PHYSIOLOGICAL MECHANISMS OF SEX DIFFERENCES IN EXERTIONAL DYSPNEA: ROLE OF NEURAL RESPIRATORY MOTOR DRIVE

by

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ABSTRACT

Dyspnea, the awareness of an increase in breathing discomfort, is commonly experienced during physical activity in healthy individuals and in patients with cardiopulmonary disease. It is well established that the intensity of perceived dyspnea is consistently higher during exercise in healthy women compared to men, regardless of age, height, and weight. However, the mechanism(s) of this sex-related difference in activity-related dyspnea is/are poorly understood and represented the primary focus of this thesis.

Compared to men, women have smaller lungs, narrower airways, and weaker breathing muscles. These anatomical differences manifest as greater mechanical constraints on ventilation, particularly during the stress of exercise when ventilatory requirements are high. In addition, the amount of work the breathing muscles must perform in order to move a given volume of air into and out of the lungs during exercise is considerably higher in women than men. It is reasonable to predict that, because of these differences, the central nervous system must activate the respiratory muscles (particularly the diaphragm) to a greater extent during exercise in women compared to men to achieve the same level of ventilation and that this higher respiratory muscle activation may account for the increased perception of activity-related dyspnea in women. While it is not feasible to directly measure the neural output of the brains' respiratory control center at rest or during exercise in humans, central neural respiratory motor drive can be assessed indirectly by quantifying the electromyogram of the crural diaphragm (EMGdi) using a special electrode catheter positioned in an individual's esophagus. To date, no previous study, in health or disease, has examined whether the combination of relatively greater dynamic mechanical ventilatory constraints and a higher EMGdi (i.e., neuromechanical uncoupling of the respiratory system) during exercise in women is responsible, at least in part, for sex differences in activity-related dyspnea. To address this important question we compared detailed assessments of EMGdi, respiratory muscle function, ventilation, breathing pattern, operating lung volumes, cardio-metabolic function, and dyspnea intensity and unpleasantness ratings during symptom-limited incremental bicycle exercise testing in 25 healthy, young (20-40 yrs) women and 25 age-matched men.

Our results demonstrated relatively greater mechanical constraints on tidal volume expansion at any given ventilation during exercise in women compared to men. The present study was the first to demonstrate that esophageal electrode catheter-derived measures of EMGdi were consistently higher at any given ventilation during exercise in women compared with men and that these differences reflected, in large part, the presence of relatively greater dynamic mechanical ventilatory constraints in women. In keeping with the results of previous studies, sensory intensity and unpleasantness ratings of dyspnea were higher at any given ventilation during submaximal exercise in women compared to men. However, in contrast to our *a priori* hypothesis, these perceptual differences could not be readily explained by greater neuromechanical uncoupling of the respiratory system, but primarily reflected the awareness of a relatively higher EMGdi (or central neural respiratory motor drive) needed to achieve any given ventilation during exercise in the setting of greater dynamic mechanical ventilatory constraints in women. These findings may have implications for our understanding of the physiological mechanisms of sex differences in activity-related dyspnea in variants of health (e.g., aging) and in patients with cardiopulmonary disease.

RÉSUMÉ

La dyspnée, définie comme la conscience d'une augmentation de gêne respiratoire, est souvent connu pendant l'activité physique chez les sujets sains ainsi que chez les patients ayant une maladie cardio-pulmonaire. Il est bien établi que l'intensité de la dyspnée perçue est systématiquement plus élevée au cours de l'exercice chez les femmes en bonne santé par rapport aux hommes, indépendamment de l'âge, de la taille et du poids. Cependant le/les mécanisme(s) de cette différence sont mal compris et la clarification de ceux-ci comportent l'objet principal de la thèse en question.

Comparativement aux hommes, les femmes ont de plus petits poumons, des voies respiratoires plus étroites et des muscles respiratoires plus faibles. Ces différences anatomiques se manifestent par de plus grandes contraintes mécaniques sur la ventilation, en particulier pendant le stress de l'exercice lorsque les besoins ventilatoires sont élevés. Par conséquent, le travail que les muscles respiratoires doivent effectuer afin de déplacer un volume défini d'air dans les poumons pendant l'exercice est considérablement plus élevé chez les femmes que chez les hommes. En raison de ces différences, nous prévoyons que le système nerveux central doit activer les muscles respiratoires (notamment le diaphragme) dans une plus grande mesure chez les femmes pour atteindre le même niveau de ventilation et que cette activation supérieure peut expliquer la perception accrue de la dyspnée liée à l'activité chez les femmes. Même s'il n'est pas possible de mesurer directement les signaux envoyés par le centre de contrôle respiratoire chez l'homme, le contrôle moteur de la respiration peut être évalué indirectement en quantifiant l'électromyogramme du diaphragme crural (EMGdi) en utilisant un cathéter à électrode spécialisée placée dans l'oesophage d'un individu. À ce jour, aucune étude n'a examiné si la combinaison de contraintes ventilatoires mécaniques plus grandes et d'un EMGdi plus élevé pendant l'exercice chez les femmes est responsable des différences de sexe dans la dyspnée liée à l'activité. Nous avons donc comparé des évaluations détaillées de EMGdi, de fonction musculaire respiratoire, de ventilation, de modèle de respiration, de volumes pulmonaires opérationnels, de fonction cardio-métabolique, et d'intensité de la dyspnée et des cotes de désagréments lors de tests d'exercice incrémental de vélo dans 25 jeunes (20-40 yrs) femmes saines et 25 hommes sains du même âge.

Nos résultats démontrent des contraintes mécaniques sur l'expansion du volume courant pendant l'exercice plus fortes chez les femmes par rapport aux hommes. La présente étude est la première à démontrer que les mesures de cathéter à électrodes œsophagiennes dérivés de EMGdi étaient systématiquement plus élevés peu importe le niveau de ventilation au cours de l'exercice chez les femmes par rapport aux hommes et que ces différences reflètent, en grande partie, la présence de contraintes ventilatoires mécaniques dynamiques relativement plus grande chez les femmes. En accord avec les résultats d'études antérieures, l'intensité sensorielle et le désagrément de dyspnée ont été supérieurs à n'importe quelle ventilation donnée au cours de l'exercice sous-maximal chez les femmes par rapport aux hommes. Cependant, contrairement à notre hypothèse a priori, ces différences de perception ne peuvent être facilement expliquées par un plus grand découplage neuromécanique du système respiratoire et reflètent la conscience d'une EMGdi relativement élevée (ou moteur d'entraînement respiratoire neural central) nécessaire pour atteindre une ventilation donnée pendant l'exercice dans le cadre de contraintes ventilatoires mécaniques dynamiques plus grande chez les femmes. Ces résultats pourraient avoir des implications dans notre compréhension des mécanismes de différences de sexe dans la dyspnée liée à l’activité dans les variantes de la santé et chez les patients ayant une maladie cardio-pulmonaire.

PREFACE & CONTRIBUTION OF AUTHORS

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Schaeffer MR was the primary author and played the principle role in identification and design of the study, data collection, data analysis, and manuscript preparation.

Mendonca CT assisted in data collection and analysis.

LeVangie MC assisted in data analysis.

Andersen RE contributed valuable resources required for data collection.

Taivassalo T contributed valuable resources required for data collection.

Jensen D assisted in identification and design of the study, contributed to data collection and analysis, and manuscript preparation.

CHAPTER I. Introduction

1. DYSPNEA

1.1. Introduction to the symptom of dyspnea and its clinical importance

Dyspnea, the "subjective experience of breathing discomfort that consists of qualitatively distinct sensations that vary in intensity," [1] is commonly experienced during physical activity in health and in patients with cardiopulmonary disease. As discussed in more detail below, the symptom of dyspnea on exertion is an independent predictor of morbidity and mortality in these populations. It is also associated with adverse health-related quality of life (HRQoL), particularly as it relates to reductions in functional capacity and the ability to perform activities of daily living. Furthermore, it has been demonstrated that dyspnea increases as a function of normative aging, with greater prevalence and severity in women [2, 3].

While dyspnea is not typically the primary symptom limiting exercise in healthy adults [4], there is evidence to support that dyspnea on exertion is an independent predictor of mortality in the general population. Bodegard et al. [5] examined the relationship between the reason(s) for stopping laboratory-based symptom-limited incremental cardiopulmonary cycle exercise testing and the risk of cardiovascular, pulmonary and all-cause mortality in 1,923 otherwise healthy middle-aged men. After a 26-year follow-up, they found that the individuals who stopped exercise due to impaired breathing (dyspnea) were at a 1.9-fold, 3.5-fold and 1.6-fold higher risk of dying from coronary heart disease (CHD), pulmonary disease, and any cause, respectively, compared to those who stopped to due to leg fatigue or a combination of the two (Fig. 1.1). Similarly, Abidov et al. [6] found that dyspnea was an independent predictor of

cardiovascular and all-cause mortality in 17,991 patients (men and women) both with and without CHD that were referred for pharmacological or exercise cardiac stress testing.

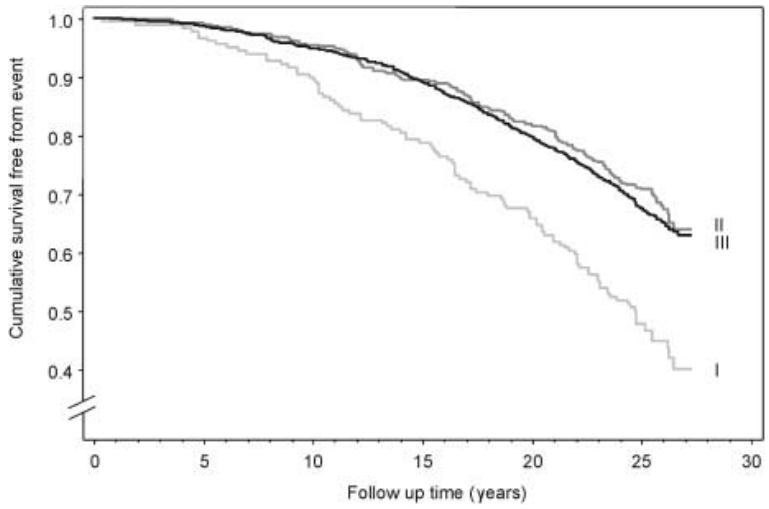

Figure 1.1. Kaplan Meier curves for total mortality related to reasons for termination of cardiopulmonary exercise testing among 1,923 healthy middle-aged men. Group I (n=178) represents men with dyspnea as the reason for terminating the exercise test, group III represents men who stopped because of exhaustion (n=1,376: impaired breathing and lower limb fatigue combined) and group II because of lower limb fatigue (n=369). Groups II and III demonstrate similar total mortality throughout the observation period. Group III demonstrates significantly higher mortality compared to both groups II and III (p<0.0001). *Adapted from Bodegard et al. [5].*

Dyspnea is also a major source of adverse health outcomes (e.g., lower health related quality of life, impaired functional capacity, loss of autonomy) in a variety of clinical populations, including, but not limited to chronic obstructive pulmonary disease (COPD), congestive heart failure (CHF), interstitial lung disease (ILD), and asthma. The clinical importance of this symptom in these patient populations is supported by a strong positive correlation between the severity of activity-related dyspnea as assessed by standardized task-based questionnaires and all-cause mortality in each of the abovementioned disease states [7-10].

Despite the high prevalence and negative functional and clinical implications of the symptom of dyspnea, the underlying physiological and pathophysiological

mechanism(s) remain poorly understood. This may reflect the fact that, historically and in contrast to objective measurement of physiological parameters (e.g., metabolic rate of oxygen consumption ($\dot{V}O_2$)), sensory responses to exercise have been largely dismissed and underappreciated by cardiorespiratory and exercise scientists due to their inherent subjectivity. As a consequence, the etiology of activity-related dyspnea remains relatively understudied despite strong evidence to support that dyspnea is a reliable and important determinant of adverse health outcomes (e.g., morbidity, mortality, HRQoL) in health and disease [11].

1.2. Principles of psychophysics

Psychophysics is the scientific study of the relationship between stimulus and sensation [12]. It is well established that humans can reliably detect (i.e., threshold detection), quantify (i.e., magnitude estimation) and discriminate qualitatively distinct sensations of dyspnea provoked by different respiratory stimuli applied experimentally or resulting from cardiopulmonary disease [1, 12-15]. The principal of psychophysics is that the linkage between stimulus and perception consists of: (1) the receptor activated by the stimulus; (2) the sensory nerves transmitting the stimulus to the central nervous system; (3) the processing of this afferent information in the central nervous system in light of previous experience and learning; and (4) the generation of the conscious sensation/perception. It follows that by examining the relationship between a well-controlled physiological stress (i.e., incremental cycle exercise) and the attendant sensory response (i.e., dyspnea) within- and/or between-subjects the mechanisms of perceived respiratory discomfort may be elucidated.

1.3. Role of cardiopulmonary exercise testing (CPET) in evaluating the mechanisms of dyspnea

In light of the above, cardiopulmonary exercise testing, which permits simultaneous examination of the ventilatory, cardiometabolic, and perceptual responses to a controlled physiological stress, is a powerful investigative tool to help better understand the physiological and pathophysiological mechanisms of activity-related dyspnea in both health and disease [16-18]. During exercise, dyspnea intensity ratings can be measured by validated scales (i.e., Borg's 0-10 category ratio scale [19]), which permits serial ratings of dyspnea to be obtained in a reliable manner [11] that can be subsequently compared to the stimulus of work (e.g., $\dot{V}O_2$, ventilation ($\dot{V}_E$), and work-rate (WR)). More specifically, to study dyspnea and its underlying mechanism(s), it is important to standardize the physiological stimulus (i.e., standardized submaximal $\dot{V}_E$, $\dot{V}O_2$, and/or WR) to allow comparisons to be made within-subjects on repeated measurements or between-subjects (i.e., young *vs.* old; health *vs.* disease; men *vs.* women; etc.).

