclists performed time to exhaustion cycle exercise at 90% of $\dot{V}O_2MAX$. The W_b was increased or decreased by respiratory muscle loading and unloading respectively. The increased W_b by respiratory muscle loading caused a 15% reduction in time to exhaustion performance compared to controls. This detrimental effect on exercise performance was attributed to the associated reduction in leg blood flow, which enhances the onset of leg fatigue and also the intensity of leg and respiratory muscle effort perceptions. When the

respiratory muscles were unloaded, there was a reduction in $\dot{V}O_2$, a reduced rate of change in dyspnoeic and limb discomfort sensations, and improvements in time to exhaustion performance by 14%. Given the findings in the present study, it is possible that endurance trained women with a high W_b may be at risk of diaphragmatic fatigue and possible decreases in endurance performance. However, this hypothesis has not been directly tested in women.

Limitations

Studies aimed at understanding sex based physiological differences are highly dependent on appropriate subject matching. This is particularly the case in respiratory exercise physiology. Body size is related to lung volumes and thus breathing mechanics. Female subjects in this study were significantly smaller in size and had a lower $\dot{V}O_2MAX$. However, subjects were of similar athletic calibre and were matched based on predicted values for $\dot{V}O_2MAX$. The purpose of this study was to assess the typical sex based differences (i.e., smaller lungs, airway diameters, etc.) that may influence breathing mechanics in the female population. Ideally, subjects would be matched for age, height, weight, body composition, lung volumes, airway diameter, $\dot{V}O_2MAX$, and several other variables. However, this is not a typical representation of the male and female athletic population. Therefore, the present study aimed at sampling a group of male and female athletes of similar aerobic capacity that possessed all of the typical sex based anatomical differences.

The NEP technique has been validated in both mechanically ventilated patients (117) and in resting subjects with chronic obstructive pulmonary disease (69). However, only three studies have been performed using the NEP technique during muscular exercise (28, 67, 86). Two of these studies have deemed the NEP technique to be a reliable method for determining EFL during exercise. However, a recent study by de Bisschop et al. (28) provide evidence that the NEP technique during exercise might misrepresent EFL taken as an index of airway flow limitation. They detected EFL in 5 out of 8 healthy subjects with a 5hPa NEP. However, when the NEP was increased to 9hPa, 4 of these 5 individuals no longer experienced EFL. These authors concluded that EFL during exercise is NEP dependent which could be related to a reflex inhibition of expiratory muscle activity, via lung and muscle receptors, leading to a decrease in the expiratory driving pressure. It is unlikely

however that EFL was falsely detected in this study because a NEP of approximately -10 cmH_2O (- 9.8 hPa) was used which should be high enough according to de Bisschop et al. (28). However, false detection of EFL cannot be completely ruled out because there was some variability in the magnitude of the NEP.

The analysis of the W_b in this study is similar to those of Milic-Emili et al. (84) and Cibella et al. (22) where the W_b was compared between two groups. In order to allow for a direct comparison, the W_b in men and women were compared in two different ways. Firstly, constants were computed from equation 1 based on the relationship between the W_b and $\dot{V}E$ as determined by Otis (93). The statistical comparison was based on the average constants for men and women. The corresponding curves can be seen in figure 10. The second method involved the comparison at specific points of $\dot{V}E$ (figure 11) in order to provide a better idea of where the differences occurred as opposed to a general comparison of a constant which essentially describes the shape of the curve. This second method has some limitations because the W_b was derived by entering different ventilations into each subject's individual equation. Although this method allowed us to compare both sexes across different points of $\dot{V}E$, the variability may have been increased because values were obtained through interpolation and extrapolation. However, it is unlikely that this had a major effect on the results since each subject fit equation 1 very closely (i.e., r^2=0.99).

The measurement of EELV was determined using IC manoeuvres. EELV may be altered by performing these IC manoeuvres which could happen as subjects prepare to perform an IC or transiently after the maneuver is performed (63). To account for any alterations in EELV, several breaths were monitored prior to the IC 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 IC manoeuvres are dependent on subject motivation, it is possible that subjects will not fully inspire to TLC when performing the IC. To account for this limitation, continuous measurements of P_{es} were made so that maximal inspiratory pressure during the IC manoeuvres could be monitored. If the maximal inspiratory pressure obtained during exercise was similar to that obtained repeatedly at full inflation (TLC) at rest, confidence can be made that TLC was reached during the manoeuvres (8, 62, 122). If subjects failed to achieve the pre-exercise target

pressures, they were required to repeat the IC maneuver during exercise. Therefore, it is unlikely that the IC was underestimated at any point during exercise.

Conclusions

The results from this study indicate that the female pulmonary system may be at a disadvantage compared to their male counterparts during intense exercise. The present study has demonstrated that female athletes tend to develop EFL more frequently than male athletes. It was also observed that women have higher relative increases in EELV and EILV at maximal exercise. Although women are clearly utilizing a greater portion of their ventilatory reserve, the physiological cost of EFL during heavy exercise may have implications on exercise performance. Finally, women tend to have a higher W_b across a wide range of ventilations compared to men. Many of the differences observed in this study are likely due to the smaller lungs and presumably the smaller diameter airways in women. A higher W_b may cause a competitive relationship between the respiratory and locomotor muscles for a limited cardiac output. Also, the higher W_b and increased susceptibility to EFL in women may lead to exercise induced diaphragmatic fatigue. However, neither of these hypotheses has been directly tested in women. Further research is necessary to gain a further understanding of the physiological and practical implications of the present findings.

APPENDIX A

Review of Literature – Pulmonary System Limitations during Exercise

Maximum oxygen uptake ($\dot{V}O_2MAX$) can be defined as the highest rate at which the body can take up and utilize oxygen during maximal exercise (11). It has received significant attention in the field of exercise physiology for a number of reasons. For example, $\dot{V}O_2MAX$ is commonly used to indicate the cardiorespiratory fitness of an individual and is often used as a tool for exercise prescription. Given its wide usage in the field of exercise physiology, significant interest has developed over the years in identifying the physiological factors that limit $\dot{V}O_2MAX$ and the corresponding effects on endurance performance. Examples of these limitations include cardiac output, maximum stroke volume, skeletal muscle vascularity, and the oxidative capacity of locomotor muscles (29). The pulmonary system also plays a key role in the oxygen transport chain and has recently attracted attention as a potential rate limiting factor to $\dot{V}O_2MAX$. Traditionally, the pulmonary system was considered relatively "overbuilt" for exercise and did not pose a significant barrier to oxygen transport. However, as one moves up the fitness continuum, the gas exchange capability of the lung and chest wall assume a more critical role as a rate limiting factor in determining $\dot{V}O_2MAX$ (29). Moving up this fitness continuum is achieved because of the metabolic, haemodynamic and cardiovascular adaptations that occur with training, while the lung and chest wall remain relatively unchanged. Therefore, the pulmonary system that was once thought to be "overbuilt" for exercise actually lags behind the other adapted organ systems and poses a significant limitation to oxygen transport (29). Examples of pulmonary system limitations that occur with exercise include exercise induced arterial hypoxaemia (EIAH), expiratory flow limitation (EFL), and exercise induced diaphragmatic fatigue. Research on these pulmonary system limitations have traditionally been focused on male subjects and only recently have researchers compared the male and female responses of the respiratory system to exercise with accumulating evidence to suggest women may be more susceptible to pulmonary system abnormalities. Accordingly, this review will focus on EIAH and EFL as it pertains to the female population with an additional review on exercise induced diaphragmatic fatigue.

Exercise Induced Arterial Hypoxaemia

It has traditionally been thought that the degree of oxygenation in arterial blood is maintained during all levels of exercise. However, a number of studies have documented arterial O_2 saturations of 3-15% below resting levels at or near maximum exercise intensities (39, 100, 109). This decline in arterial oxygenation has been termed exercise induced arterial hypoxaemia and has been characterized as mild (SaO_2: 93-95%), moderate (SaO_2: 88-93%), and severe (SaO_2: $\leq$ 88%) (32).

To determine the prevalence of EIAH, Powers et al. (98) studied sixty-eight males with varying fitness levels. Subjects were divided into untrained, moderately trained or highly trained groups. All subjects performed an incremental cycle test to exhaustion and SaO_2 was determined using pulse oximetry. None of the subjects in the untrained and moderately trained groups demonstrated EIAH (defined as <91%) whereas 52% of the highly trained subjects developed significant EIAH.

A number of recent studies have attempted to determine the prevalence of EIAH in women with conflicting results. Harms et al. (42) studied 29 healthy young females with a wide range of fitness levels ($\dot{V}O_2MAX$ range: 35-70ml/kg/min) during the early follicular phase of their menstrual cycle. Of these 29 women, 22 (76%) had significant desaturation during treadmill running to exhaustion. The work of Harms et al. (42) claims a higher prevalence of EIAH compared to men and that it occurs in women with lower aerobic fitness levels. Despite these findings, Hopkins et al. (51) found that only 24% of fit female subjects (n=17) developed EIAH during treadmill running to exhaustion. It is difficult to determine why there is such a large discrepancy between these studies but it may be due in part to the role of the menstrual cycle and circulating sex hormones. Harms et al. (42) standardized testing to the follicular phase of the menstrual cycle while Hopkins et al. (51) tested subjects randomly throughout the menstrual cycle.