1.4. The "normal" ventilatory response to exercise

During exercise in health, several acute physiological adaptations help to ensure that the respiratory system fulfills its primary role of matching alveolar ventilation and pulmonary gas exchange to muscle metabolic demand [20, 21], despite dramatic increases in central neural respiratory motor command output (i.e., ventilatory demand >10 time resting values). These adaptations include: (1) the precise control of dynamic operating lung volumes, including end-expiratory (EELV) and end-inspiratory (EILV) lung volumes; (2) reflex bronchodilatation with attendant reductions in airway resistance; (3) breathing pattern optimization; and (4) enhanced matching of pulmonary ventilation

and perfusion [22, 23]. Collectively, these adaptations serve to maximize pulmonary gas exchange efficiency; maintain arterial blood gas/acid-base status near resting levels; and ensure that tidal volume (V_T) expands within the most linear (compliant) portion of the respiratory systems' sigmoid pressure-volume relation (Fig. 1.2) where (i) the work of breathing is minimized, (ii) the relationship between increased contractile respiratory muscle pressure development (and presumably also neural respiratory motor drive (NRMD)) and the simultaneous tidal volume (V_T) expansion/response is preserved (i.e., neuromechanical coupling of the respiratory system), and (iii) the intensity of perceived respiratory discomfort (dyspnea) is minimized [20].

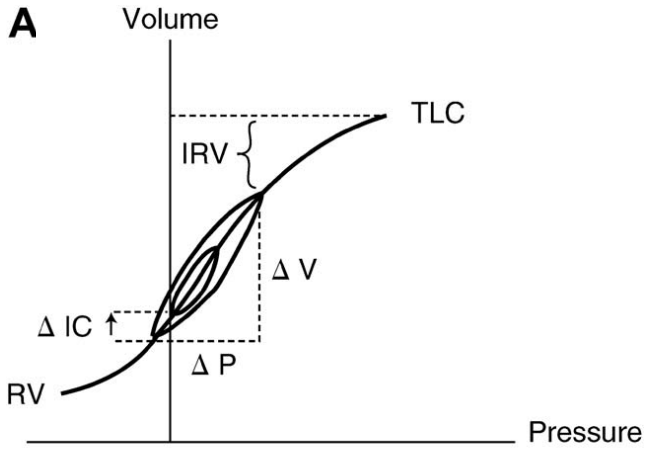

Figure 1.2. The static sigmoidal pressure-volume relationship of the respiratory system in healthy subjects. Superimposed are the tidal pressure-volume loops at rest (smaller loops) and during exercise (larger loops). Breathing rest and during exercise (as the inspiratory capacity (IC) increases) occurs in the central linear portion of the pressure-volume relationship such that relatively small changes in tidal pressure (ΔP) will produce comparatively large changes in tidal volume (ΔV). RV, residual volume IRV, inspiratory reserve volume; TLC, total lung capacity. *Adapted from O'Donnell et al. [24].*

During exercise in health (that is, in the setting of neuromechanical coupling), dyspnea intensity ratings have been shown to increase in direct proportion with measures of exercise intensity and ventilatory output, such as $\dot{V}O_2$, WR, and $\dot{V}_E$ [2, 20]. It follows that any condition associated with an exaggerated NRMD, impaired respiratory

mechanics (increased dynamic mechanical constraints on V_T expansion), respiratory muscle weakness, or a combination thereof may be associated with a greater magnitude increase in the awareness of dyspnea during exercise. For example, breathing on the upper alinear extreme of the pressure-volume relation, such as occurs during exercise in patients with chronic obstructive pulmonary disease due to dynamic lung hyperinflation (Fig. 1.3), will result in shortening and functional weakening of the inspiratory muscles (i.e., the diaphragm) which, according to basic principles of muscle physiology and length-tension relationships, will necessitate an increased NRMD to generate the respiratory muscle pressure and inspiratory flow rates required to achieve or maintain a given $\dot{V}_E$ [25].

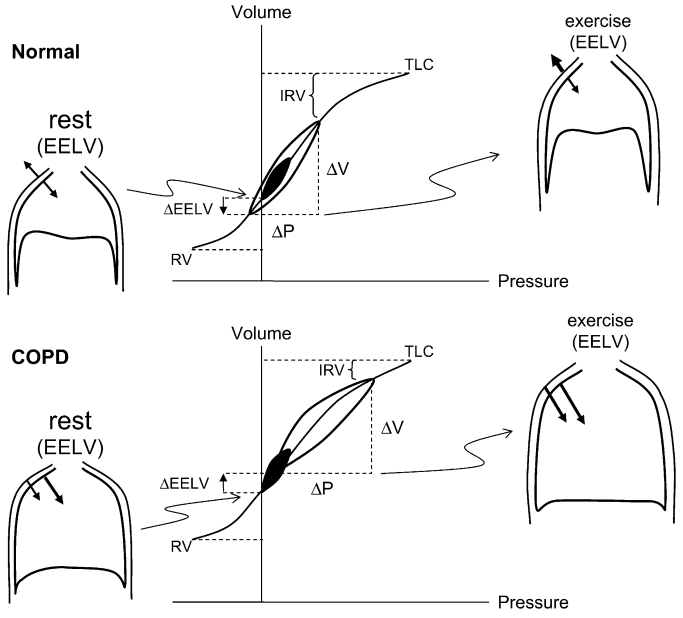

Figure 1.3. Pressure-volume (P-V) relationships of the total respiratory system in health and in chronic obstructive pulmonary disease (COPD). Tidal pressure-volume curves during rest (filled loop) and exercise (open loop) are illustrated. In COPD, because of resting and dynamic hyperinflation (a further increased end-expiratory lung volume (EELV), tidal volume (ΔV) encroaches on the upper, alinear extreme of the respiratory system's P-V curve where there is increased elastic loading. Therefore, the ability to further expand tidal volume in COPD is reduced (i.e. inspiratory reserve volume (IRV) is diminished). The recoil pressure of the lungs and chest wall due to hyperinflation is inwardly directed (see arrows), which results in increased elastic loading and functional weakening of the inspiratory muscles, including the diaphragm. Rv, residual volume; TLC, total lung capacity. *Adapted from O'Donnell et al. [26].*

1.5. Proposed neurophysiological mechanisms of exertional dyspnea

The neurophysiological mechanisms of exertional dyspnea are complex, multifactorial and include alterations in both central and peripheral sensory inputs and their integration within higher brain centers (Figs. 1.4 & 1.5). Jensen et al. [20] recently proposed that the somatosensory cortex calibrates and interprets the appropriateness of the mechanical/muscular response of the respiratory system (conveyed *via* multiple sensory afferents in the lungs, airways and respiratory musculature) to the prevailing level of increased NRMD during exercise, which may or may not be directly perceived by a corresponding increase in central corollary discharge (or "efferent copy").

As illustrated in Fig. 1.4, when the mechanical/muscular response of the respiratory system is matched with the prevailing level of increased NRMD (i.e., neuromechanical coupling of the respiratory system), dyspnea intensity ratings are likely to increase in direct proportion to indices of NRMD; however, while intense, these sensations need not be unpleasant and need not elicit a negative emotional or behavioral compensation response, such as exercise cessation. Such indices of NRMD include ventilation ($\dot{V}_E$, an index of ventilatory output), tidal esophageal pressure swings (Pes,tidal, an index of contractile respiratory muscle effort) and the electromyogram of the diaphragm (EMGdi, an index of neural respiratory motor drive) [16, 20]. Indeed, Jensen et al. [27] recently demonstrated that increasing central respiratory motor command output *via* dead space loading increased (compared to control) dyspnea intensity ratings in direct proportion to concurrent increases in $\dot{V}_E$, Pes,tidal, and EMGdi during symptom-limited incremental cycle exercise in healthy, older adults. Neuromechanical coupling of the respiratory system in these healthy subjects, however,

remained largely preserved throughout exercise in the presence of an added dead space, as evidenced by no demonstrable change in the inter-relationships between EMGdi, Pes,tidal and $\dot{V}_E$ during exercise with versus without an added dead space.

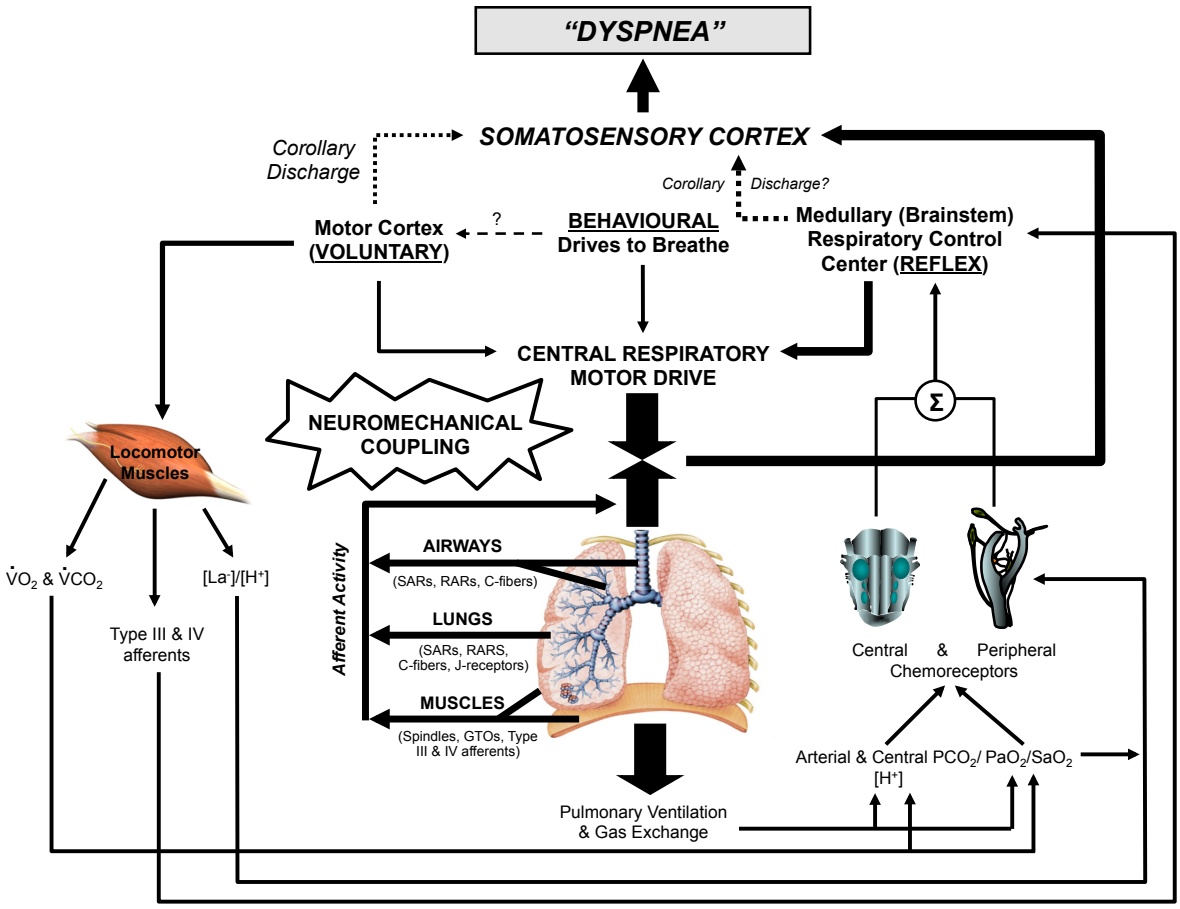

Figure 1.4. Neurophysiological underpinnings of perceived respiratory discomfort (dyspnea) during exercise in healthy humans: a working hypothesis. $\dot{V}O_2$ and $\dot{V}CO_2$, metabolic rates of oxygen consumption and carbon dioxide production, respectively; Type III and IV mechano- and metabosensitive afferents in the peripheral locomotor (and respiratory) muscles and their vasculature; SARs, slowly adapting receptors; RARs, rapidly adapting receptors; C-fibers, bronchopulmonary C-fibers; J-receptors, juxtapulmonary capillary receptors; GTOs, Golgi tendon organs; PCO_2, partial pressure of carbon dioxide; $[H^+]$, hydrogen ion concentration; $[La^-]$, lactate ion concentration; PaO_2 , arterial partial pressure of oxygen; SaO_2 , arterial blood oxygen saturation. *Adapted from Jensen et al. [20].*

In contrast to the above, and as illustrated in Fig. 1.5, if the mechanical/muscular response of the respiratory system is constrained below the prevailing level of increased NRMD, dyspnea intensity ratings are likely to increase in direct proportion to the widening disparity between NRMD and dynamic respiratory mechanics (i.e., neuromechanical uncoupling of the respiratory system). Furthermore, in the setting of neuromechanical uncoupling (e.g., COPD) it has been demonstrated that increases in dyspnea intensity ratings are accompanied by concomitant increases in sensory unpleasantness (perhaps due to increased limbic system activation) with attendant behavioral compensation, such as termination of exercise prior to achieving physiological maxima. Accordingly, imposing a mechanical constraint to V_T expansion by chest wall strapping has been shown to markedly increase (compared to control) dyspnea intensity ratings at a standardized submaximal WR and $\dot{V}_E$ during exercise in healthy men [28, 29]. More specifically, O'Donnell et al. [30] found that, under these circumstances, exercise tolerance was impaired and dyspnea intensity ratings increased in conjunction with the Pes,tidal-to-tidal volume ratio (a crude index of neuromechanical uncoupling of the respiratory system) and that sensory unpleasantness ratings increased with increasing constraints on V_T expansion (i.e., inability to expand the thorax) at any given submaximal WR and $\dot{V}_E$ during exercise with *vs.* without chest wall strapping.

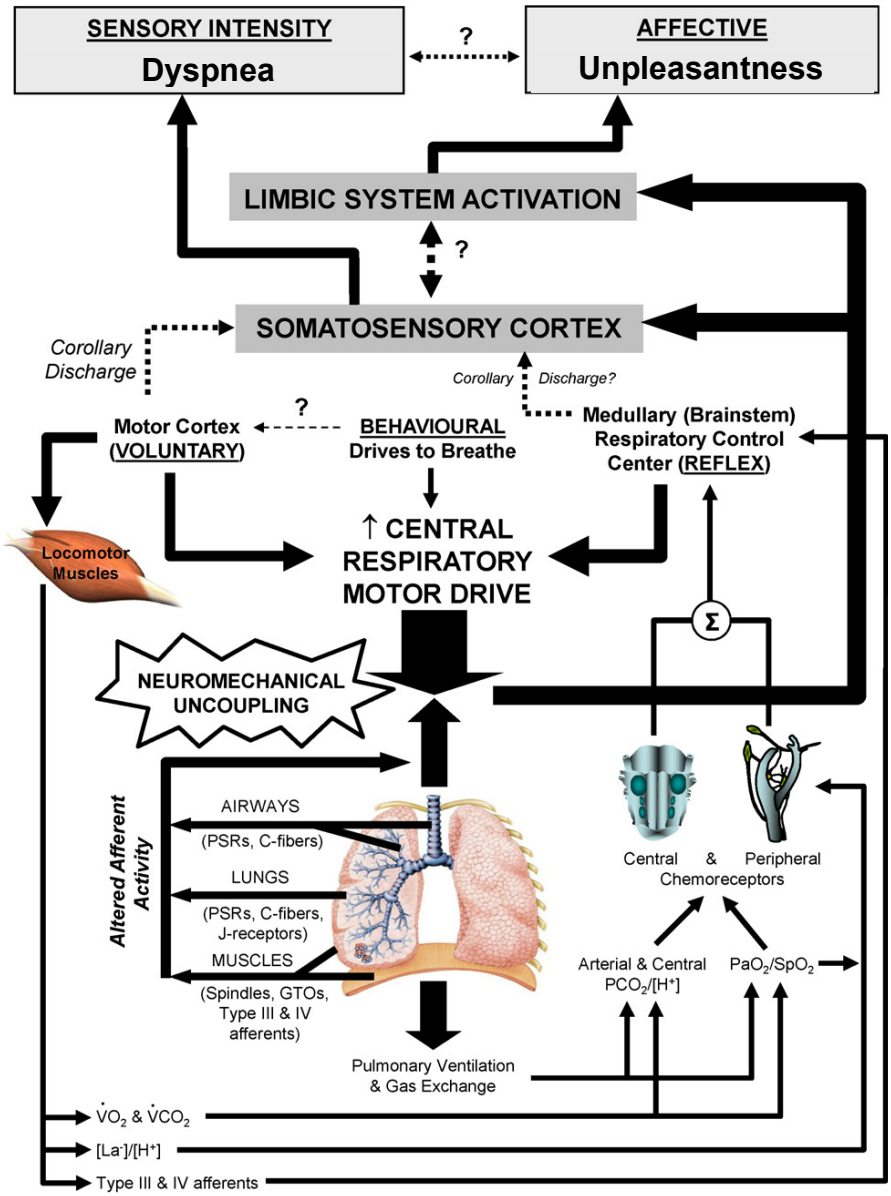

Figure 1.5. Neurophysiological underpinnings of perceived respiratory discomfort (dyspnea) during exercise in patients with pulmonary disease: a working hypothesis. Refer to Fig. 1.4 footnote for definition of abbreviations. *Adapted from O'Donnell et al. [16].*

1.6. Sex differences in exertional dyspnea

In light of the information cited above, it is reasonable to assume that any condition associated with an exaggerated NRMD and/or greater dynamic mechanical constraints on V_T expansion has the potential to increase the intensity and unpleasantness of exertional dyspnea. As described in more detail below, the primary focus of this thesis is to

examine the physiological mechanisms of sex differences in the intensity and unpleasantness of exertional dyspnea. Available evidence suggests that, in both health and disease, the symptom of dyspnea on exertion is more prevalent and severe in women compared to men, independent of age and disease severity. Based on the neurophysiological constructs of activity-related dyspnea presented above (Figs. 1.4 and 1.5), neuromechanical uncoupling may be a potential but hitherto unexplored contributor to the abovementioned sex differences in activity-related dyspnea.

Killian et al. [2] were among the first to demonstrate that the intensity of perceived dyspnea is consistently higher at any given absolute submaximal WR during symptom-limited incremental cycle exercise in healthy women compared to men, regardless of age, height and weight. Subsequent studies have confirmed and expanded these observations to incremental treadmill exercise [3]. Furthermore, the established ventilatory and respiratory abnormalities associated with chronic pulmonary diseases such as COPD (i.e., increased NRMD, increased airways resistance, higher mechanical work of breathing, greater dynamic mechanical constraints on V_T expansion, etc.), have been shown to have greater negative physiological and perceptual consequences (Fig. 1.6) [31] and result in poorer HRQoL and greater activity limitation [32, 33] in women compared to men matched for age and disease severity. Despite the consistency in and clinical significance of these observations, ***the physiological mechanism(s) of sex differences in activity-related dyspnea remain poorly understood, largely understudied and represent the primary focus of the current thesis.*** Based on the proposed neurophysiological constructs of exertional dyspnea (Figs. 1.4 & 1.5), the question remains whether sex-related differences in activity-related dyspnea reflect differences in

NRMD, dynamic respiratory mechanical/muscular function, or their interaction (i.e., neuromechanical uncoupling)?

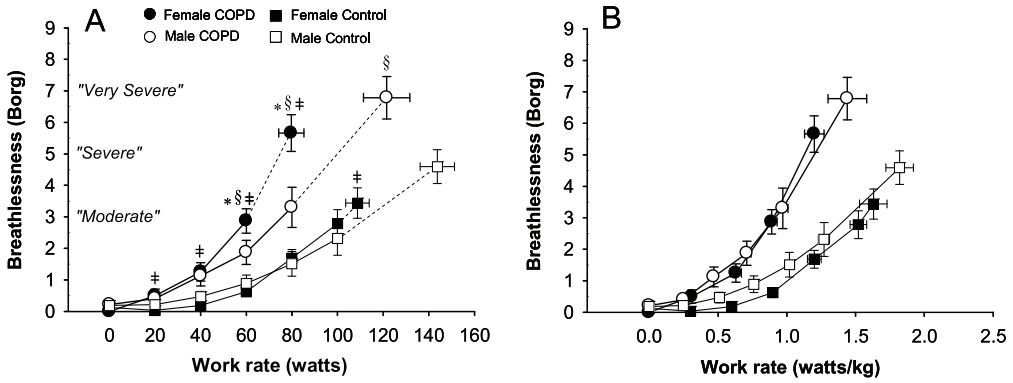

Figure 1.6. Sensory intensity ratings of breathlessness *vs.* absolute work rate (Panel A) and work rate normalized for sex differences in body mass (Panel B) in male and female patients with mild COPD (n=16, each) and healthy age-matched male and female controls (n=16, each). For a given work rate, females with COPD reported higher breathlessness ratings compared to males with similar disease severity; these differences disappear after correction for differences in body mass. *Adapted from Guenette et al. [31].*

2. SEX DIFFERENCES IN RESPIRATORY ANATOMY

It is well established that, even after correction for differences in body height, women have smaller lungs, narrower airways, and weaker breathing muscles than age-matched men [34]. There is direct anatomical evidence supporting sex differences in airway growth and development. Results of a study by Thurlbeck et al. [35], whereby the lungs of 36 boys and 20 girls aged 6 weeks to 14 years were obtained and examined post-mortem, showed that boys have significantly larger lungs compared to girls matched for age and body-height beginning around the age of 2 years. Beyond having smaller lungs than their male counterparts, women have also been shown to have disproportionately narrower airways relative to lung volume; this disparate growth pattern between lung volume and airway diameter is referred to as "dysanapsis" [36]. Previously, computed tomography (CT) was used to assess and compare tracheal dimensional properties in 130

children and adolescents. The results of this study demonstrated that while there were no significant differences in tracheal dimensions between boys and girls in childhood (ages 0-16 years), there were appreciable sex differences as the children approached maturity (age 16-20) independent of height, such that only the females experienced a post-adolescent plateau in tracheal growth. It was suggested that when males stop growing in height, their tracheas may stop growing in length, but not in diameter [37]. Indeed, a recent CT imaging study of 25 older women and 25 age- and lung volume-matched men by Sheel at al. [38] illustrated that the tracheal areas were 14-25% larger in the latter.

Additionally, respiratory muscle strength has been shown to be volume-dependent. As a consequence, women also exhibit significantly lower maximal inspiratory and expiratory mouth pressures, indices of respiratory muscle strength, compared to men independent of age [3, 39].

As illustrated in Figs. 1.7 and 1.8, these sex differences in pulmonary structure and function translate into women having (i) reduced total lung capacities (TLC) and vital capacities (VC) compared to men matched for body height and (ii) reduced peak inspiratory and expiratory flow rates (PIFR and PEFR, respectively) and forced expiratory volume after 1 second (FEV_1) independent of age and even after correction for differences in lung volume [3, 21, 40]. As described in more detail below, these sex differences have important (negative) implications for the ventilatory and perceptual response to exercise, as well as the clinical manifestations of pulmonary disease.

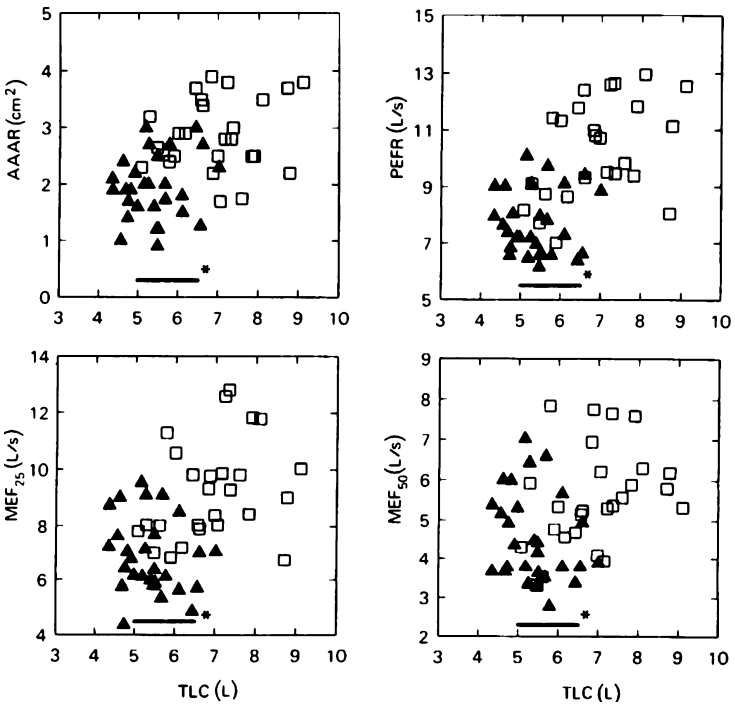

Figure 1.7. Indices of airway size versus total lung capacity (TLC) in female (white squares, n=28) and male subjects (black triangles, n=26). When matched for TLC, tracheal airway area acoustic reflectance (AAAR) was 40% greater in males *vs.* females. Peak expiratory flow rates (PEFR) and maximal expiratory flow rates after 25% (MEF_{25}) and 50% (MEF_{50}) of vital capacity were 23% and 11% greater in males *vs.* females, respectively. Airway size was related to sex but not to lung size. *Adapted from Martin et al. [40].*

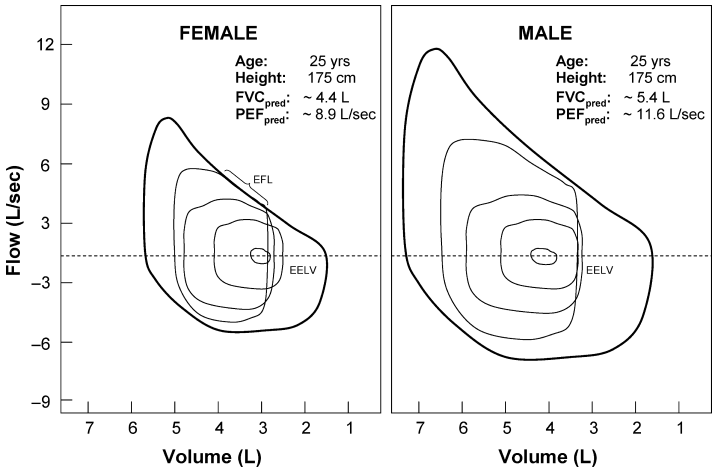

Figure 1.8. Theoretical response to progressive exercise (based on predictive equations) in age- and height-matched men and women. Women have a smaller predicted force vital capacity (FVC_{pred}, represented by the distanced spanned on the x-axis by the thick-lined outer, or maximal, flow-volume loop) and predicted peak expiratory flow rate (PEF_{pred}). The figure demonstrates increasing tidal volumes and the presence of expiratory flow limitation (EFL) in women, where the expiratory tidal flow-volume loop intersects the maximal flow-volume loop. At maximal exercise, there is a greater increase in end-expiratory lung volume (EELV) in women compared to men. The leftwards shift in EELV in women indicates dynamic hyperinflation. *Adapted from Sheel et al. [21].*

3. SEX DIFFERENCES IN THE VENTILATORY RESPONSE TO EXERCISE

The abovementioned anatomical sex differences of the lungs, airways, and breathing muscles appear to have important (negative) physiological and perceptual consequences, particularly when considered in the context of exercise. As described in more detail below, women compared to men during exercise: (i) have a relatively reduced maximal ventilatory capacity; (ii) are at higher risk to develop expiratory flow limitation (EFL) and dynamic hyperinflation; (iii) adopt a more rapid and shallow breathing pattern; and (iv) have a higher mechanical work of breathing (WOB) with evidence of relatively greater dynamic mechanical constraints on V_T expansion [21, 31, 41-47].

As a result of having a lower maximal ventilatory capacity and peak expiratory flow rates, women are more susceptible to developing EFL during exercise compared to men, despite achieving relatively lower peak levels of $\dot{V}_E$ (Fig. 1.8) [21, 48], where EFL is characterized by a plateau, or no further increase, in expiratory flow rate with increasing transpulmonary pressure (i.e., tidal expiratory flow meets or exceeds maximal expiratory flow rates). Since airflow resistance is inversely proportional to the airway radius raised to the fourth power, even small differences in airway diameter (e.g. as a result of dysanapsis), translate into substantial increases in airflow resistance. Indeed, a study of 20 healthy young women by Dominelli et al. [49] found that women who experienced EFL during symptom-limited incremental cycle exercise had (i) a significantly smaller average VC, and (ii) evidence of significantly greater dysanapsis (i.e., narrower airways for any given lung volume) compared to the women who were not flow-limited (Fig. 1.9), suggesting that EFL depends, at least in part, on anatomical differences in lung size, airway diameter, and their interaction.