Most recently, Richards et al. (101) determined the prevalence of EIAH in 52 young women of varying fitness levels using pulse oximetry. EIAH occurred at a higher prevalence (67%) and at relatively lower fitness levels in females compared to that previously reported for males. Richards et al. (101) also observed moderate EIAH in women who had a $\dot{V}O_2MAX$ that was within 15-20% of their predicted value. This is an interesting finding because males

who experience EIAH have a $\dot{V}O_2MAX$ that is often ≥ 150% of their predicted value. The findings of Richards et al. (101) claims a prevalence of EIAH similar to that of Harms et al. (42), suggesting that women may be more susceptible to EIAH compared to males. More importantly, in both studies on women, EIAH occurred at lower levels of $\dot{V}O_2MAX$ compared to what is normally seen in the male population. Therefore, some of the sex differences in pulmonary structure and function may influence gas exchange and contribute to the EIAH seen in women.

The aforementioned results are supported by physiological evidence that women may be more vulnerable to pulmonary limitations during exercise (81, 83, 105, 114). For example, there are a number of important sex differences in pulmonary structure and resting pulmonary function that may influence gas exchange and the ventilatory response to exercise. 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. Lower maximal expiratory flow rates are due to smaller airway diameter whereas a lowered diffusing capacity is due to a smaller surface area resulting from fewer alveoli (81, 83, 105). Some of the sex differences seen in resting lung function are partially explained by differences in sitting height, which may serve as a surrogate for chest volume (105). Recent work confirms that women have significantly smaller lung volumes and lower maximal expiratory flow rates compared with predicted values for men at the same age and standing height (42, 81).

Mechanisms of EIAH

EIAH occurs when PaO_2 is reduced below resting levels due to a widened alveolar to arterial oxygen difference ($AaDO_2$) and an insufficient increase in PAO_2. Further reductions in SaO_2 occur because of a rightward shift in the HbO_2 dissociation curve due to metabolic acidosis and increased temperature. Although a number of studies have been conducted in an attempt to elucidate the mechanism underlying EIAH, the causes are not clearly understood and remain a topic of debate. Several mechanisms have been identified as likely contributors to the development of EIAH, however their relative importance is currently unknown. Possible mechanisms include relative alveolar hypoventilation, ventilation-perfusion

mismatching and diffusion limitation. This review will focus on these mechanisms as they pertain to healthy females.

The onset of metabolic acidosis and arterial hypoxaemia is generally compensated through a hyperventilatory response that occurs during heavy exercise. However, those who experience EIAH may have an inadequate hyperventilatory response. If an individual does not adequately ventilate in the face of a widened $AaDO_2$, then alveolar PO_2, SaO_2 and ultimately arterial O_2 content may be compromised. An inadequate hyperventilatory response may be due to EFL, a blunted respiratory drive and/or hormonal fluctuations.

The role of EFL in women was first examined by McClaran et al. (81). These authors asked whether the smaller lung volumes and lower maximal expiratory flow rates in women would alter the degree of expiratory flow limitation and thereby affect the ventilatory response to exercise. To answer this question, they studied 29 healthy females of varying fitness levels. They found that EFL constrains the ability to compensate for inadequate alveolar-to-arterial O_2 exchange and thus exacerbates EIAH. The work of Walls et al. (119) extends these findings by demonstrating a strong correlation between end exercise saturation and EFL ($r = -0.71$) in 8 healthy women exercising to exhaustion. Despite these findings, the specific contribution of increased EFL to EIAH remains unclear (52).

An inadequate hyperventilatory response may also be related to a blunted drive to breathe. A blunted drive to breath is determined by ones chemoresponsiveness, which can be measured by performing a resting hypoxic ventilatory response (HVR) test. A low HVR has been correlated to EIAH by some investigators (33, 43) and not by others (53). Most recently, Guenette et al. (39) compared the HVR between sexes and found no difference in resting chemoresponsiveness in trained and untrained men and women. Furthermore, their findings extend those of Hopkins and McKenzie (53) that show no correlation between chemoresponsiveness and EIAH.

Finally, it has been postulated that inadequate hyperventilation may be due to hormonal fluctuations. Progesterone combined with estrogen raises both $\dot{V}_A$ and chemosensitivity via central (13) and peripheral (113) receptor-mediated mechanisms. The role of circulating progesterone throughout the menstrual cycle on EIAH and the ventilatory response to exercise remains unclear (52). While there is evidence that an inadequate

hyperventilatory response may contribute to the pulmonary gas exchange impairment in women during exercise, it appears that $\dot{V}_E$ alone cannot fully compensate for the excessively widened $AaDO_2$.

An excessively widened $AaDO_2$ may also be attributable in part, to a heterogeneous $\dot{V}_A/\dot{Q}$ distribution combined with a reduced mixed venous O_2 content. The work of Olfert et al. (91) is the only study that has attempted to assess the relative contribution of $\dot{V}_A/\dot{Q}$ inequality and diffusion limitation to the $AaDO_2$ during exercise in women compared to men. Furthermore, this is the only study to compare the effects of sex, independent of the effects of lung size on pulmonary gas exchange. Subjects were matched on the basis of age, height, lung volumes and $\dot{V}_{O_2MAX}$. They exercised men and women in both normoxia and hypoxia to assess the contribution of both $\dot{V}_A/\dot{Q}$ matching and O_2 diffusion limitation to pulmonary gas exchange during light, moderate and heavy exercise. The data showed no differences in $AaDO_2$ based on sex. This data revealed less $\dot{V}_A/\dot{Q}$ inequality during exercise in females compared to males. Female subjects did not experience greater O_2 diffusion limitation during exercise suggesting the importance of absolute lung size or aerobic fitness in determining susceptibility to EIAH rather than sex *per se.* Furthermore, the lower maximal expiratory flow rates and smaller diameter airways in women may be a contributing factor to some of the exercise induced pulmonary system abnormalities found in women. To determine the role of lung size, airway diameter, and flow rates to EIAH in women, an in depth examination of ventilatory limitations and lung mechanics is necessary.

Mechanical Work of Breathing

The work necessary to ventilate the lungs can be divided into elastic and non-elastic work components. Elastic work is composed of work that must be done against lung elastic recoil, chest wall recoil, and surface tension. The non-elastic component refers to the effort required to overcome airway resistance but includes a small contribution from tissue resistance. The W_b can be further subdivided into sub-components including inertial forces, gravitational forces, and distorting forces of the chest wall. The total mechanical work done during breathing is the sum of all elastic work and work against gravity done during inspiration and /or expiration, all flow resistive work except that done by previously stored

elastic energy and all negative work (92). With progressive exercise to exhaustion, the increase in minute $\dot{V}_E$ results in a disproportionate increase in the work and O_2 cost of breathing (61).

Breathing Pattern during Exercise

Efficient gas exchange during exercise is dependent on the regulation and coordination of the respiratory musculature to increase $\dot{V}_A$. Increases in $\dot{V}_A$ are mediated by changes in both breathing frequency (F_b) and tidal volume (V_T). During light to moderate intensity exercise, increases in $\dot{V}_E$ are primarily accomplished by increases in V_T. Tidal volume changes are achieved by increasing end inspiratory lung volume and decreasing end expiratory lung volume (EELV); thus encroaching on both inspiratory and expiratory reserve volumes. As V_T approaches 50 to 60% of vital capacity, any further increase in $\dot{V}_E$ occurs by an increase in F_b by reducing time on inspiration and expiration. This typical breathing pattern has several advantages related to gas exchange efficiency and the functionality of the inspiratory muscles: (1) it limits the amount of dead space $\dot{V}_E$; (2) it reduces the W_b by avoiding volume increases to levels where there is increased lung stiffness; (3) the reduced EELV occurs by active expiatory muscle effort thereby assisting inspiratory muscle effort, and (4) the reduction in lung volume means that the diaphragm and other inspiratory muscles are lengthened and therefore operate at a more optimal position for generating force (44). Although this typical breathing pattern can be considered metabolically efficient, there are exceptions when the W_b becomes very high during strenuous exercise.

Ventilatory Constraint

The constraint to $\dot{V}_E$ that occurs during exercise has traditionally been assessed using a measure of breathing reserve. Comparing one's maximal exercise $\dot{V}_E$ to his or her maximal voluntary ventilation (MVV) may provide an estimate of ventilatory capacity and mechanical constraints to exercise hyperpnoea (63). However, this technique provides little information regarding the source or type of ventilatory constraint (e.g., EFL, inspiratory flow limitation, or high inspiratory elastic load) (63). Using a voluntary measure of $\dot{V}_E$ at rest and comparing it to $\dot{V}_E$ during exercise may be problematic because of breathing pattern differences. For

example, Klas and Dempsey (66) demonstrated that if the MVV is performed above the resting functional residual capacity, EILV approaches total lung capacity, and expiratory flows reach maximum even at the highest lung volumes. This pattern of breathing is different from the pattern of breathing that occurs during exercise. Furthermore, they demonstrated that the W_b associated with the MVV far exceeds the W_b during exercise. For these reasons, breathing reserve may not be an ideal measure for assessing mechanical constraints to exercise hyperpnoea. More accepted techniques involve the measurement of exercise tidal flow-volume loops (FVL) and placing them within a maximum flow-volume loop (MFVL) or using the negative expiratory pressure (NEP) technique.

The MFVL defines the limits of the airways and respiratory muscles to produce flow and volume during exercise. Thus, when the exercise tidal FVL is placed within the MFVL, the presence and extent of EFL can be determined (figure 1a). Using the exercise FVL not only provides a visual index of ventilatory demand versus capacity but it allows the quantification of ventilatory constraint. More specifically, as summarized by Johnson et al. (63), this technique provides the following information: (1) the degree of EFL; (2) breathing strategy; (3) elastic load; (4) inspiratory flow reserve and (5) a theoretical estimate of the ventilatory capacity. Although this is a widely used technique, some have criticized its validity for two primary reasons. Firstly, the thoracic gas compression artefact of the MFVL may lead to a false detection or overestimation of EFL (54). The second limitation is based on differences in the volume and time history that precede tidal expiration and forced expiratory manoeuvres (86). An alternative method to detect EFL is to apply a negative expiratory pressure (NEP) at the mouth, and the flow volume curve during the ensuing expiration is compared with the preceding breath (86, 117). Unlike the previously mentioned technique, the NEP technique does not require forced expiratory efforts or the correction for gas compression and the volume history of the control expiration and the subsequent expiration with the NEP is the same. However, this technique has its limitations because the NEP may cause upper airway collapse resulting in a false comparison with spontaneous expirations (72). Other limitations include the inability to detect changes in EELV and the "all or none" quantification of EFL which provides little information regarding the degree of ventilatory constraint (58). Perhaps the most appropriate technique is to combine the

measurement of the exercise tidal FVL with the application of the NEP. This would avoid complications of performing a MFVL yet preserving the ability to quantify changes in EELV and determine the degree of EFL.

In most healthy individuals, the pulmonary system is capable of meeting the ventilatory demand of intense exercise, since ventilations up to 120-130 L/min are typically within the limits of the normal MFVL (61). However, in some individuals, the increase in $\dot{V}_E$ that occurs during progressive exercise may cause a portion of the tidal breathing loop to intersect the MFVL. This intersection is known as EFL and is defined as the percent of the tidal volume that meets or exceeds the expiratory boundary of the MFVL (59, 60). The presence of EFL may cause reflex inhibition of the hyperventilatory response and/or a significant alteration in breathing pattern. With the onset of EFL, EELV may increase back to resting values resulting in dynamic hyperinflation (figure 1b) (61, 62). This dynamic hyperinflation permits increases in flow rate (96) at the expense of an increased elastic work because lung compliance is reduced as lung volume increases. 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 maximal capacity of the muscles to generate force (104). This will reduce inspiratory muscle length and may substantially increase the work and O_2 cost of breathing, thus decreasing inspiratory muscle endurance time (116). Secondary to the hyperinflation induced fatigue, a relative ischemia to the diaphragm may also result in diaphragm fatigue (14). However, increases in EELV have been documented in the absence of EFL when using the NEP technique (86). These authors showed that 9 out of 10 elite level cyclists did not develop EFL despite a significant increase in EELV. As such, increases in EELV could not be attributed to EFL. Instead they propose a possible reflex mechanism, which is triggered when an individual approaches EFL as a strategy to avoid dynamic compression of the airways.

Expiratory flow limitation may have a significant affect on exercise hyperpnoea as evidenced by breathing low density gas such as a helium O_2 (HeO_2) mixture during exercise. Breathing HeO_2 (79% He and 21% O_2) increases maximal expiratory flow rates and therefore reduces EFL during intense exercise (81). These changes result in an increase in $\dot{V}_E$ but only when HeO_2 is administered to individuals that experience EFL during normoxic exercise

(81). Thus, in the absence of EFL, no change in $\dot{V}_E$ occurs while breathing HeO_2. Although this demonstrates the importance of EFL on $\dot{V}_E$, Johnson et al (61) has shown that ventilatory constraint is not sufficient enough to cause a retention of CO_2. Instead, EFL may limit the hyperventilatory response to long-term exercise, keeping arterial partial pressure of carbon dioxide ($PaCO_2$) at normal exercise levels and lowering alveolar, and therefore, arterial partial pressure of O_2 (PaO_2) with a potential consequence to gas exchange.

Mechanisms of Expiratory Flow limitation

The underlying mechanisms of EFL and the regulation of EELV in healthy humans still requires further investigation. However, EFL and the regulation of EELV during exercise may be related to dynamic compression of the airways. According to Olafsson and Hyatt (90), when expiratory flow during tidal breathing is increased to levels at which EFL occurs, and if the expiratory muscles generate a transpulmonary pressure that exceeds the minimal pressure necessary to produce maximal flow, then the airways downstream from the flow-limited segment undergo dynamic compression. This dynamic compression may change breathing pattern and terminate expiration prematurely, thus increasing EELV (95). The gas compression artefact also plays an important role when obtaining a MFVL to be used in the assessment of EFL. The true capacity for airflow generation at a given lung volume may be underestimated, particularly over the effort-independent portion of the MFVL due to gas compression. This problem may be corrected by having individuals perform their MFVL in a volume displacement plethysmograph which uses a separate flow sensor to compensate for the gas compression when measured at the mouth. This technique is rarely practiced as it requires subjects to exercise in a confined space. A more practical option to correct for gas compression is to have subjects perform a series of three to five expiratory manoeuvres at different efforts from TLC to RV and taking the highest flow obtained at each lung volume (54, 55, 90). Another consideration when measuring EFL is the role of bronchodilation. Maximal flows may be increased during exercise because of significant bronchodilation and therefore flows measured during exercise could exceed the MFVL measured at rest. Therefore, a post-exercise MFVL may provide the simplest estimate of maximal available flows and volume during exercise (120).

Metabolic Costs of a High Work of Breathing

As one would expect, the W_b will increase when $\dot{V}E$ is increased and therefore the energy demand by the respiratory muscles is presumably increased as well. It has been shown that the metabolic and circulatory costs of a high W_b during high levels of $\dot{V}E$ amount to 10% of the $\dot{V}O_2MAX$ in the untrained person, and up to 15-16% of $\dot{V}O_2MAX$ and maximal cardiac output in the highly trained person (3, 45). Although respiratory muscle blood flow can not be directly obtained in humans, animal work shows major increases in blood flow to the muscles of respiration (35, 77, 87). The remarkable cardiac output requirements of the respiratory muscles during maximal exercise is consistent with the work using radionuclide-labeled microspheres to study blood flow to the respiratory muscles in ponies at rest and during maximal treadmill exercise (77).

Respiratory Muscle Fatigue

The human diaphragm is a large dome-shaped muscle that separates the abdominal and thoracic cavities and is the primary muscle involved in active inspiration. Other muscles of inspiration include the external intercostals and accessory muscles (i.e., scalenes and sternoncledomastoid) which become recruited to a greater degree as $\dot{V}E$ increases. The recruitment of the diaphragm during exercise can be indirectly obtained through the measurement of trans-diaphragmatic pressure (P_{di}) and surface electromyography (EMG). Using these techniques, Bye et al. (19) showed that the diaphragm is recruited roughly in proportion to the increasing exercise $\dot{V}E$ that occurs with increasing work loads. However, others have shown that P_{di} plateaus despite increases in $\dot{V}E$, suggesting that the diaphragm contribution to total pressure of the inspiratory muscles diminishes because of accessory muscle recruitment (57). Despite this discrepancy, what remains important is that the diaphragm must sustain extremely high levels of force output and requires a high proportion of cardiac output during intense exercise. Given the significant demand for O_2 and the high W_b that occurs during maximal exercise, it seems possible that the diaphragm, like other skeletal muscle, can be susceptible to fatigue.

Fatigue has been defined as any exercise-induced reduction in the ability to exert muscle force or power, regardless of whether or not the task can be sustained (16). However,

due to the potential clinical significance of respiratory muscle fatigue, a more appropriate definition is "a loss in the capacity for developing force and/or velocity of a muscle, resulting from muscle activity under load and which is reversible by rest" (2). It is important to consider that fatigue refers not only to a physiological or pathological state in which muscles perform below their expected maximum, but to a symptom reported by subjects in whom there may be no obvious defect in muscle performance (36).

Although the techniques used in the assessment of diaphragm fatigue are indirect, there is sufficient evidence to suggest that the muscles of inspiration (primarily diaphragm) can be fatigued with high levels of respiratory muscle work. For fatigue to occur, the diaphragm must sustain extremely high levels of force output (9, 14) and during heavy endurance exercise, the magnitude of fatigue and the likelihood of its occurrence increases as the relative intensity exceeds 85% of $\dot{V}O_2MAX$ (57).