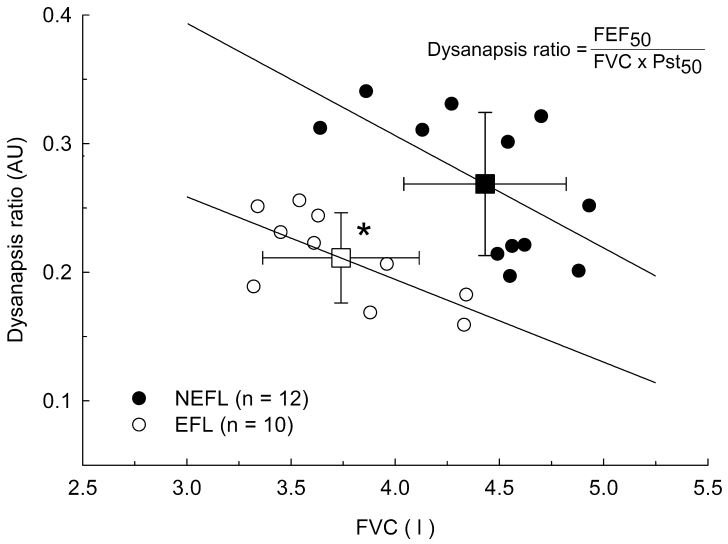

Figure 1.9. The relationship between the dysanapsis ratio (an index of airway size) and forced vital capacity (FVC, an index of lung size) for 2 subject groups of healthy young women: non-expiratory flow-limitation (NEFL, n=12) and expiratory flow-limitation (EFL, n=10). Both groups demonstrated a decrease in the dysanapsis ratio with an increase in FVC. The NEFL group had a larger average FVC and dysanapsis ratio compared to the EFL group, and more specifically, a disproportionately larger dysanapsis ratio for a given FVC. Dysanapsis ratio, the relationship between estimated airway size (maximal expiratory flow at 50% of FVC (FEF_{50})) corrected for the static recoil pressure at FEF_{50} (Pst_{50}) and FVC; AU, arbitrary units. *Adapted from Dominelli et al. [49].*

In light of the above, at a given absolute $\dot{V}_E$ during exercise, women, on average, are breathing at a greater percentage of their maximal breathing capacity, and therefore with less ventilatory reserve, compared to men. One means of achieving a $\dot{V}_E$ above resting levels is through V_T expansion, which occurs *via* reductions in inspiratory reserve volume (IRV) and increases in inspiratory capacity (IC), secondary to increases in EILV and decreases in EELV, respectively. In health, as V_T approaches 50-60% of the VC during exercise, further increases in $\dot{V}_E$ are accomplished *via* increases in breathing frequency (f_b), which is commonly referred to as a tachypneic breathing pattern. This adaptation is dependent on and varies with differences in airway geometry [38], and due to the abovementioned sex differences in pulmonary anatomy, this breathing pattern

modification occurs at lower absolute levels of $\dot{V}_E$ during exercise in women *vs.* men, regardless of age (Fig. 1.10).

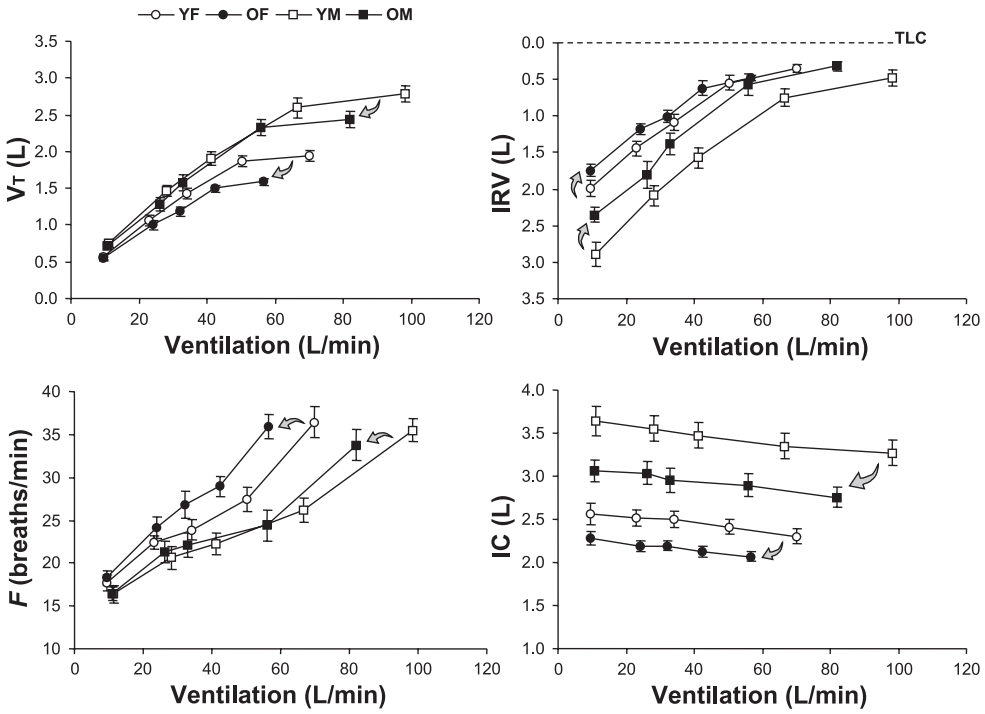

Figure 1.10. Breathing pattern and operating lung volume measurements plotted against ventilation for each subject group (younger men (YM, n=18), older men (OM, n=16), younger females (YF, n=16), older females (OF, n=18)) during symptom limited incremental treadmill exercise. For a given ventilation, women compared to men demonstrate a reduced (V_T), a reduced inspiratory reserve volume (IRV), a higher breathing frequency (*F*), and a reduced inspiratory capacity (IC) independent of age. Values are means ± SE for measurements at rest, the first 2 stages of exercise, the V_T/ventilation inflection point, and peak exercise. TLC, total lung capacity; gray arrows, direction of age-related effects. *Adapted from Ofir et al. [3].*

Exercise-induced increases in f_b are achieved by reducing inspiratory (T_I) and expiratory (T_E) time, with a greater decrease in T_E compared to T_I during progressive exercise. In the setting of narrower airways in women *vs.* men (i.e., greater expiratory flow resistance), the relatively greater reduction in T_E *vs.* T_I during heavier exercise may result in an increase in EELV (or decrease in IC) in the former, such that the EELV may return to or exceed resting levels (Fig. 1.11). In adopting a more rapid and shallow

breathing pattern (Fig. 1.10) women are able to minimize the resistive WOB and achieve the higher flow rates available at higher lung volumes [42, 50]. However, it is well established that breathing at relatively higher lung volumes (that is, closer to TLC) decreases dynamic lung compliance and functionally weakens the inspiratory pump muscles (namely the diaphragm) with attendant increases in the inspiratory elastic WOB [47].

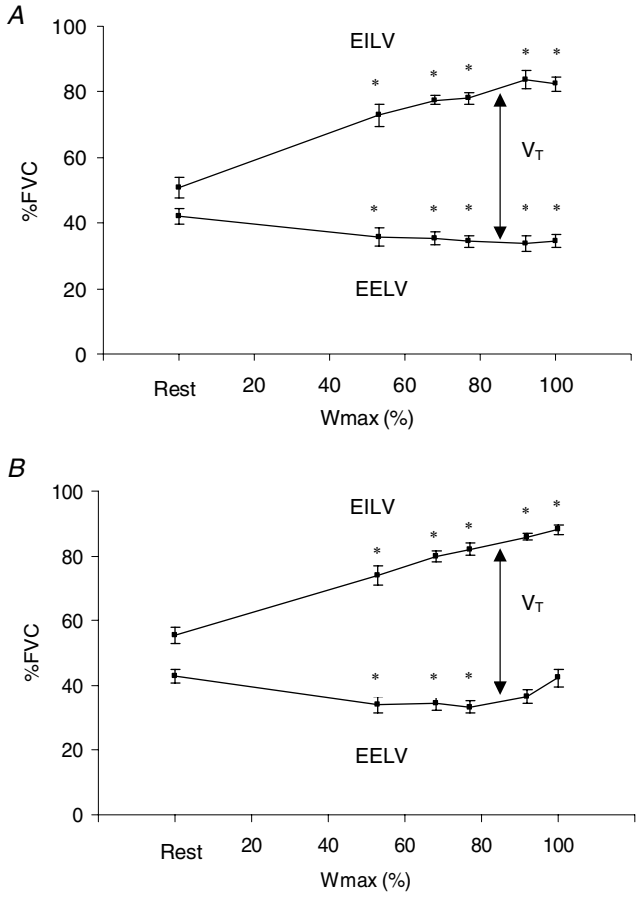

Figure 1.11. Subdivision of lung volume, expressed as a percentage of forced vital capacity (FVC) at rest and during progressive exercise to maximal workload (W_{max}) in men (n=8, panel *A*) and women (n=10, panel *B*). End-expiratory lung volume (EELV) remained significantly below resting EELV in men throughout exercise. Women initially reduced and maintained EELV below resting EELV during exercise, however, EELV was no longer significantly different rest at intensities ≥89%W_{max}. At W_{max}, relative EELV was significantly higher in women compared to men. End-inspiratory lung volume (EILV) continued to increase from rest throughout exercise, but plateaued at W_{max} between 90-100%. EILV increased from rest until 100% W_{max}. At W_{max}, relative EILV was significant;y higher in women compared to men. V_T, tidal volume. Values are means ± SEM. *Significantly different from rest (p<0.05). *Adapted from Guenette et al. [45]*.

As previously demonstrated by Ofir et al. [3], there are relatively greater dynamic mechanical constraints on V_T expansion during exercise in healthy young and older women compared to men as evidenced by relatively higher V_T%IC and V_T%VC ratios, and relatively lower IC and IRV (Figs. 1.10 and 1.12); however, there were no sex differences found when measurements of V_T and IRV were expressed as percentages of predicted VC and TLC, respectively. Collectively, these differences indicate a higher ventilatory demand-to-capacity ratio in women compared to men, such that for any given absolute $\dot{V}_E$ during exercise, V_T in the former is positioned on a relatively higher portion of the respiratory system's sigmoid pressure-volume relation, where there is increased inspiratory elastic loading and functional weakening of the diaphragm. Indeed, several recent studies have demonstrated that, at any given absolute $\dot{V}_E$ during exercise, the WOB in women is disproportionately higher than that of men, suggesting that the oxygen cost of moving a given amount of air into and out of the lungs becomes progressively and substantially higher in the former [21, 38, 42]. More specifically, and as illustrated in Fig. 1.13, sex differences in the WOB during exercise have been shown to become significant at levels of $\dot{V}_E \geq 50$ l min^{-1}, and the WOB becomes as much as 2-fold greater in women *vs.* men at an exercise $\dot{V}_E$ of 90 l min^{-1} [45]. In addition, it has been demonstrated that the observed sex differences in the WOB during exercise could be largely attributed to differences in the resistive WOB (Fig. 1.14), which is directly dependent on airway size [45].

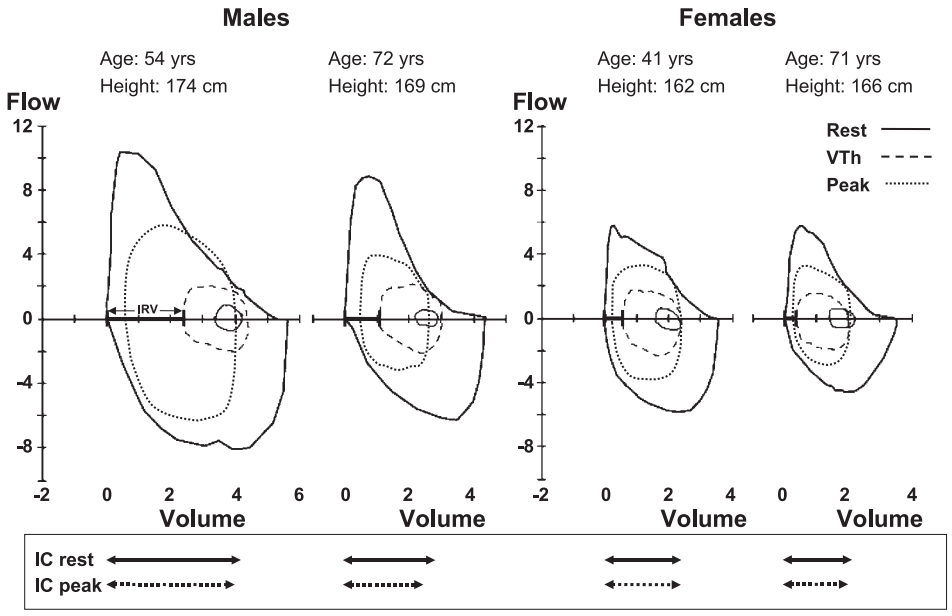

Figure 1.12. Tidal flow-volume loops at rest, the ventilatory threshold (VTh), and at peak exercise are plotted within the respective maximal loops in representative subjects from each group (younger men (YM, n=18), older men (OM, n=16), younger females (YF, n=16), older females (OF, n=18)) during symptom limited incremental treadmill exercise. Inspiratory capacity (IC) reduces with age, thereby reducing inspiratory reserve volume (IRV) and limiting the V_T response to exercise, with this mechanical restriction greater in women compared to men. *Adapted from Ofir et al. [3]*.