Early work by Loke et al (74) showed that the maximal inspiratory pressure (PI_{max}) measured after a marathon was significantly lower than the pre-marathon values. This study along with others (17, 21, 94) showed significant decreases in respiratory muscle strength post-exercise, suggesting potential respiratory muscle fatigue. Using PI_{max} may provide a meaningful measurement of global inspiratory muscle function but its use in the assessment of respiratory muscle fatigue is debatable. For example, PI_{max} tests are volitional and require full motivation of the subject. Accordingly, a low result may be due to a lack of motivation and does not necessarily indicate reduced inspiratory or expiratory muscle strength (1). Therefore, a more appropriate method for assessing diaphragm fatigue is the bilateral phrenic nerve stimulation (BPNS) technique. This technique involves the bilateral and supramaximal stimulation of the phrenic nerves via electrical or magnetic stimulation while measuring P_{di} (difference between gastric and oesophageal pressure) both pre and post exercise. Since the phrenic nerve innervates the diaphragm, the BPNS technique allows for the investigation of the diaphragm without interference from other respiratory muscles. Furthermore, the artificial stimulation of the phrenic nerves eliminates the control of the central nervous system and is therefore independent of motivation. The BPNS technique also allows for the quantification of fatigue at a range of stimulation frequencies. Several studies utilizing this technique in healthy individuals have shown that the diaphragm fatigues during heavy

endurance exercise (10, 57, 75, 76). These studies demonstrate that P_{di} is reduced immediately post exercise and remains below normal capacity for more than 1 hour post exercise. There is ample evidence to support the theory that the diaphragm fatigues during heavy exercise. However, it is important to consider the other muscles involved in breathing that are recruited during high levels of respiratory muscle work. These accessory muscles prevent hypoventilation even in the face of major reductions in diaphragmatic force production (10, 57). Since $\dot{V}_A$ is not compromised during respiratory muscle fatigue, the practical application and influence on exercise performance is questionable. A more probable association between respiratory muscle work, diaphragm fatigue, and exercise performance is based on the reflexive effects of respiratory muscle fatigue which increases sympathetic vasoconstrictor outflow and reduces limb blood flow during prolonged exercise (31).

To test the effect of respiratory muscle fatigue on sympathetic outflow and limb blood flow, Harms et al. (41, 45) altered the W_b during high intensity cycle exercise which resulted in marked changes in leg blood flow, cardiac output and whole-body and active limb O_2 uptake. More specifically, they used graded resistive loads and a pressure-assist ventilator to manipulate the W_b via respiratory muscle loading and unloading and found a reflexive vasoconstriction or dilation respectively. Respiratory muscle loading resulted in a reduction in limb locomotor blood flow, as evidenced by changes in limb vascular resistance and noradrenaline spillover. The changes in leg blood flow indicate a competitive relationship between muscles of locomotion and the muscles of respiration for a limited cardiac output. In a similar study performed during submaximal exercise (up to 75% $\dot{V}O_2MAX$), respiratory muscle loading and unloading significantly affected $\dot{V}O_2$ and cardiac output with no apparent change in limb vascular resistance (121). This is an interesting finding considering diaphragm fatigue does not occur until relative exercise intensity exceeds 85% of $\dot{V}O_2MAX$ (57). As such, the reflexes from the respiratory muscles are most likely elicited during high levels of respiratory muscle work and when cardiac output is limited in its availability. It was later hypothesized that a high W_b will influence locomotor muscle performance and thus whole body endurance performance (46). To test this hypothesis, cyclists performed time to exhaustion cycle exercise at 90% of $\dot{V}O_2MAX$. The W_b was increased or decreased by

respiratory muscle loading and unloading respectively. The increased W_b by respiratory muscle loading caused a 15% reduction in time to exhaustion performance compared to controls. This detrimental effect on exercise performance was attributed to the associated reduction in leg blood flow, which enhances the onset of leg fatigue and also the intensity of leg and respiratory muscle effort perceptions. When the respiratory muscles were unloaded, there was a reduction in $\dot{V}O_2$, a reduced rate of change in dyspnoeic and limb discomfort sensations, and improvements in time to exhaustion performance by 14%. It should be noted that the improvement in exercise performance with respiratory muscle unloading is not a universal finding (70, 78). Earlier work by Marcniuk et al. (78) demonstrated that respiratory muscle unloading during submaximal endurance exercise, had no affect on $\dot{V}E$, tidal volume, breathing frequency and endurance time compared to a control trial. The authors concluded that central respiratory muscle fatigue does not play a role in determining endurance performance, sense of respiratory effort, or breathing pattern. The work of Krishnan et al. (70) later confirmed these findings by testing healthy male subjects during two constant work rate cycle tests at ~80% of maximal power under respiratory muscle mechanical unloading and control conditions. Despite a reduction in respiratory muscle load during the unloaded condition, there was no change in $\dot{V}E$, breathing pattern or $\dot{V}O_2$. They suggest that the load on the respiratory muscles has a minor role in the regulation of exercise $\dot{V}E$ and that unloading implies that respiratory muscle function does not limit endurance performance. It is difficult to reconcile the findings of Harms et al. (46) with those of Krishnan et al. (70) and Marciniuk et al. (78). Potential differences may be due, in part, to the magnitude of respiratory unloading achieved, the exercise intensities used and the changes in $\dot{V}O_2$ obtained.

Several recent investigations have attempted to elucidate the mechanisms underlying the interactions among the respiratory muscles, the autonomic nervous system and the cardiovascular system. St. Croix et al. (110) hypothesized that reflexes arising from working respiratory muscles can elicit increases in sympathetic vasoconstrictor outflow to limb skeletal muscle. To test this hypothesis, they used a high resistance, prolonged inspiratory duty cycle at rest, which was sufficient enough to cause an inferred reduction in diaphragm blood flow and cause fatigue leading to an increase in muscle sympathetic nerve activity (MSNA) in the leg muscle. This increase in MSNA was independent of central respiratory

motor output, suggestive of a reflex origin. Furthermore, MSNA was unaltered during the first 1-2 min but increased over time. They attributed the temporal nature of this MSNA response to a reflex arising from a diaphragm that was accumulating metabolic end products in the face of high force output in addition to compromised blood flow. This work in conjunction with the finding of increased neural activity in type IV afferents from the diaphragm during fatiguing contractions in the anaesthetized rat (50), are consistent with the idea of a metaboreflex that increases sympathetic vasoconstrictor outflow. Furthermore, Sheel et al. (108) asked whether inspiratory muscle fatigue in humans would cause vasoconstriction and reduced resting limb blood flow in humans. These authors established that this apparent respiratory muscle-limb reflex has the ability to significantly reduce resting limb blood flow and vascular conductance.

Most recently, lactic acid was injected into the phrenic and deep circumflex iliac arteries to activate the diaphragm and abdominal expiratory muscle metaboreflexes to determine the blood pressure, cardiac output and blood flow response in awake dogs at rest and during exercise (103). At rest, there was a reduction in cardiac output and blood flow to the hind limbs and an increase mean arterial blood pressure. During steady state exercise, the lactic acid elicited pressor responses and reduced hind limb blood flow and vascular conductance. It was concluded that the activation of the respiratory muscle metaboreflex may increase sympathetic tone and causes a redistribution of blood flow during exercise. This work in combination with previous findings (56, 108, 110) provides convincing evidence for the existence of a respiratory muscle metaboreflex. However, a number of unanswered questions remain as pointed out by Seals (106). For example, the potential physiological consequences of the W_b in limiting maximal aerobic capacity and perhaps human performance has yet to be determined.

Potential Sex Differences

As previously mentioned, EFL may lead to an increased work and O_2 cost of breathing. Aaron et al. (3, 4) demonstrated that the highest W_b occurred in those subjects with the greatest degree of EFL. There was one female subject in this study (n=8) and she had the greatest W_b compared to the group mean (544.1 J/min vs. 391.8 J/min) despite having

a lower maximal $\dot{V}_E$ relative to the group (139 L/min vs. 147 L/min). Furthermore, her O_2 cost of breathing was higher at 70% and 100% of $\dot{V}_{O_2MAX}$ compared to the group mean. The findings in this study are consistent with those of McClaran et al. (81) that claim a higher prevalence of EFL in females and are consistent with the work of Topin et al. (115) that claim a higher O_2 cost of breathing in females compared to males. Presumably, the increased prevalence of EFL in women may cause greater diaphragm fatigue compared to men. However, a recent study by Ozkaplan et al. (94) found no sex difference in inspiratory muscle fatigue by measuring PI_{max} both pre and post maximal exercise in moderately trained males and females. Further evidence for a lack of sex differences in diaphragm function is seen in the recent work of Guenette et al. (40). They found that men and women adapt in a similar fashion to 5 weeks of inspiratory muscle training with significant improvements in PI_{max} being unrelated to cycle time to exhaustion performance. It still remains possible that diaphragm fatigue could happen more frequently in women compared to men because of their increased susceptibility to EFL (81). However, to accurately assess the possibility of sex differences, respiratory muscle fatigue should be assessed using bilateral magnetic phrenic nerve stimulation with continuous measures of flow, volume, and pressure so that the W_b and extent of EFL can be quantified.

APPENDIX B – Raw Data

Table 8. Individual descriptive and anthropometric data. BMI = body mass index; BSA = body surface area

Subject	Sex	Age (years)	Height (cm)	Weight (kg)	BMI ($kg \cdot m^2$)	BSA (m^2)
1	M	23	192	95	25.8	2.25
2	M	24	181	65	19.7	1.83
3	M	24	188	74	21.2	1.99
4	M	22	187	77	21.9	2.01
5	M	24	191	85	23.4	2.14
6	M	31	178	75	23.7	1.92
7	M	23	173	66	22.2	1.79
8	M	36	183	76	22.8	1.97
9	F	23	175	71	23.3	1.86
10	F	25	167	64	23.0	1.72
11	F	25	166	63	22.9	1.70
12	F	21	166	61	22.1	1.68
13	F	22	173	62	20.8	1.73
14	F	26	172	66	22.4	1.78
15	F	26	164	61	22.7	1.65
16	F	31	169	66	23.2	1.75
17	F	23	161	55	21.4	1.58
18	F	25	174	64	21.0	1.77

Table 9. Individual pulmonary function data. FVC = forced vital capacity; $FEV_{1.0}$ = forced expiratory volume in 1 second; PEF = peak expiratory flow; MVV = maximal voluntary ventilation; pred = predicted.

Subject	Sex	FVC (L)	FVC (%pred)	$FEV_{1.0}$ (L)	$FEV_{1.0}$ (%pred)	$FEV_{1.0}$/FVC	$FEV_{1.0}$/FVC (%pred)	PEF ($L \cdot sec^{-1}$)	PEF (%pred)	MVV (L/min)	MVV (%pred)
1	M	7.2	117	6.4	120	88.6	104	14.8	137	224	145
2	M	5.0	90	4.0	82	78.7	92	11.4	113	194	141
3	M	5.9	99	5.2	101	88.0	103	12.9	122	208	140
4	M	6.7	116	5.9	117	87.2	102	13.8	136	209	143
5	M	6.4	104	5.6	104	87.0	102	12.5	116	212	139
6	M	4.5	84	3.5	80	78.3	95	10.4	109	189	107
7	M	5.3	106	4.7	108	87.8	102	12.0	130	206	164
8	M	6.9	125	6.0	133	86.6	106	13.1	133	219	123
9	F	4.8	107	4.1	108	85.7	100	7.7	106	167	136
10	F	4.4	108	3.8	107	85.8	99	8.6	126	169	147
11	F	5.4	134	4.5	131	84.0	97	10.0	148	196	171
12	F	3.8	92	3.5	98	91.8	105	7.2	105	137	117
13	F	4.1	92	3.0	80	74.3	86	6.5	91	129	106
14	F	4.7	110	3.9	107	81.9	96	7.8	111	147	124
15	F	4.7	119	3.8	112	80.7	93	7.8	118	142	127
16	F	3.9	98	3.6	107	92.4	109	9.4	140	162	144
17	F	4.0	105	3.3	99	82.5	93	6.8	103	138	124
18	F	5.1	116	4.4	118	86.1	101	7.8	109	143	119

Table 10. Individual resting ventilatory data. PET_{O_2} = partial pressure of end tidal CO_2; V_T = tidal volume; F_b = breathing frequency; $\dot{V}_E$ = minute ventilation

Subject	Sex	PET_{CO2} (mmHg)	V_T (L)	F_b (breaths/min)	$\dot{V}_E$ (L/min)
1	M	42.5	0.91	16	13.4
2	M	40.2	0.51	21	9.8
3	M	42.9	0.87	16	11.7
4	M	44.1	0.93	15	12.7
5	M	44.7	0.86	17	13.2
6	M	44.9	0.63	16	9.4
7	M	46.0	0.83	15	11.3
8	M	41.6	0.76	17	11.7
9	F	38.7	0.9	16	13.6
10	F	37.9	0.72	16	10.4
11	F	41.2	0.93	15	12.0
12	F	41.8	0.52	19	9.0
13	F	41.0	0.68	13	7.5
14	F	43.9	0.64	11	6.3
15	F	41.5	0.66	19	11.3
16	F	42.6	0.76	15	10.2
17	F	41.2	0.64	17	9.9
18	F	37.4	1.27	10	10.9

Table 11. Individual metabolic and performance data at maximal exercise on Day 1. F_b = breathing frequency; V_T = tidal volume; $\dot{V}_E$ = minute ventilation; $\dot{V}O_2$ = oxygen consumption; $\dot{V}CO_2$ = carbon dioxide production; RER = respiratory exchange ratio; $\dot{V}_E/\dot{V}O_2$ = ventilatory equivalent for oxygen; $\dot{V}_E/\dot{V}CO_2$ = ventilatory equivalent for carbon dioxide; HR = heart rate; Peak Power = Last completed exercise workload.

Name	Sex	F_b (breaths/min)	V_T (L)	$\dot{V}_E$ (L/min)	$\dot{V}O_2$ (L/min)	$\dot{V}CO_2$ (L/min)	RER	$\dot{V}_E/\dot{V}O_2$	$\dot{V}_E/\dot{V}CO_2$	$\dot{V}O_2$ (ml/kg/min)	HR (bpm)	Exercise Duration (sec)	Peak Power (Watts)
1	M	68	3.1	190.7	5.56	6.52	1.17	34.3	29.3	58.5	188	1459	380
2	M	65	2.4	145.3	4.61	5.04	1.09	31.5	28.8	71.3	187	1260	380
3	M	61	3.1	171.0	6.30	6.29	1.00	27.1	27.2	84.7	189	1257	380
4	M	68	3.2	193.0	4.79	5.72	1.19	40.3	33.8	62.5	204	1142	350
5	M	56	3.3	164.6	6.26	6.40	1.02	26.3	25.7	73.3	180	1625	440
6	M	56	2.8	139.4	5.00	5.21	1.04	27.9	26.7	67.0	183	1277	380
7	M	45	2.9	120.3	4.63	4.97	1.07	26.0	24.2	69.8	197	1080	350
8	M	50	3.7	165.2	5.21	5.49	1.05	31.7	30.1	68.8	181	1080	350
9	F	61	2.6	145.4	4.23	4.82	1.14	34.4	30.2	59.2	166	1294	280
10	F	64	2.2	125.9	3.53	3.70	1.05	35.7	34.0	55.0	203	1080	250
11	F	56	2.9	149.7	4.05	4.19	1.03	37.0	35.8	64.2	196	1146	250
12	F	67	1.8	109.8	3.65	3.71	1.02	30.1	29.6	59.8	185	1277	280
13	F	50	2.3	102.1	3.19	3.59	1.13	32.0	28.4	51.6	205	1000	220
14	F	61	1.9	109.1	3.74	3.85	1.03	29.1	28.3	56.4	186	1142	250
15	F	55	2.3	115.0	3.94	4.14	1.05	29.2	27.8	65.1	197	1359	280
16	F	57	2.4	121.1	4.09	4.45	1.09	29.6	27.2	62.1	186	1408	280
17	F	56	1.9	94.8	3.24	3.41	1.05	29.3	27.8	58.4	198	1085	250
18	F	58	2.3	124.4	4.23	4.70	1.11	29.4	26.5	66.5	194	1500	310

Table 12. Minute ventilation ($\dot{V}E$) and the corresponding work of breathing (W_b) at the end of each workload in individual male subjects.

	Subject 1		Subject 2		Subject 3		Subject 4	
Workload (Watts)	$\dot{V}E$ (L/min)	W_b (J/min)	$\dot{V}E$ (L/min)	W_b (J/min)	$\dot{V}E$ (L/min)	W_b (J/min)	$\dot{V}E$ (L/min)	W_b (J/min)
0	14.0	4.2	14.0	2.6	12.6	2.1	8.8	0.9
200	49.9	39.4	69.1	49.7	61.2	50.6	61.5	53.7
230	70.0	65.8	80.9	75.4	75.0	89.5	73.9	86.5
260	59.3	50.5	87.1	83.8	94.1	121.4	83.5	106.3
290	82.9	89.0	90.3	85.1	104.5	185.8	89.0	125.2
320	91.8	116.1	109.0	138.0	130.6	286.2	115.7	209.9
350	124.2	215.5	128.2	206.6	150.4	371.6	178.4	546.0
380	146.6	285.9	138.0	237.9	172.4	569.7	202.0	1055.8
410	206.0	686.3						
440								

	Subject 5		Subject 6		Subject 7		Subject 8	
Workload (Watts)	$\dot{V}E$ (L/min)	W_b (J/min)	$\dot{V}E$ (L/min)	W_b (J/min)	$\dot{V}E$ (L/min)	W_b (J/min)	$\dot{V}E$ (L/min)	W_b (J/min)
0	9.7	1.7	6.4	0.8	9.0	1.4	10.4	1.4
200	59.8	30.9	82.4	110.8	53.1	35.2	66.2	34.2
230	68.4	46.7	84.2	109.3	60.1	42.8	81.7	54.4
260	80.2	68.3	81.0	112.4	88.4	86.6	98.7	89.0
290	100.4	107.9	84.2	137.1	84.5	92.1	101.4	86.0
320	109.8	137.7	105.5	264.9	92.1	114.0	137.4	194.0
350	119.2	162.0	137.9	465.8	122.4	223.9	164.4	313.9
380	158.5	302.2	136.6	496.8	144.6	342.6	179.3	446.0
410	167.4	335.9						
440	208.5	583.1						

Table 13. Minute ventilation ($\dot{V}E$) and the corresponding work of breathing (W_b) at the end of each workload in individual female subjects.