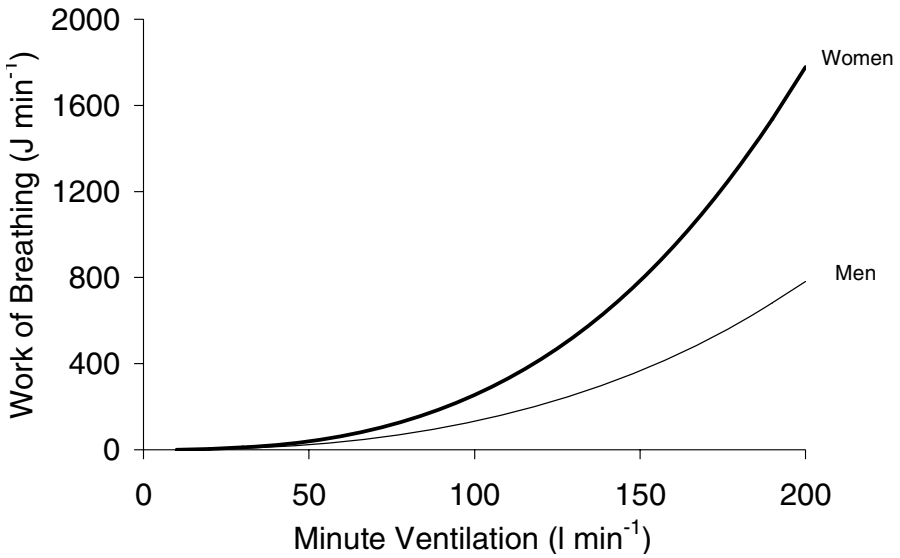

Figure 1.13. Mean curves relating the total work of breathing to minute ventilation in endurance-trained men (n=8) and women (n=10). For a given absolute minute ventilation, women have a higher work of breathing compared to men. *Adapted from Guenette et al. [45]*.

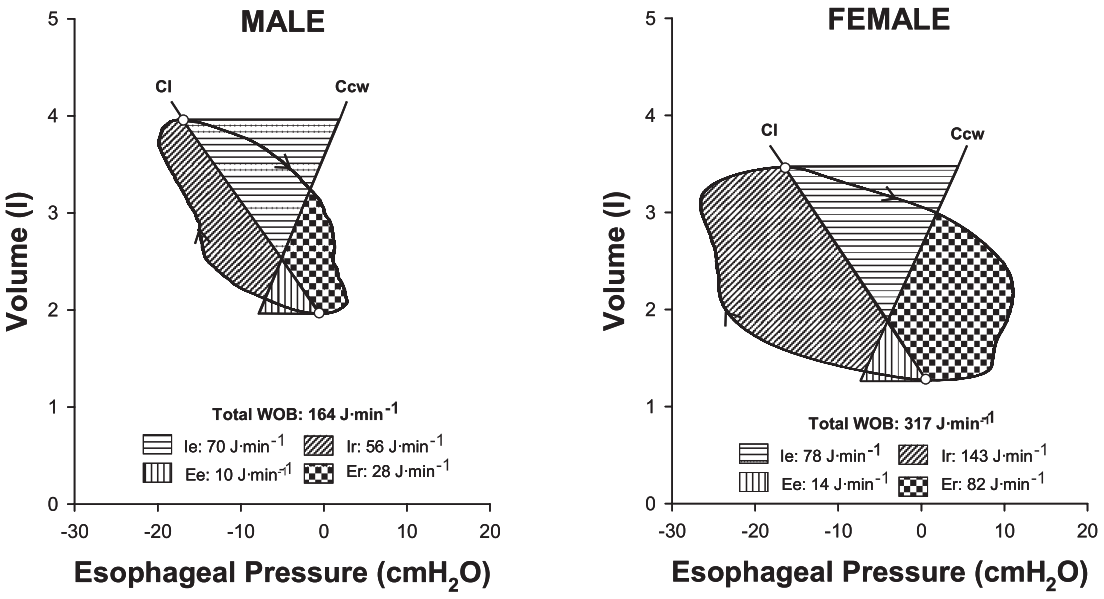

Figure 1.14. Modified Campbell diagrams from an individual male and female subject during incremental cycle exercise matched for ventilation, tidal volume, breathing frequency, age, and mass. Significantly higher pressures are generated in the female compared to the male to achieve the same absolute ventilatory load, resulting in a much higher inspiratory resistive (Ir) and expiratory resistive (Er) work of breathing (WOB), with little difference in inspiratory elastic (Ie) and expiratory elastic (Ee) WOB. CL, dynamic lung compliance Ccw, chest wall compliance. *Adapted from Guenette et al [47].*

In the setting of these relatively greater dynamic respiratory mechanical constraints, a higher NRMD would be required to generate the higher respiratory pressures needed to achieve any given $\dot{V}_E$ during exercise in women compared to men [47]. Accordingly, neuromechanical uncoupling, or an imbalance between NRMD and the simultaneous mechanical/muscular response of the respiratory system (Fig. 5), could presumably be occurring during exercise in women compared to men at any given $\dot{V}_E$, $\dot{V}O_2$, or WR, and may explain the higher sensory intensity ratings of activity-related dyspnea in the former. The mechanistic studies outlined in this thesis are the first to examine the contributory of role of neuromechanical uncoupling of the respiratory system (and its physiological determinants) in sex differences in exertional dyspnea.

In support of this hypothesis, Ofir et al. [3] recently demonstrated, first, that the slope of the relationship between increasing dyspnea intensity ratings and increasing $\dot{V}_E$ during symptom-limited incremental treadmill exercise was significantly higher in women compared to men, independent of age and, second, that these sex differences disappeared when $\dot{V}_E$ was normalized for differences in maximal breathing capacity. Collectively, these findings indicate that respiratory mechanical muscular factors (secondary to differences in lung size, airway diameter and respiratory muscle strength) are likely responsible, at least in part, for sex differences in exertional dyspnea (Fig. 1.15-1.16).

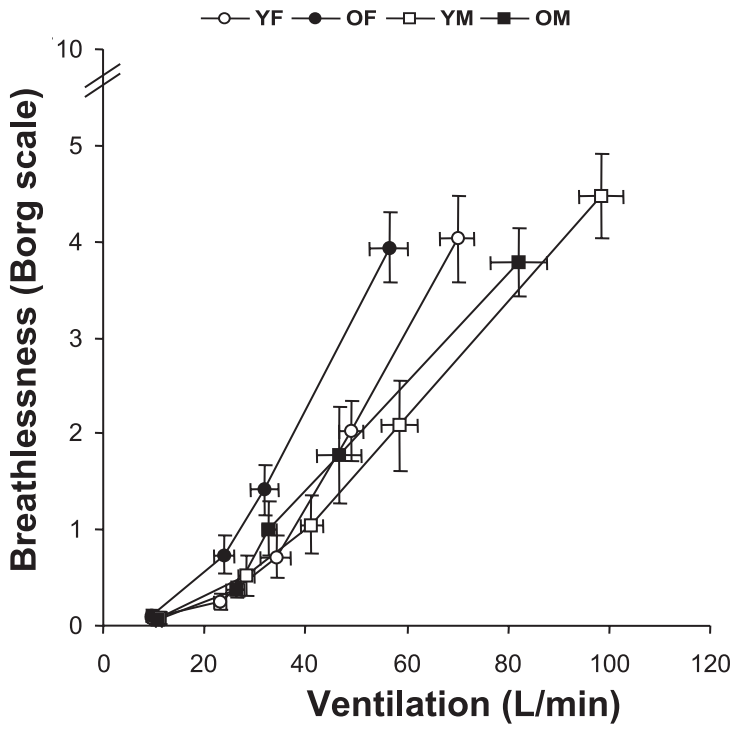

Figure 1.15. Breathlessness plotted against ventilation for each subject group (younger men (YM, n=18), older men (OM, n=16), younger females (YF, n=16), older females (OF, n=18)) during symptom limited incremental treadmill exercise. Breathlessness ventilation slopes were significantly steeper in women compared to men ($p<0.05$). Values are means ± SE for measurements at rest, during each stage of exercise, and peak exercise. *Adapted from Ofir et al. [3]*.

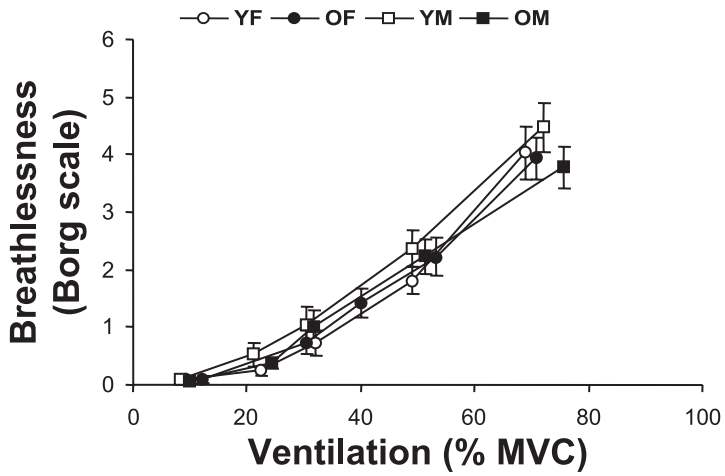

Figure 1.16. Breathlessness plotted against ventilation expressed as a % of the maximal ventilatory capacity (MVC) for each subject group (younger men (YM, n=18), older men (OM, n=16), younger females (YF, n=16), older females (OF, n=18). Breathlessness intensity was not different across groups. *Adapted from Ofir et al. [3]*.

4. POSITION OF THE PROBLEM

There appears to be a respiratory mechanical effect of sex that translates into relatively greater perceived dyspnea during exercise in women compared to men. Based on the proposed neurophysiological construct of dyspnea (Fig. 1.5), it is reasonable to postulate that neuromechanical uncoupling of the respiratory may form the physiological basis of sex differences in exertional dyspnea. In other words, the combination of a higher NRMD and relatively greater dynamic mechanical ventilatory constraints during exercise may manifest as higher sensory intensity and unpleasantness ratings of dyspnea in women compared to men.

Therefore, the primary objective of the present study was to test the hypothesis that neuromechanical uncoupling of the respiratory system forms the physiological basis of sex differences in exertional dyspnea. To this end, we compared detailed assessments of $\dot{V}_E$, breathing pattern, dynamic operating lung volumes, contractile respiratory muscle function, diaphragm electromyography (EMGdi), and sensory intensity and

unpleasantness ratings of dyspnea during symptom-limited incremental cardiopulmonary cycle exercise testing (CPET) in 25 healthy, young, women and 25 age-matched men. Sex differences in physiological and perceptual responses to CPET were examined, first, at standardized absolute submaximal levels of $\dot{V}_E$ in order to provide mechanistic insight into the abovementioned sex differences in the ventilatory response to exercise and, second, after normalization of $\dot{V}_E$ to account for known sex differences in exercising muscle mass. To our knowledge, the present study is the first to examine sex differences in (1) NRMD as assessed by quantifying the EMGdi using a multipair esophageal electrode catheter [27, 51] and (2) the affective dimension (i.e., unpleasantness) of dyspnea during symptom-limited CPET in a relatively large group of healthy, young adults.

CHAPTER II. Physiological mechanisms of sex differences in exertional dyspnea: role of neural respiratory motor drive

ABSTRACT

The purpose of this study was to elucidate the physiological mechanisms of sex differences in exertional dyspnea. We compared detailed measures of neural respiratory motor drive (diaphragm EMG expressed as a % of maximal EMGdi [EMGdi%max]), breathing pattern, operating lung volumes, dynamic respiratory mechanics (tidal esophageal [Pes,tidal%peak], and transdiaphragmatic [Pdi,tidal%peak] pressure swings expressed as a % of their respective peak values), and sensory intensity and unpleasantness ratings of dyspnea during symptom-limited incremental cycle exercise in healthy young women (n=25) and men (n=25). The tidal volume to forced vital capacity ratio (V_T%FVC), breathing frequency, EMGdi%max, Pes,tidal%peak, Pdi,tidal%peak, and sensory intensity and unpleasantness ratings of dyspnea were higher, while dynamic inspiratory capacity and inspiratory reserve volume (IRV) were lower at a standardized absolute ventilation ($\dot{V}_E$) of 55 l min^{-1} during submaximal exercise in women *vs.* men (all $p<0.05$). By contrast, sex had no demonstrable effect on the inter-relationships between exercise-induced increases in V_T%FVC, EMGdi%max, and sensory intensity and unpleasantness ratings of dyspnea. The results of this study suggest that sex differences in the intensity and unpleasantness of exertional dyspnea in health reflect the awareness of a relatively higher neural respiratory motor drive (or EMGdi%max) needed to achieve any given $\dot{V}_E$ during exercise in the setting of relatively greater dynamic mechanical constraints on V_T expansion in women.

INTRODUCTION

It is well established that, even after correction for differences in body height, women have smaller lungs, narrower airways, and weaker respiratory musculature than age-matched men [3, 21, 35-37, 39, 40, 52, 53]. As a result of these differences, women compared to men during symptom-limited incremental cycle exercise: (i) have a relatively reduced maximal ventilatory capacity; (ii) are at higher risk to develop expiratory flow limitation and dynamic lung hyperinflation; (iii) adopt a more rapid and shallow breathing pattern; and (iv) have a higher mechanical work of breathing with evidence of relatively greater dynamic mechanical constraints on tidal volume (V_T) expansion [21, 38, 42, 45, 47, 48]. Collectively, these sex differences in dynamic respiratory mechanical/muscular function would be expected to result in relatively greater neural respiratory motor drive and contractile respiratory muscle effort requirements to achieve any given ventilation ($\dot{V}_E$) during exercise in women compared to men, with attendant greater negative sensory consequences in the former. Indeed, sensory intensity ratings of dyspnea are consistently higher at any given submaximal *absolute* work rate, oxygen uptake ($\dot{V}O_2$), and $\dot{V}_E$ during symptom-limited incremental cycle and treadmill exercise in healthy women *vs.* men, regardless of age [2, 3]. However, the physiological mechanisms of sex-differences in activity-related dyspnea remain poorly understood and represent the primary focus of this study.