	Subject 9		Subject 10		Subject 11		Subject 12		Subject 13	
Workload (Watts)	$\dot{V}E$ (L/min)	W_b (J/min)	$\dot{V}E$ (L/min)	W_b (J/min)	$\dot{V}E$ (L/min)	W_b (J/min)	$\dot{V}E$ (L/min)	W_b (J/min)	$\dot{V}E$ (L/min)	W_b (J/min)
0	14.8	2.3	5.9	0.9	10.5	1.8	9.6	1.2	9.0	3.7
100	48.4	30.6	43.2	36.4	57.1	31.8	38.1	30.2	37.7	41.3
130	57.1	44.3	51.2	52.0	61.5	41.8	45.8	47.1	47.9	68.2
160	63.0	51.3	56.4	64.5	64.1	42.3	56.2	76.5	57.5	93.6
190	81.3	90.4	73.9	128.7	92.8	92.5	75.1	162.2	69.8	147.0
220	91.4	120.4	98.5	255.3	108.7	127.6	95.7	263.4	86.0	239.4
250	113.3	223.9	123.5	449.3	141.7	261.4	104.3	366.9	91.1	431.7
280	140.8	436.8			177.3	513.3	106.3	401.4		
310	149.7	659.1								
340										

	Subject 14		Subject 15		Subject 16		Subject 17		Subject 18	
Workload (Watts)	$\dot{V}E$ (L/min)	W_b (J/min)	$\dot{V}E$ (L/min)	W_b (J/min)	$\dot{V}E$ (L/min)	W_b (J/min)	$\dot{V}E$ (L/min)	W_b (J/min)	$\dot{V}E$ (L/min)	W_b (J/min)
0	10.0	2.0	8.3	0.8	8.1	0.9			3.6	0.2
100	33.8	21.0	44.6	30.6	38.4	34.5			31.8	18.4
130	39.3	24.4	49.9	40.2	40.6	34.4			40.4	32.6
160	54.9	52.7	53.9	49.6	46.6	50.6			59.4	61.9
190	66.0	77.7	59.6	61.2	52.3	57.7	NA		63.9	75.7
220	87.3	138.1	68.9	87.4	61.2	74.7			73.1	119.2
250	106.7	237.6	83.0	136.2	72.1	111.9			89.5	165.9
280	106.3	250.7	110.2	298.0	99.8	226.3			87.5	171.0
310			127.3	468.7	119.5	379.4			113.9	346.4
340									117.9	387.9

APPENDIX C – QUESTIONAIRES

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 pas 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:

_____

Physical Activity History

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

REFERENCES

1. ATS/ERS Statement on respiratory muscle testing. *Am J Respir Crit Care Med* 166: 518-624, 2002.

2. NHLBI Workshop summary. Respiratory muscle fatigue. Report of the Respiratory Muscle Fatigue Workshop Group. *Am Rev Respir Dis* 142: 474-480, 1990.

3. **Aaron EA, Johnson BD, Seow CK, and Dempsey JA**. Oxygen cost of exercise hyperpnea: measurement. *J Appl Physiol* 72: 1810-1817, 1992.

4. **Aaron EA, Seow KC, Johnson BD, and Dempsey JA**. Oxygen cost of exercise hyperpnea: implications for performance. *J Appl Physiol* 72: 1818-1825, 1992.

5. **Andrew GM, Becklake MR, Guleria JS, and Bates DV**. Heart and lung functions in swimmers and nonathletes during growth. *J Appl Physiol* 32: 245-251, 1972.

6. **Armour J, Donnelly PM, and Bye PT**. The large lungs of elite swimmers: an increased alveolar number? *Eur Respir J* 6: 237-247, 1993.

7. **ATS**. Lung function testing: selection of reference values and interpretative strategies. American Thoracic Society. *Am Rev Respir Dis* 144: 1202-1218, 1991.

8. **Babb TG, Viggiano R, Hurley B, Staats B, and Rodarte JR**. Effect of mild-to-moderate airflow limitation on exercise capacity. *J Appl Physiol* 70: 223-230, 1991.

9. **Babcock MA, Johnson BD, Pegelow DF, Suman OE, Griffin D, and Dempsey JA**. Hypoxic effects on exercise-induced diaphragmatic fatigue in normal healthy humans. *J Appl Physiol* 78: 82-92, 1995.

10. **Babcock MA, Pegelow DF, McClaran SR, Suman OE, and Dempsey JA**. Contribution of diaphragmatic power output to exercise-induced diaphragm fatigue. *J Appl Physiol* 78: 1710-1719, 1995.

11. **Bassett DR, Jr. and Howley ET**. Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med Sci Sports Exerc* 32: 70-84, 2000.

12. **Baydur A, Behrakis PK, Zin WA, Jaeger M, and Milic-Emili J**. A simple method for assessing the validity of the esophageal balloon technique. *Am Rev Respir Dis* 126: 788-791, 1982.

13. **Bayliss DA and Millhorn DE**. Central neural mechanisms of progesterone action: application to the respiratory system. *J Appl Physiol* 73: 393-404, 1992.

14. **Bellemare F and Grassino A**. Effect of pressure and timing of contraction on human diaphragm fatigue. *J Appl Physiol* 53: 1190-1195, 1982.

15. **Belman MJ, Botnick WC, and Shin JW**. Inhaled bronchodilators reduce dynamic hyperinflation during exercise in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 153: 967-975, 1996.

16. **Bigland-Ritchie B and Woods JJ**. Changes in muscle contractile properties and neural control during human muscular fatigue. *Muscle Nerve* 7: 691-699, 1984.

17. **Boussana A, Matecki S, Galy O, Hue O, Ramonatxo M, and Le Gallais D**. The effect of exercise modality on respiratory muscle performance in triathletes. *Med Sci Sports Exerc* 33: 2036-2043, 2001.

18. **Briscoe WA and Dubois AB**. The relationship between airway resistance, airway conductance and lung volume in subjects of different age and body size. *J Clin Invest* 37: 1279-1285, 1958.

19. **Bye PT, Esau SA, Walley KR, Macklem PT, and Pardy RL**. Ventilatory muscles during exercise in air and oxygen in normal men. *J Appl Physiol* 56: 464-471, 1984.

20. **Calverley PM and Koulouris NG**. Flow limitation and dynamic hyperinflation: key concepts in modern respiratory physiology. *Eur Respir J* 25: 186-199, 2005.

21. **Chevrolet JC, Tschopp JM, Blanc Y, Rochat T, and Junod AF**. Alterations in inspiratory and leg muscle force and recovery pattern after a marathon. *Med Sci Sports Exerc* 25: 501-507, 1993.

22. **Cibella F, Cuttitta G, Romano S, Grassi B, Bonsignore G, and Milic-Emili J**. Respiratory energetics during exercise at high altitude. *J Appl Physiol* 86: 1785-1792, 1999.

23. **Clanton TL, Dixon GF, Drake J, and Gadek JE**. Effects of swim training on lung volumes and inspiratory muscle conditioning. *J Appl Physiol* 62: 39-46, 1987.

24. **Coast JR, Rasmussen SA, Krause KM, O'Kroy JA, Loy RA, and Rhodes J**. Ventilatory work and oxygen consumption during exercise and hyperventilation. *J Appl Physiol* 74: 793-798, 1993.

25. **Collett PW and Engel LA**. Influence of lung volume on oxygen cost of resistive breathing. *J Appl Physiol* 61: 16-24, 1986.

26. **D'Angelo E, Prandi E, Marazzini L, and Milic-Emili J**. Dependence of maximal flow-volume curves on time course of preceding inspiration in patients with chronic obstruction pulmonary disease. *Am J Respir Crit Care Med* 150: 1581-1586, 1994.

27. **D'Angelo E, Prandi E, and Milic-Emili J**. Dependence of maximal flow-volume curves on time course of preceding inspiration. *J Appl Physiol* 75: 1155-1159, 1993.

28. **de Bisschop C, Montandon G, and Guenard H**. Expiratory muscles modulate negative expiratory pressure-induced flow during muscular exercise. *Respir Physiol Neurobiol*, 2006 (In Press).

29. **Dempsey JA**. J.B. Wolffe memorial lecture. Is the lung built for exercise? *Med Sci Sports Exerc* 18: 143-155, 1986.

30. **Dempsey JA, Hanson PG, and Henderson KS**. Exercise-induced arterial hypoxaemia in healthy human subjects at sea level. *J Physiol* 355: 161-175, 1984.

31. **Dempsey JA, Sheel AW, Haverkamp HC, Babcock MA, and Harms CA**. [The John Sutton Lecture: CSEP, 2002]. Pulmonary system limitations to exercise in health. *Can J Appl Physiol* 28 Suppl: S2-24, 2003.

32. **Dempsey JA and Wagner PD**. Exercise-induced arterial hypoxemia. *J Appl Physiol* 87: 1997-2006, 1999.

33. **Derchak PA, Stager JM, Tanner DA, and Chapman RF**. Expiratory flow limitation confounds ventilatory response during exercise in athletes. *Med Sci Sports Exerc* 32: 1873-1879., 2000.

34. **Dolmage TE and Goldstein RS**. Repeatability of inspiratory capacity during incremental exercise in patients with severe COPD. *Chest* 121: 708-714, 2002.

35. **Fixler DE, Atkins JM, Mitchell JH, and Horwitz LD**. Blood flow to respiratory, cardiac, and limb muscles in dogs during graded exercise. *Am J Physiol* 231: 1515-1519, 1976.