Based on our current understanding of the neurophysiology of exertional dyspnea in health and in pulmonary diseases [1, 16, 20, 26], it is reasonable to postulate that the higher sensory intensity ratings of dyspnea during exercise at a given submaximal *absolute* work rate, $\dot{V}O_2$, and $\dot{V}_E$ in women *vs.* men may reflect relatively greater

neuromechanical uncoupling of the respiratory system, as manifest by the combination of a relatively higher neural respiratory motor drive and relatively greater dynamic mechanical constraints on V_T expansion. Therefore, the purpose this study was to test the hypothesis that neuromechanical uncoupling of the respiratory system forms the mechanistic basis of sex differences in activity-related dyspnea. To this end, we compared detailed measures of breathing pattern, dynamic operating lung volumes, multipair esophageal electrode catheter-derived measures of the diaphragm electromyogram (EMGdi, an index of neural respiratory motor drive), esophageal (Pes), gastric (Pga), and transdiaphragmatic (Pdi) pressure-derived measures of contractile respiratory muscle function, and sensory intensity and unpleasantness ratings of dyspnea during symptom-limited incremental cycle exercise in healthy, young men and women.

METHODS

Subjects. Study participants included 50 healthy, habitually active, non-smoking, non-obese men (n=25) and women (n=25) aged 19-38 years with normal lung function according to routine spirometry: forced expiratory volume in 1-sec (FEV_1) ≥80% predicted and FEV_1-to-forced vital capacity ratio (FEV_1/FVC) >70% [54]. Exclusion criteria included: history of cardiovascular, vascular, respiratory, renal, liver, musculoskeletal, endocrine, neuromuscular, and/or metabolic disease/dysfunction that could make it unsafe for them to exercise and/or that could interfere with proper conduct of the exercise tests; a body mass index (BMI) <18.5 kg m^{-2} and >30 kg m^{-2}; were unable to perform exercise and/or pulmonary function testing; were taking doctor prescribed medications other than oral contraceptives; and/or had an allergy to latex. Neither menstrual cycle phase [55, 56] nor oral contraceptive use [57] affects the ventilatory

response to exercise in healthy young women. Thus, no attempt was made to control for menstrual cycle status and/or oral contraceptive use in our female volunteers. Subjects were recruited from Montréal and surrounding areas by word-of-mouth and online postings in the McGill University and Concordia University classifieds.

Study design. This was a single-center, cross-sectional study wherein subjects visited the laboratory on 2 separate occasions over a period of approximately 2 weeks. The study protocol and consent form received approval by the Institutional Review Board of the Faculty of Medicine at McGill University in accordance with the *Declaration of Helsinki.* Written informed consent was obtained from all subjects prior to study participation.

Visit 1 included a thorough medical history; pulmonary function testing; and a symptom-limited incremental cardiopulmonary cycle exercise test (CPET) for familiarization purposes. *Visit 2* included a dual-energy x-ray absorptiometry (DXA) scan; pulmonary function testing; and a symptom-limited incremental CPET with added measurements of EMGdi, Pes, Pga and Pdi using a combined esophageal electrode-balloon catheter. Subjects were told to entirely avoid alcohol, caffeine and strenuous exercise on each test day, which were separated by ≥24 hours.

Pulmonary function tests. Routine spirometry and a 15-sec maximal voluntary ventilation maneuver (MVV_{15}) were performed with subjects in the sitting position using automated testing equipment (SensorMedics Vs229d; SensorMedics, Yorba Linda, CA) at each visit in accordance with recommended techniques [58]. Spirometric parameters were standardized as percentages of predicted normal values [59]; predicted normal

values for inspiratory capacity (IC) were calculated as predicted total lung capacity (TLC) minus predicted functional residual capacity.

Body composition. Whole and regional body composition was measured using the General Electric Lunar iDXA scanner (GE Healthcare, USA). Lean body mass (LBM) and lean leg mass (LLM) were determined using regions of interest within General Electric's Lunar Encore™ software (V.11.20).

Cardiopulmonary exercise testing. Exercise tests were performed at each visit in accordance with clinical exercise testing guidelines [18] and conducted on an electronically braked cycle ergometer (VIAsprint 150P; Ergoline, Bitz, Germany) by use of an automated CPET system (Vs229d; SensorMedics). Exercise tests consisted of a steady-state resting period of at least 6-min of quiet resting breathing followed by 25-watt increases in work rate (starting at 25-watts) every 2-min to the point of symptom-limitation. Subjects maintained a pedaling cadence between 50-90 revolutions/min and were verbally encouraged to exercise to the point of symptom-limitation.

Standard metabolic, respiratory, breathing pattern, and gas exchange parameters were collected on a breath-by-breath basis while subjects breathed through a mouthpiece and low-resistance flow transducer with nasal passages occluded by a nose clip. Heart rate (HR) was monitored using a Polar® heart rate monitor.

Operating lung volumes. Changes in end-expiratory lung volume (EELV) were estimated from IC maneuvers performed at rest, at the end of every 2-min stage during exercise, and at end-exercise. Assuming that TLC does not change during exercise [60], changes in IC and inspiratory reserve volume (IRV = IC – V_T) reflect changes in dynamic

EELV and end-inspiratory lung volume (EILV), respectively. Satisfactory technique and reproducibility of IC maneuvers performed at rest and during exercise were confirmed for each subject by evaluating the consistency of peak inspiratory Pes (Pes,inspir) measurements [61]. Inspiratory capacity and IRV were expressed as *absolute* values (i.e., liters) and as a % of FVC for each subject.

Multidimensional evaluation of perceived dyspnea. Using Borg's 0-10 category ratio scale [19], subjects provided ratings to the following questions at rest, within the first 30-sec of every 2-min stage during exercise, and at end-exercise: How *intense* is your sensation of breathing overall? How *unpleasant* or *bad* does your breathing make you feel? How *intense* is your sensation of leg discomfort? The sensation of "breathing overall" (hereafter referred to as dyspnea) was defined to each subject as the "global awareness of your breathing." Prior to each exercise test, subjects were familiarized with the Borg scale and its endpoints were anchored such that "0" represented "no intensity (or unpleasantness) at all" and "10" represented "the most severe intensity (or unpleasantness) you have ever experienced or could ever imagine experiencing." Prior to each exercise test, a script derived from Price et al. [62] and used previously by Banzett et al. [63] was read to each subject to help them distinguish between the *intensity* and *unpleasantness* of perceived dyspnea.

Symptom ratings preceded IC maneuvers by several (~3-5) breaths to avoid interference with pre-IC breathing patterns and the possible influence that performance of an IC maneuver might have on intensity and/or unpleasantness ratings of dyspnea. Immediately at the end of exercise, subjects were asked to: (i) verbalize their main reason(s) for stopping (i.e., dyspnea, leg discomfort, combination of dyspnea and leg

discomfort or other); (ii) quantify the relative contribution of dyspnea and leg discomfort to exercise cessation; and (iii) select the most appropriate qualitative descriptors of dyspnea experienced at the limits of tolerance from a list of 15 phrases [30].

Electromyogram of the diaphragm (EMGdi) measurement and analysis. The electromyogram of the crural diaphragm (EMGdi) was recorded from a multipair esophageal electrode catheter (Guangzhou Yinghui Medical Equipment Co. Ltd, Guangzhou, China) and used as an index of neural respiratory motor drive [51]. The shape and configuration of the combined esophageal electrode-balloon catheter has been described in detail by Luo et al. [64]. Briefly, the catheter consists of ten 1-cm silver coils that form five consecutive EMGdi recording pairs and an esophageal and gastric balloon for measurement of respiratory muscle pressure generation.

After 'numbing' of the nasal and pharyngeal passages with a 2% endotracheal lidocaine spray (Lidodan™ Endotracheal Non Aerosol Spray; Odan Laboratories Ltd., Montréal, QC, Canada), the electrode-balloon catheter was passed through the nose and positioned at the crus of the diaphragm based on the strength of EMGdi recorded simultaneously from different pairs of electrodes during spontaneous breathing [27, 51]. The raw EMGdi signals were sampled at a rate of 2,000 Hz using a PowerLab 16/30 analog-to-digital converter (model ML880) running LabChart Pro Version 5.4 software (ADInstruments, Castle Hill, Australia); amplified and band-pass filtered between 20 and 1,000 Hz (bio-amplifier model RA-8, Guangzhou Yinghui Medical Equipment Co. Ltd, Guangzhou, China); and displayed in real time and saved for further off-line analysis.

The raw EMGdi signals were converted to root mean square (RMS) using a time constant of 100-msec and a moving window (LabChart Pro Version 5.4 software,

ADInstruments, Castle Hill, Australia). The maximum RMS value during 100-msec subdivisions of each inspired breath was determined by manually selecting EMGdi signals falling between QRS complexes so as to avoid the influence of ECG artifact on EMGdi. The RMS selected was that from the electrode pair with the largest EMGdi amplitude for each breath. Maximum voluntary EMGdi (EMGdi,max) was identified as the largest of all the RMS values obtained from IC maneuvers performed either at rest or during exercise, as previously described [25, 27, 64, 65]. In both health and disease, EMGdi,max values obtained from IC maneuvers are consistently higher than those obtained during either maximal inspiratory sniff maneuvers from functional residual capacity or maximal isovolumetric contractions at functional residual capacity [64, 65]. The ratio of EMGdi to EMGdi,max (EMGdi%max) was used as an index of neural respiratory motor drive. The ratio of EMGdi%max to thoracic volume displacement (V_T expressed as a % of FVC) was used as an index of neuromechanical coupling of the respiratory system, where a higher EMGdi%max:V_T%FVC in one group *vs.* another would indicate relatively greater neuromechanical uncoupling.

Esophageal, gastric and transdiaphragmatic pressure measurement and analysis. The esophageal and gastric balloons were filled with 1.0 ml and 1.2 ml of air, respectively. Esophageal and gastric pressures were collected on a breath-by-breath basis and sampled continuously at a rate of 200 Hz using a PowerLab 16/30 analog-to-digital converter (model ML880) running LabChart Pro Version 5.4 software (ADInstruments, Castle Hill, Australia); a differential pressure transducer (model DP15-34, Validyne Engineering, Northridge, CA, USA); and a signal conditioner (model CD280-4, Validyne Engineering, Northridge, CA, USA). The continuous flow signal from the Vs229d CPET system was

simultaneously input into this data-acquisition system and sampled at a rate of 200 Hz. Flow, Pes, Pga, and Pdi (calculated as the difference between Pga and Pes) signals were displayed in real time and saved for further off-line analysis.

Tidal Pes (Pes,tidal), Pga (Pga,tidal), and Pdi (Pdi,tidal) swings were calculated as the difference between peak tidal inspiratory and peak tidal expiratory Pes, Pga, and Pdi, respectively. The ratio of Pes,tidal, Pga,tidal, and Pdi,tidal to their respective symptom-limited peak exercise values was used as an index of global respiratory muscle effort (Pes,tidal%peak), expiratory muscle effort (Pga,tidal%peak), and diaphragm muscle effort (Pdi,tidal%peak).

Analysis of exercise end-points. All physiological parameters measured breath-by-breath were averaged in 30-sec intervals at rest and during exercise. These parameters, collected over the first 30-sec period of every 2nd minute during exercise, were linked with symptom ratings and IC measurements collected in the latter 30-sec of the same minute so as to avoid contamination of averaged breath-by-breath data by subject-experimenter interaction and irregular breaths surrounding IC maneuvers. Measured parameters were compared at rest, at a standardized *absolute* submaximal $\dot{V}_E$ of 55 l min^{-1} during exercise (iso-$\dot{V}_E$), and at peak exercise. Pre-exercise *rest* was defined as the average of the last 30-sec of the steady-state period after at least 2-min of quiet breathing on the mouthpiece while seated quietly on the stationary bicycle before the start of exercise. *Iso-*$\dot{V}_E$ was defined as the highest equivalent *absolute* submaximal $\dot{V}_E$ achieved during incremental cycle tests performed by all subjects: cardiorespiratory and esophageal electrode-balloon catheter-derived parameters, IC measurements and

symptom ratings at iso-$\dot{V}_E$ and at standardized *absolute* submaximal levels of $\dot{V}_E$ of 25 l min^{-1}, 35 l min^{-1}, and 45 l min^{-1} were calculated by linear interpolation between adjacent measurement points for each subject. *Peak exercise* was defined as the average of the last 30-sec of loaded pedaling. Peak work rate was defined as the highest cycle work rate that the subject was able to sustain for ≥30-sec, while exercise endurance time was defined as the duration of loaded pedaling.

During exercise, $\dot{V}_E$ is tightly coupled to the metabolic rate of CO_2 production ($\dot{V}CO_2$), which in turn varies as a function of exercising muscle mass. Thus, to account for known sex differences in exercising muscle mass during cycle ergometry, physiological and perceptual parameters were compared between men and women after adjusting $\dot{V}_E$ for DXA-derived estimates of LLM ($\dot{V}_{E,LLM}$).

Statistical analysis. Using an unpaired subject formula (SigmaStat® for Windows Version 3.10; Systat® Software Inc., San Jose, CA), we estimated, *a priori*, that a minimum sample size of 16 subjects/group would provide >80% power to detect a ±1 Borg 0-10 scale unit difference in sensory intensity ratings of dyspnea [66] between sexes during CPET, assuming an α of 0.05, a two-tailed test of significance and a within-subject standard deviation of ±1 Borg 0-10 scale unit based on unpublished data from our laboratory.

Unpaired *t*-tests were used to examine the effect of sex (male *vs.* female) on physical characteristics; pulmonary function test parameters; cardiorespiratory and esophageal electrode-balloon catheter-derived parameters; operating lung volumes; and symptom responses at each measurement time (Microsoft Excel for Mac 2011 Version

14.1.0, Redmond, WA). Qualitative descriptors of dyspnea at end-exercise and the reasons for stopping exercise were analyzed using Fisher's exact test. A $p<0.05$ level of statistical significance was used for all analyses. Data are presented as mean ± SEM.

RESULTS

Subjects. Baseline subject characteristics are summarized in Table 2.1. Other than anticipated differences in body height, mass, and composition, men and women were well matched for age, BMI, and % predicted spirometric pulmonary function test parameters.