36. **Gandevia SC**. Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev* 81: 1725-1789, 2001.

37. **George JD, Stone WJ, and Burkett LN**. Non-exercise VO2max estimation for physically active college students. *Med Sci Sports Exerc* 29: 415-423, 1997.

38. **Grimby G, Saltin B, and Wilhelmsen L**. Pulmonary flow-volume and pressure-volume relationship during submaximal and maximal exercise in young well-trained men. *Bull Physiopathol Respir (Nancy)* 7: 157-172, 1971.

39. **Guenette JA, Diep TT, Koehle MS, Foster GE, Richards JC, and Sheel AW**. Acute hypoxic ventilatory response and exercise-induced arterial hypoxemia in men and women. *Respir Physiol Neurobiol* 143: 37-48, 2004.

40. **Guenette JA, Martens AM, Lee AL, Tyler GD, Richards JC, Foster GE, Warburton DE, and Sheel AW**. Variable effects of respiratory muscle training on cycle exercise performance in men and women. *Appl Physiol Nutr Metab* 31: 159-166, 2006.

41. **Harms CA, Babcock MA, McClaran SR, Pegelow DF, Nickele GA, Nelson WB, and Dempsey JA**. Respiratory muscle work compromises leg blood flow during maximal exercise. *J Appl Physiol* 82: 1573-1583, 1997.

42. **Harms CA, McClaran SR, Nickele GA, Pegelow DF, Nelson WB, and Dempsey JA**. Exercise-induced arterial hypoxaemia in healthy young women. *J Physiol* 507 (Pt 2): 619-628, 1998.

43. **Harms CA and Stager JM**. Low chemoresponsiveness and inadequate hyperventilation contribute to exercise-induced hypoxemia. *J Appl Physiol* 79: 575-580., 1995.

44. **Harms CA, T.J. Wetter and J.A. Dempsey**. *Rehabilitation of the Patient with Respiratory Disease. Breathing In Exercise*. New York, NY: McGraw-Hill Publishers, 1999.

45. **Harms CA, Wetter TJ, McClaran SR, Pegelow DF, Nickele GA, Nelson WB, Hanson P, and Dempsey JA**. Effects of respiratory muscle work on cardiac output and its distribution during maximal exercise. *J Appl Physiol* 85: 609-618, 1998.

46. **Harms CA, Wetter TJ, St Croix CM, Pegelow DF, and Dempsey JA**. Effects of respiratory muscle work on exercise performance. *J Appl Physiol* 89: 131-138, 2000.

47. **Henke KG, Arias A, Skatrud JB, and Dempsey JA**. Inhibition of inspiratory muscle activity during sleep. Chemical and nonchemical influences. *Am Rev Respir Dis* 138: 8-15, 1988.

48. **Henke KG, Dempsey JA, Badr MS, Kowitz JM, and Skatrud JB**. Effect of sleep-induced increases in upper airway resistance on respiratory muscle activity. *J Appl Physiol* 70: 158-168, 1991.

49. **Henke KG, Sharratt M, Pegelow D, and Dempsey JA**. Regulation of end-expiratory lung volume during exercise. *J Appl Physiol* 64: 135-146, 1988.

50. **Hill JM**. Discharge of group IV phrenic afferent fibers increases during diaphragmatic fatigue. *Brain Res* 856: 240-244, 2000.

51. **Hopkins SR, Barker RC, Brutsaert TD, Gavin TP, Entin P, Olfert IM, Veisel S, and Wagner PD**. Pulmonary gas exchange during exercise in women: effects of exercise type and work increment. *J Appl Physiol* 89: 721-730, 2000.

52. **Hopkins SR and Harms CA**. Gender and pulmonary gas exchange during exercise. *Exerc Sport Sci Rev* 32: 50-56, 2004.

53. **Hopkins SR and McKenzie DC**. Hypoxic ventilatory response and arterial desaturation during heavy work. *J Appl Physiol* 67: 1119-1124., 1989.

54. **Ingram RH, Jr. and Schilder DP**. Effect of gas compression on pulmonary pressure, flow, and volume relationship. *J Appl Physiol* 21: 1821-1826, 1966.

55. **Ingram RH, Jr. and Schilder DP**. Effect of thoracic gas compression on the flow-volume curve of the forced vital capacity. *Am Rev Respir Dis* 94: 56-63, 1966.

56. **Jammes Y and Balzamo E**. Changes in afferent and efferent phrenic activities with electrically induced diaphragmatic fatigue. *J Appl Physiol* 73: 894-902, 1992.

57. **Johnson BD, Babcock MA, Suman OE, and Dempsey JA**. Exercise-induced diaphragmatic fatigue in healthy humans. *J Physiol* 460: 385-405, 1993.

58. **Johnson BD, Beck KC, Zeballos RJ, and Weisman IM**. Advances in pulmonary laboratory testing. *Chest* 116: 1377-1387, 1999.

59. **Johnson BD, Reddan WG, Pegelow DF, Seow KC, and Dempsey JA**. Flow limitation and regulation of functional residual capacity during exercise in a physically active aging population. *Am Rev Respir Dis* 143: 960-967, 1991.

60. **Johnson BD, Reddan WG, Seow KC, and Dempsey JA**. Mechanical constraints on exercise hyperpnea in a fit aging population. *Am Rev Respir Dis* 143: 968-977, 1991.

61. **Johnson BD, Saupe KW, and Dempsey JA**. Mechanical constraints on exercise hyperpnea in endurance athletes. *J Appl Physiol* 73: 874-886, 1992.

62. **Johnson BD, Scanlon PD, and Beck KC**. Regulation of ventilatory capacity during exercise in asthmatics. *J Appl Physiol* 79: 892-901, 1995.

63. **Johnson BD, Weisman IM, Zeballos RJ, and Beck KC**. Emerging concepts in the evaluation of ventilatory limitation during exercise: the exercise tidal flow-volume loop. *Chest* 116: 488-503, 1999.

64. **Kiers A, van der Mark TW, Woldring MG, and Peset R**. Determination of the functional residual capacity during exercise. *Ergonomics* 23: 955-959, 1980.

65. **Kilbride E, McLoughlin P, Gallagher CG, and Harty HR**. Do gender differences exist in the ventilatory response to progressive exercise in males and females of average fitness? *Eur J Appl Physiol* 89: 595-602, 2003.

66. **Klas JV and Dempsey JA**. Voluntary versus reflex regulation of maximal exercise flow: volume loops. *Am Rev Respir Dis* 139: 150-156, 1989.

67. **Koulouris NG, Dimopoulou I, Valta P, Finkelstein R, Cosio MG, and Milic-Emili J**. Detection of expiratory flow limitation during exercise in COPD patients. *J Appl Physiol* 82: 723-731, 1997.

68. **Koulouris NG, Rapakoulias P, Rassidakis A, Dimitroulis J, Gaga M, Milic-Emili J, and Jordanoglou J**. Dependence of forced vital capacity manoeuvre on time course of preceding inspiration in patients with restrictive lung disease. *Eur Respir J* 10: 2366-2370, 1997.

69. **Koulouris NG, Valta P, Lavoie A, Corbeil C, Chasse M, Braidy J, and Milic-Emili J**. A simple method to detect expiratory flow limitation during spontaneous breathing. *Eur Respir J* 8: 306-313, 1995.

70. **Krishnan B, Zintel T, McParland C, and Gallagher CG**. Lack of importance of respiratory muscle load in ventilatory regulation during heavy exercise in humans. *J Physiol* 490 (Pt 2): 537-550, 1996.

71. **Lebrun CM, McKenzie DC, Prior JC, and Taunton JE**. Effects of menstrual cycle phase on athletic performance. *Med Sci Sports Exerc* 27: 437-444., 1995.

72. **Liistro G, Veriter C, Dury M, Aubert G, and Stanescu D**. Expiratory flow limitation in awake sleep-disordered breathing subjects. *Eur Respir J* 14: 185-190, 1999.

73. **Lind F and Hesser CM**. Breathing pattern and lung volumes during exercise. *Acta Physiol Scand* 120: 123-129, 1984.

74. **Loke J, Mahler DA, and Virgulto JA**. Respiratory muscle fatigue after marathon running. *J Appl Physiol* 52: 821-824, 1982.

75. **Mador MJ and Dahuja M**. Mechanisms for diaphragmatic fatigue following high-intensity leg exercise. *Am J Respir Crit Care Med* 154: 1484-1489, 1996.

76. **Mador MJ, Magalang UJ, Rodis A, and Kufel TJ**. Diaphragmatic fatigue after exercise in healthy human subjects. *Am Rev Respir Dis* 148: 1571-1575, 1993.

77. **Manohar M**. Blood flow to the respiratory and limb muscles and to abdominal organs during maximal exertion in ponies. *J Physiol* 377: 25-35, 1986.

78. **Marciniuk D, McKim D, Sanii R, and Younes M**. Role of central respiratory muscle fatigue in endurance exercise in normal subjects. *J Appl Physiol* 76: 236-241, 1994.

79. **Margaria R, Milic-Emili G, Petit JM, and Cavagna G**. Mechanical work of breathing during muscular exercise. *J Appl Physiol* 15: 354-358, 1960.