Table 2.1. Subject characteristics and pulmonary function test parameters

Parameter	Men (n=25)	Women (n=25)
Age, yrs	24.2 ± 0.9	22.5 ± 0.5
BMI, kg m^{-2}	23.2 ± 0.5	22.2 ± 0.6
Total Body Mass, kg	73.6 ± 1.9	57.0 ± 1.9‡
Lean Body Mass, kg	56.6 ± 1.7	38.4 ± 0.9‡
Fat Mass, kg	14.0 ± 1.1	16.3 ± 1.2
Leg Lean Mass, kg	19.6 ± 0.6	13.0 ± 0.4‡
Pulmonary function test parameters		
FEV_1, l	4.40 ± 0.15	3.20 ± 0.10‡
FEV_1, % predicted	99.6 ± 2.7	102.6 ± 2.8
FVC, l	5.54 ± 0.19	3.82 ± 0.10‡
FVC, % predicted	99.0 ± 2.7	99.0 ± 2.3
FEV_1/FVC, %	79.8 ± 1.4	83.8 ± 1.5
PEFR, l s^{-1}	10.53 ± 0.36	7.47 ± 0.28‡
PEFR, % predicted	106.6 ± 3.6	114.2 ± 4.8
$FEF_{25-75\%}$, l s^{-1}	4.16 ± 0.25	3.51 ± 0.23
$FEF_{25-75\%}$, % predicted	88.6 ± 5.0	94.7 ± 5.9
MVV_{15}, l min^{-1}	181.4 ± 7.3	129.5 ± 5.3‡

Values are means ± SEM. BMI, body mass index; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; PEFR, peak expiratory flow rate; FEF25–75%, forced expiratory flow between 25 and 75% of the FVC maneuver; MVV15, maximal voluntary ventilation in 15-sec; *, $p<0.05$ vs. men; ‡, $p<0.001$ *vs.* men.

Physiological responses to exercise. Physiological responses at rest, during exercise at iso-$\dot{V}_E$, and at peak exercise are summarized in Table 2.2. Compared to men, women achieved a significantly lower symptom-limited peak cycle work rate (expressed in watts)

and $\dot{V}O_2$ (expressed in l min^{-1} and ml kg^{-1} min^{-1}). However, these sex differences disappeared when the data were adjusted for DXA-derived measures of LBM and LLM, indicating that men and women were well matched for aerobic working capacity.

Table 2.2. Sex differences in physiological and perceptual parameters at rest, during exercise at a standardized submaximal absolute ventilation of 55 l min^{-1} (iso-$\dot{V}_E$) and at peak exercise.

Parameter	Rest		Iso-$\dot{V}_E$		Peak	
	Men	Women	Men	Women	Men	Women
Work rate, watts	- -	- -	145.6 ± 4.3	129.9 ± 4.6*	251.0 ± 10.7	172.0 ± 7.0‡
Work rate, watts (kg LBM)$^{-1}$	- -	- -	2.62 ± 0.11	3.38 ± 0.09‡	4.43 ± 0.12	4.48 ± 0.15
Work rate, watts (kg LLM)$^{-1}$	- -	- -	7.64 ± 0.34	10.03 ± 0.29‡	12.89 ± 0.44	13.28 ± 0.46
EET, min	- -	- -	- -	- -	19.1 ± 0.8	13.1 ± 0.5‡
Perceptual parameters, Borg 0-10 scale units						
Intensity of *"Breathing Overall" (dyspnea)*	0.1 ± 0.1	0.2 ± 0.1	2.7 ± 0.4	4.0 ± 0.3†	7.4 ± 0.4	6.9 ± 0.4
"*Unpleasantness*" of Breathing	0.2 ± 0.1	0.4 ± 0.2	1.8 ± 0.3	3.4 ± 0.5*	6.2 ± 0.5	5.9 ± 0.5
Leg Discomfort	0.2 ± 0.1	0.1 ± 0.1	2.9 ± 0.4	4.7 ± 0.4†	8.1 ± 0.5	7.9 ± 0.3
Cardiometabolic and gas exchange parameters						
$\dot{V}O_2$, l min^{-1}	0.38 ± 0.02	0.25 ± 0.01‡	2.33 ± 0.05	1.99 ± 0.06‡	3.85 ± 0.17	2.50 ± 0.10‡
$\dot{V}O_2$, ml min^{-1} kg^{-1}	4.94 ± 0.18	4.44 ± 0.17*	31.96 ± 0.99	34.68 ± 1.09	52.20 ± 2.07	44.16 ± 1.82†
$\dot{V}O_2$, ml min^{-1} (kg LBM)$^{-1}$	6.69 ± 0.33	6.57 ± 0.22	41.74 ± 1.20	52.15 ± 1.40‡	67.71 ± 2.05	65.09 ± 2.12
$\dot{V}O_2$, ml min^{-1} (kg LLM)$^{-1}$	19.43 ± 0.97	19.55 ± 0.76	121.54 ± 4.23	154.89 ± 5.00‡	197.39 ± 7.24	193.33 ± 7.38
$\dot{V}CO_2$, l min^{-1}	0.41 ± 0.12	0.21 ± 0.01	2.23 ± 0.03	2.06 ± 0.04†	4.25 ± 0.17	2.85 ± 0.11‡
$\dot{V}CO_2$, ml kg^{-1} min^{-1}	5.45 ± 1.48	3.67 ± 0.18	31.07 ± 0.79	35.95 ± 1.07†	58.70 ± 2.24	49.51 ± 1.92†
$\dot{V}CO_2$, ml min^{-1} (kg LBM)$^{-1}$	6.94 ± 1.82	5.52 ± 0.26	40.25 ± 1.23	54.11 ± 1.36‡	74.96 ± 1.80	74.37 ± 2.46
$\dot{V}CO_2$, ml min^{-1} (kg LLM)$^{-1}$	20.15 ± 5.30	16.42 ± 0.85	117.09 ± 4.06	160.73 ± 4.96‡	218.36 ± 6.60	220.89 ± 8.44
RER	0.79 ± 0.02	0.85 ± 0.03	0.97 ± 0.02	1.04 ± 0.02†	1.12 ± 0.02	1.14 ± 0.01
HR, beats min^{-1}	77.2 ± 2.4	79.9 ± 2.2	143.0 ± 3.1	163.9 ± 2.2‡	184.3 ± 1.8	182.2 ± 2.2
$\dot{V}E/\dot{V}CO_2$	40.7 ± 1.8	45.2 ± 1.4	24.7 ± 0.3	26.9 ± 0.5†	31.1 ± 0.7	32.7 ± 1.0
$PETCO_2$, mmHg	34.9 ± 0.5	33.6 ± 0.9	42.9 ± 0.6	39.8 ± 0.8†	35.0 ± 0.8	33.4 ± 0.9
Ventilatory, breathing pattern, and operating lung volume parameters						
$\dot{V}_E$, l min^{-1}	12.1 ± 0.6	9.5 ± 0.6†	55.0 ± 0.0	55.0 ± 0.0	132.2 ± 6.1	93.1 ± 4.4‡
$\dot{V}_{E,LLM}$, l min^{-1} (kg LLM)$^{-1}$	0.62 ± 0.02	0.74 ± 0.05*	1.68 ± 0.07	2.50 ± 0.20‡	6.78 ± 0.25	7.23 ± 0.34
$\dot{V}_T$, l	0.78 ± 0.05	0.57 ± 0.02†	2.21 ± 0.07	1.73 ± 0.06‡	2.91 ± 0.10	1.92 ± 0.07‡
V_T, %FVC	14.4 ± 1.2	15.2 ± 0.6	40.6 ± 1.8	45.6 ± 1.6*	53.0 ± 1.7	50.5 ± 1.9
f_R, breaths min^{-1}	17.1 ± 0.8	17.2 ± 0.8	26.1 ± 0.9	33.1 ± 1.4‡	46.1 ± 1.9	49.7 ± 2.4
IC, l	3.40 ± 0.10	2.54 ± 0.10‡	3.59 ± 0.12	2.60 ± 0.12‡	3.74 ± 0.16	2.56 ± 0.11‡
IC, %FVC	61.7 ± 1.6	66.1 ± 1.3*	65.3 ± 2.1	67.5 ± 2.1	67.6 ± 2.2	66.6 ± 1.7
ΔIC from rest, l	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.07	0.06 ± 0.07	0.34 ± 0.09	0.02 ± 0.04†
IRV, l	2.62 ± 0.11	1.88 ± 0.13‡	1.38 ± 0.11	0.85 ± 0.11†	0.83 ± 0.13	0.66 ± 0.10
IRV, %FVC	47.3 ± 1.6	48.7 ± 2.7	24.7 ± 1.8	21.3 ± 2.5	14.6 ± 2.1	16.5 ± 2.3
T_I, sec	0.27 ± 0.03	0.30 ± 0.01	0.08 ± 0.00	0.11 ± 0.00‡	0.07 ± 0.01	0.11 ± 0.01‡
T_E, sec	1.79 ± 0.15	1.64 ± 0.13	1.13 ± 0.04	0.89 ± 0.03‡	0.69 ± 0.03	0.62 ± 0.03
T_I/T_{tot}, %	44.0 ± 1.1	42.3 ± 1.9	46.6 ± 0.5	46.9 ± 0.5	50.3 ± 0.5	48.7 ± 0.5*
T_E/T_{tot}, %	55.3 ± 1.1	56.5 ± 1.4	52.9 ± 0.5	54.1 ± 1.5	49.4 ± 0.5	52.3 ± 1.7
V_T/T_I, l sec^{-1}	0.48 ± 0.03	0.39 ± 0.02*	1.98 ± 0.02	1.97 ± 0.02	4.41 ± 0.21	3.20 ± 0.15‡
V_T/T_E, l sec^{-1}	0.36 ± 0.02	0.29 ± 0.02*	1.73 ± 0.02	1.74 ± 0.02	4.43 ± 0.20	3.06 ± 0.16‡

(Table 2.2 continued on next page)

(Table 2.2 continued from previous page)

Esophageal electrode-balloon catheter derived parameters																						
EMGdi, μV	20.0	±	3.8	13.1	±	1.2	70.4	±	6.2	81.0	±	7.4	141.1	±	9. 3	115.8	±	7.1*				
EMGdi, %EMGdi,max	10.5	±	1.8	7.5	±	0.7	35.6	±	2.7	46.4	±	4.3*	69.6	±	3.0	66.4	±	4.2				
EMGdi%max:V_T%FVC	0.7	±	0.1	0.5	±	0.0	0.9	±	0.1	1.0	±	0.1	1.3	±	0.1	1.3	±	0.1				
Pes,tidal, cmH_2O	4.5	±	0.4	4.9	±	0.5	14.3	±	0.9	17.8	±	0.9†	35.5	±	2.1	31.7	±	3.0				
Pes,tidal, %peak	13.3	±	0.9	17.5	±	2.0*	43.3	±	3.3	62.6	±	4.1†	100.0	±	0.0	100.0	±	0.0				
Pdi,tidal, cmH_2O	8.8	±	0.6	8.0	±	0.6	15.7	±	1.0	14.8	±	0.8	23.7	±	1.5	21.0	±	1.3				
Pdi,tidal, %peak	39.7	±	3.9	40.6	±	3.5	68.3	±	3.1	73.5	±	4.0*	100.0	±	0.0	100.0	±	0.0				
Pga,tidal, cmH_2O	5.3	±	0.4	4.0	±	0.3*	9.0	±	0.6	9.5	±	0.6	18.7	±	1.1	16.7	±	1.9				
Pga,tidal, %peak	31.1	±	3.1	32.8	±	5.8	50.6	±	3.6	66.0	±	5.1*	100.0	±	0.0	100.0	±	0.0				

Values are means ± SEM. EET, exercise endurance time; LBM, lean body mass; LLM, lean leg mass; $\dot{V}O_2$ and $\dot{V}CO_2$, metabolic rate of oxygen uptake and carbon dioxide output, respectively; RER, respiratory exchange ratio; HR, heart rate; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent for carbon dioxide; $PETCO_2$, partial pressure of end-tidal CO_2; $\dot{V}_E$, minute ventilation; V_T, tidal volume; f_R, breathing frequency; IC, inspiratory capacity; Δ, change; FVC, forced vital capacity; IRV, inspiratory reserve volume; T_I and T_E, inspiratory and expiratory time, respectively; T_I/T_{tot} and T_E/T_{tot}, inspiratory and expiratory duty cycle, respectively; V_T/T_I and V_T/T_E, mean tidal inspiratory and expiratory flow rate, respectively; EMGdi, root mean square of the diaphragm electromyogram; EMGdi,max, maximal voluntary root mean square of the diaphragm electromyogram; Pes,tidal, Pdi,tidal and Pga,tidal, tidal esophageal, transdiaphragmatic and gastric pressure swing, respectively; *, $p<0.05$ vs. men; †, $p<0.01$ vs. men; ‡, $p<0.001$ *vs.* men.

Ventilatory, breathing pattern, and dynamic operating lung volume responses to exercise are shown in Fig. 2.1. Compared with men, women adopted a more shallow (Fig. 2.1*A*) and rapid (Fig. 2.1*B*) breathing pattern, particularly towards higher levels of $\dot{V}_E$ during submaximal exercise. Tidal volume expressed as a % of FVC was significantly higher in women compared to men at any given $\dot{V}_E$ during submaximal exercise (Table 2.2, Fig. 2.1*C*), whereas the relationship between increasing breathing frequency (f_R) and increasing V_T%FVC was similar throughout exercise in men and women (Fig. 2.1*D*). Mean values of IC and IRV were significantly lower at rest (by 0.86 l and 0.74 l, respectively; both $p<0.001$) and at any given $\dot{V}_E$ during submaximal exercise (e.g., by 0.99 l and 0.53 l at iso-$\dot{V}_E$, respectively; both $p<0.01$) in women compared with men (Table 2.2, Fig. 2.1*E*); however, these sex differences disappeared when IC and IRV were expressed as a % of FVC (Fig. 2.1*F*). Both groups achieved a similar IRV (expressed in l

and as a % of FVC) at end-exercise, but women at a much lower peak $\dot{V}_E$ than men (Table 2.2, Figs. 2.1*E* and 2.1F). As illustrated in Figs. 2.1*E* and 2.1*F*, IC increased by 0.34±0.09 l from rest to end-exercise in men, which is contrast to women whose IC remained at or near resting levels throughout much of exercise (Table 2.2).