80. **Martin DE and May DF**. Pulmonary function characteristics in elite women distance runners. *Int J Sports Med* 8 Suppl 2: 84-90, 1987.

81. **McClaran SR, Harms CA, Pegelow DF, and Dempsey JA**. Smaller lungs in women affect exercise hyperpnea. *J Appl Physiol* 84: 1872-1881., 1998.

82. **McClaran SR, Wetter TJ, Pegelow DF, and Dempsey JA**. Role of expiratory flow limitation in determining lung volumes and ventilation during exercise. *J Appl Physiol* 86: 1357-1366, 1999.

83. **Mead J**. Dysanapsis in normal lungs assessed by the relationship between maximal flow, static recoil, and vital capacity. *Am Rev Respir Dis* 121: 339-342, 1980.

84. **Milic-Emili G, Petit JM, and Deroanne R**. Mechanical work of breathing during exercise in trained and untrained subjects. *J Appl Physiol* 17: 43-46, 1962.

85. **Milic-Emili J, Mead J, Turner JM, and Glauser EM**. Improved Technique For Estimating Pleural Pressure From Esophageal Balloons. *J Appl Physiol* 19: 207-211, 1964.

86. **Mota S, Casan P, Drobnic F, Giner J, Ruiz O, Sanchis J, and Milic-Emili J.** Expiratory flow limitation during exercise in competition cyclists. *J Appl Physiol* 86: 611-616, 1999.

87. **Musch TI, Friedman DB, Pitetti KH, Haidet GC, Stray-Gundersen J, Mitchell JH, and Ordway GA.** Regional distribution of blood flow of dogs during graded dynamic exercise. *J Appl Physiol* 63: 2269-2277, 1987.

88. **Newman F, Smalley BF, and Thomson ML.** Effect of exercise, body and lung size on CO diffusion in athletes and nonathletes. *J Appl Physiol* 17: 649-655, 1962.

89. **O'Donnell DE, Lam M, and Webb KA.** Measurement of symptoms, lung hyperinflation, and endurance during exercise in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 158: 1557-1565, 1998.

90. **Olafsson S and Hyatt RE.** Ventilatory mechanics and expiratory flow limitation during exercise in normal subjects. *J Clin Invest* 48: 564-573, 1969.

91. **Olfert IM, Balouch J, Kleinsasser A, Knapp A, Wagner H, Wagner PD, and Hopkins SR.** Does gender affect human pulmonary gas exchange during exercise? *J Physiol* 557: 529-541, 2004.

92. **Otis AB.** The work of breathing. In: *Handbook of Physiology.* Washington, DC: Am. Physiol. Soc., 1964, p. 463-476.

93. **Otis AB, Fenn WO, and Rahn H.** Mechanics of breathing in man. *J Appl Physiol* 2: 592-607, 1950.

94. **Ozkaplan A, Rhodes EC, Sheel AW, and Taunton JE.** A comparison of inspiratory muscle fatigue following maximal exercise in moderately trained males and females. *Eur J Appl Physiol*, 2005.

95. **Pellegrino R, Brusasco V, Rodarte JR, and Babb TG.** Expiratory flow limitation and regulation of end-expiratory lung volume during exercise. *J Appl Physiol* 74: 2552-2558, 1993.

96. **Pellegrino R, Violante B, Nava S, Rampulla C, Brusasco V, and Rodarte JR.** Expiratory airflow limitation and hyperinflation during methacholine-induced bronchoconstriction. *J Appl Physiol* 75: 1720-1727, 1993.

97. **Peslin R, da Silva JF, Chabot F, and Duvivier C.** Respiratory mechanics studied by multiple linear regression in unsedated ventilated patients. *Eur Respir J* 5: 871-878, 1992.

98. **Powers SK, Dodd S, Lawler J, Landry G, Kirtley M, McKnight T, and Grinton S.** Incidence of exercise induced hypoxemia in elite endurance athletes at sea level. *Eur J Appl Physiol Occup Physiol* 58: 298-302, 1988.

99. **Reuschlein PS, Reddan WG, Burpee J, Gee JB, and Rankin J**. Effect of physical training on the pulmonary diffusing capacity during submaximal work. *J Appl Physiol* 24: 152-158, 1968.

100. **Rice AJ, Thornton AT, Gore CJ, Scroop GC, Greville HW, Wagner H, Wagner PD, and Hopkins SR**. Pulmonary gas exchange during exercise in highly trained cyclists with arterial hypoxemia. *J Appl Physiol* 87: 1802-1812, 1999.

101. **Richards JC, McKenzie DC, Warburton DE, Road JD, and Sheel AW**. Prevalence of exercise-induced arterial hypoxemia in healthy women. *Med Sci Sports Exerc* 36: 1514-1521, 2004.

102. **Road J, Newman S, Derenne JP, and Grassino A**. In vivo length-force relationship of canine diaphragm. *J Appl Physiol* 60: 63-70, 1986.

103. **Rodman JR, Henderson KS, Smith CA, and Dempsey JA**. Cardiovascular effects of the respiratory muscle metaboreflexes in dogs: rest and exercise. *J Appl Physiol* 95: 1159-1169, 2003.

104. **Roussos C, Fixley M, Gross D, and Macklem PT**. Fatigue of inspiratory muscles and their synergic behavior. *J Appl Physiol* 46: 897-904, 1979.

105. **Schwartz J, Katz SA, Fegley RW, and Tockman MS**. Sex and race differences in the development of lung function. *Am Rev Respir Dis* 138: 1415-1421, 1988.

106. **Seals DR**. Robin Hood for the lungs? A respiratory metaboreflex that "steals" blood flow from locomotor muscles. *J Physiol* 537: 2, 2001.

107. **Sharratt MT, Henke KG, Aaron EA, Pegelow DF, and Dempsey JA**. Exercise-induced changes in functional residual capacity. *Respir Physiol* 70: 313-326, 1987.

108. **Sheel AW, Derchak PA, Morgan BJ, Pegelow DF, Jacques AJ, and Dempsey JA**. Fatiguing inspiratory muscle work causes reflex reduction in resting leg blood flow in humans. *J Physiol* 537: 277-289, 2001.

109. **Sheel AW, Edwards MR, and McKenzie DC**. Relationship between decreased oxyhaemoglobin saturation and exhaled nitric oxide during exercise. *Acta Physiol Scand* 169: 149-156, 2000.

110. **St Croix CM, Morgan BJ, Wetter TJ, and Dempsey JA**. Fatiguing inspiratory muscle work causes reflex sympathetic activation in humans. *J Physiol* 529 Pt 2: 493-504, 2000.

111. **Stubbing DG, Pengelly LD, Morse JL, and Jones NL**. Pulmonary mechanics during exercise in normal males. *J Appl Physiol* 49: 506-510, 1980.

112. **Tantucci C, Duguet A, Ferretti A, Mehiri S, Arnulf I, Zelter M, Similowski T, Derenne JP, and Milic-Emili J**. Effect of negative expiratory pressure on respiratory system flow resistance in awake snorers and nonsnorers. *J Appl Physiol* 87: 969-976, 1999.

113. **Tatsumi K, Pickett CK, Jacoby CR, Weil JV, and Moore LG**. Role of endogenous female hormones in hypoxic chemosensitivity. *J Appl Physiol* 83: 1706-1710, 1997.

114. **Thurlbeck WM**. Postnatal human lung growth. *Thorax* 37: 564-571, 1982.

115. **Topin N, Mucci P, Hayot M, Prefaut C, and Ramonatxo M**. Gender influence on the oxygen consumption of the respiratory muscles in young and older healthy individuals. *Int J Sports Med* 24: 559-564, 2003.

116. **Tzelepis G, McCool FD, Leith DE, and Hoppin FG, Jr.** Increased lung volume limits endurance of inspiratory muscles. *J Appl Physiol* 64: 1796-1802, 1988.

117. **Valta P, Corbeil C, Lavoie A, Campodonico R, Koulouris N, Chasse M, Braidy J, and Milic-Emili J**. Detection of expiratory flow limitation during mechanical ventilation. *Am J Respir Crit Care Med* 150: 1311-1317, 1994.

118. **Volta CA, Ploysongsang Y, Eltayara L, Sulc J, and Milic-Emili J**. A simple method to monitor performance of forced vital capacity. *J Appl Physiol* 80: 693-698, 1996.

119. **Walls J, Maskrey M, Wood-Baker R, and Stedman W**. Exercise-induced oxyhaemoglobin desaturation, ventilatory limitation and lung diffusing capacity in women during and after exercise. *Eur J Appl Physiol* 87: 145-152, 2002.

120. **Warren JB, Jennings SJ, and Clark TJ**. Effect of adrenergic and vagal blockade on the normal human airway response to exercise. *Clin Sci (Lond)* 66: 79-85, 1984.

121. **Wetter TJ, Harms CA, Nelson WB, Pegelow DF, and Dempsey JA**. Influence of respiratory muscle work on VO(2) and leg blood flow during submaximal exercise. *J Appl Physiol* 87: 643-651, 1999.

122. **Younes M and Kivinen G**. Respiratory mechanics and breathing pattern during and following maximal exercise. *J Appl Physiol* 57: 1773-1782, 1984.