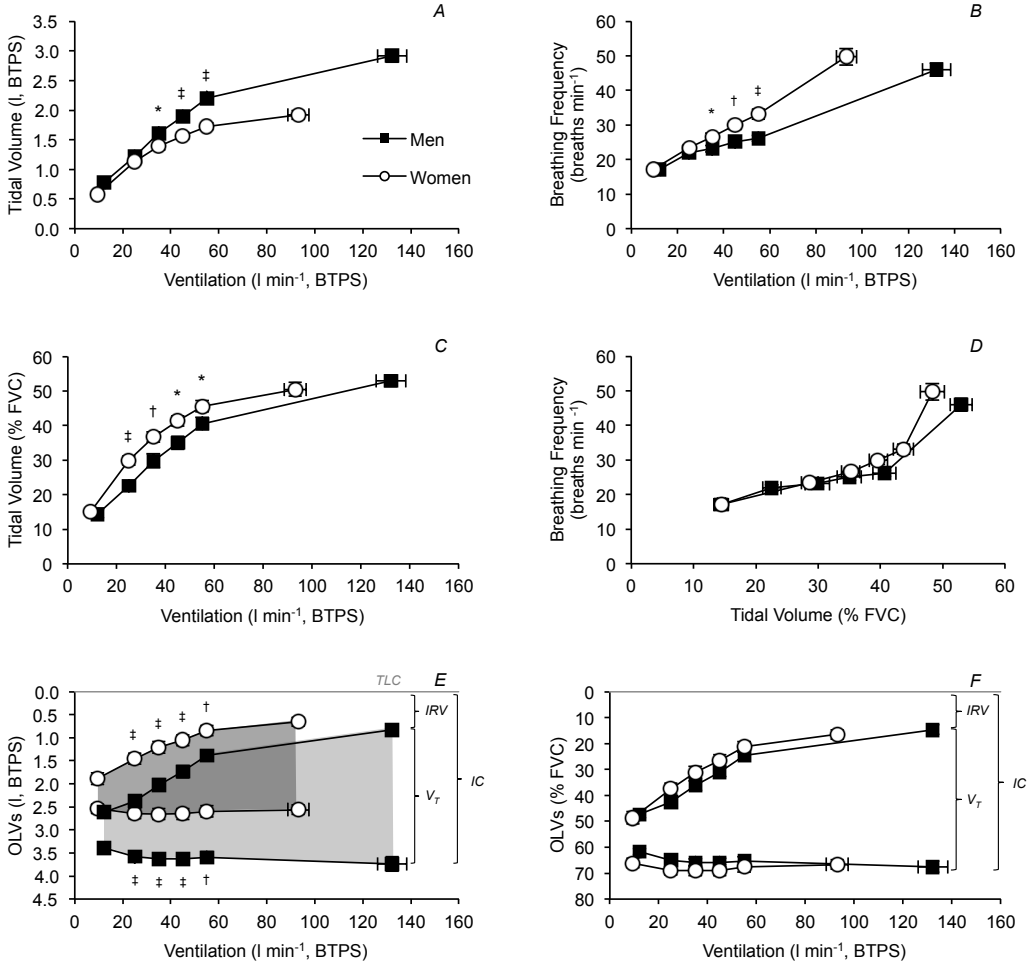

Figure. 2.1. Breathing pattern and operating lung volumes during symptom-limited incremental cycle exercise in healthy, young men (n=25) and women (n=25). Data points are mean ± SEM for measurements at rest, standardized submaximal ventilations during exercise, and at peak exercise. FVC, forced vital capacity; OLVs, operating lung volumes; TLC, total lung capacity; IRV, inspiratory reserve volume; V_T, tidal volume; IC, inspiratory capacity; *, $p<0.05$ *vs.* men; †, $p<0.01$ *vs.* men; ‡, $p<0.001$ *vs.* men.

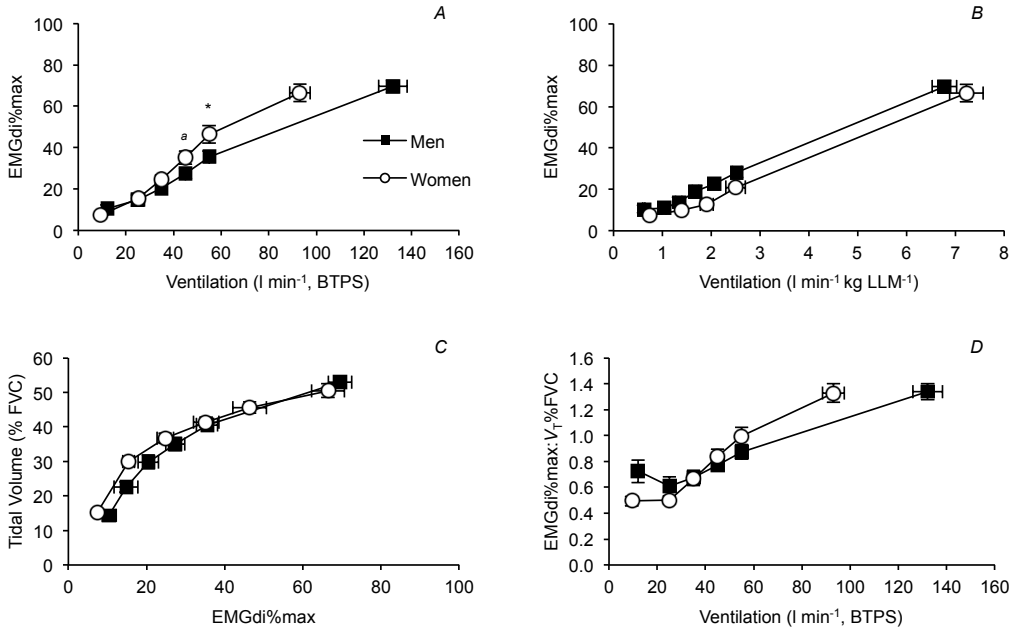

Figure 2.2. Inter-relationships among neural respiratory motor drive, ventilation, and tidal volume expansion during symptom-limited incremental cycle exercise in healthy, young men (n=25) and women (n=25). Data points are mean ± SEM for measurements at rest, standardized submaximal ventilations during exercise, and at peak exercise. EMGdi%max, root mean square of the diaphragm electromyogram (EMGdi,rms) expressed as a % of maximum EMGdi,rms; FVC, forced vital capacity; V_T, tidal volume; *, $p<0.05$ *vs.* men.

Mean values of EMGdi,max were lower in women compared with men (177.7±7.2 *vs.* 204.9±1.8 μV; $p=0.054$); however, these differences were not statistically significant. Both groups achieved a similar EMGdi%max at end-exercise, despite significant between-group differences in peak $\dot{V}_E$ (Table 2.2, Fig. 2.2*A*). As illustrated in Fig. 2.2*A*, EMGdi%max was significantly higher at iso-$\dot{V}_E$ in women *vs.* men (46.4±4.3 *vs.* 35.6±2.7 %EMGdi,max; $p=0.038$) (Table 2.2); however, this difference disappeared when EMGdi%max was examined in relation to $\dot{V}_{E,LLM}$ (Fig. 2.2*B*). Sex had no demonstrable effect on the relationship between increasing EMGdi%max and increasing V_T%FVC during exercise (Fig. 2.2*C*). It follows that the EMGdi%max:V_T%FVC ratio was not significantly different at any given $\dot{V}_E$ during submaximal exercise in women

compared to men (Table 2.2, Fig. 2.2*D*). Mean values of Pes,tidal%peak, Pdi,tidal%peak and Pga,tidal%peak were consistently higher for a given $\dot{V}_E$ during submaximal exercise in women *vs.* men (Figs. 2.3*A*-*C*); however, these differences were not significant when examined in relation to $\dot{V}_{E.LLM}$ (Figs. 2.3*D*-*F*).

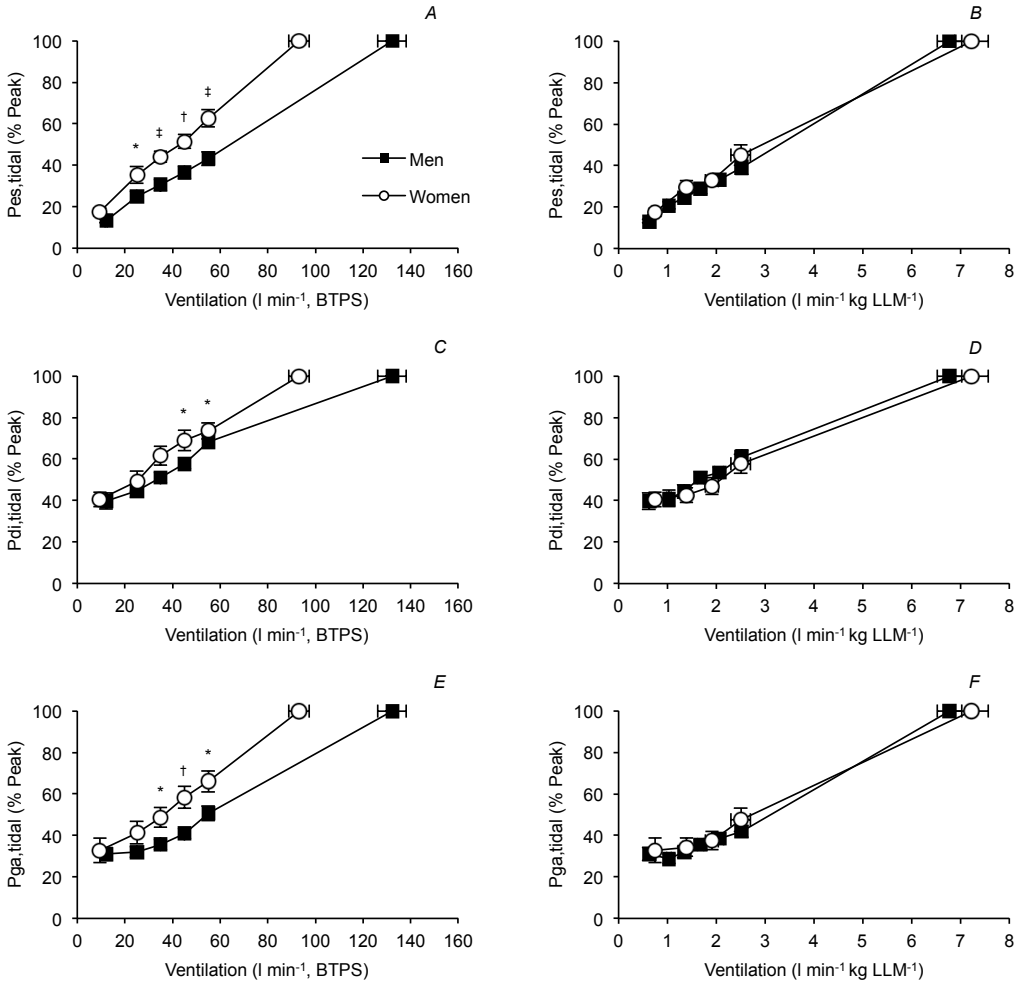

Figure 2.3. Tidal esophageal (Pes,tidal), transdiaphragmatic (Pdi,tidal), and gastric (Pga,tidal) pressure swings expressed as a % of their respective end-exercise values during symptom-limited incremental cycle exercise in healthy, young men (n=25) and women (n=25). Data points are mean ± SEM for measurements at rest, standardized submaximal ventilations and work rates during exercise, and at peak exercise. LLM, lean leg mass; *, $p<0.05$ *vs.* men; †, $p<0.01$ *vs.* men; ‡, $p<0.001$ *vs.* men.

Perceptual responses to exercise. Sensory intensity ratings of leg discomfort were similar at end-exercise in women compared to men, despite significant between-group differences in symptom-limited peak work rate and exercise endurance time (Table 2.2). Neither the intensity (Fig. 2.4*A*) nor the unpleasantness (Fig. 2.4*B*) of dyspnea was significantly different between men and women at end-exercise (Table 2.2). Sensory intensity (Fig. 2.4*A*) and unpleasantness (Fig. 2.4*B*) ratings of dyspnea were significantly higher in women *vs.* men at any given $\dot{V}_E$ during submaximal exercise (e.g., by 1.3 and 1.6 Borg 0-10 scale units at iso-$\dot{V}_E$, respectively; both $p \leq 0.011$) (Table 2.2); however, these differences disappeared when dyspnea intensity (Fig. 2.4*C*) and unpleasantness (Fig. 2.4*D*) ratings were examined in relation to $\dot{V}_{E,LLM}$. Sex had no demonstrable effect on the relationship between increasing dyspnea intensity and unpleasantness ratings and each of V_T%FVC (Figs. 2.4*E* and 2.4*F*, respectively) and EMGdi%max (Figs. 2.4*G* and 2.4*H*, respectively) during symptom-limited incremental cycle exercise.

The distribution of reasons for stopping exercise was not significantly different between sexes: 1 woman *vs.* 0 men stopped due to dyspnea alone; 8 women *vs.* 13 men stopped due to leg discomfort alone; and 16 women *vs.* 12 men stopped due to a combination of dyspnea and leg discomfort. Similarly, the relative contribution of dyspnea (Women, 30.0±4.0% *vs.* Men, 33.6±4.4%; $p=0.552$) and leg discomfort (Women, 65.8±4.3% *vs.* Men, 68.4±3.8%; $p=0.652$) to exercise cessation was not significantly different between-groups. Finally, the selection frequencies of the 15 qualitative phrases used to describe the perception of dyspnea at end-exercise were not significantly different between sexes (data not shown), with the majority of all subjects self-selecting descriptor phrases alluding to a heightened sense of "work/effort of

breathing" at end-exercise; for example, "My breathing is heavy" *(*Women, 96% *vs.* Men, 84%), "My breathing requires more work" *(*Women, 88% *vs.* Men, 80%) and "Breathing in requires effort" (Women, 80% *vs.* Men, 72%).

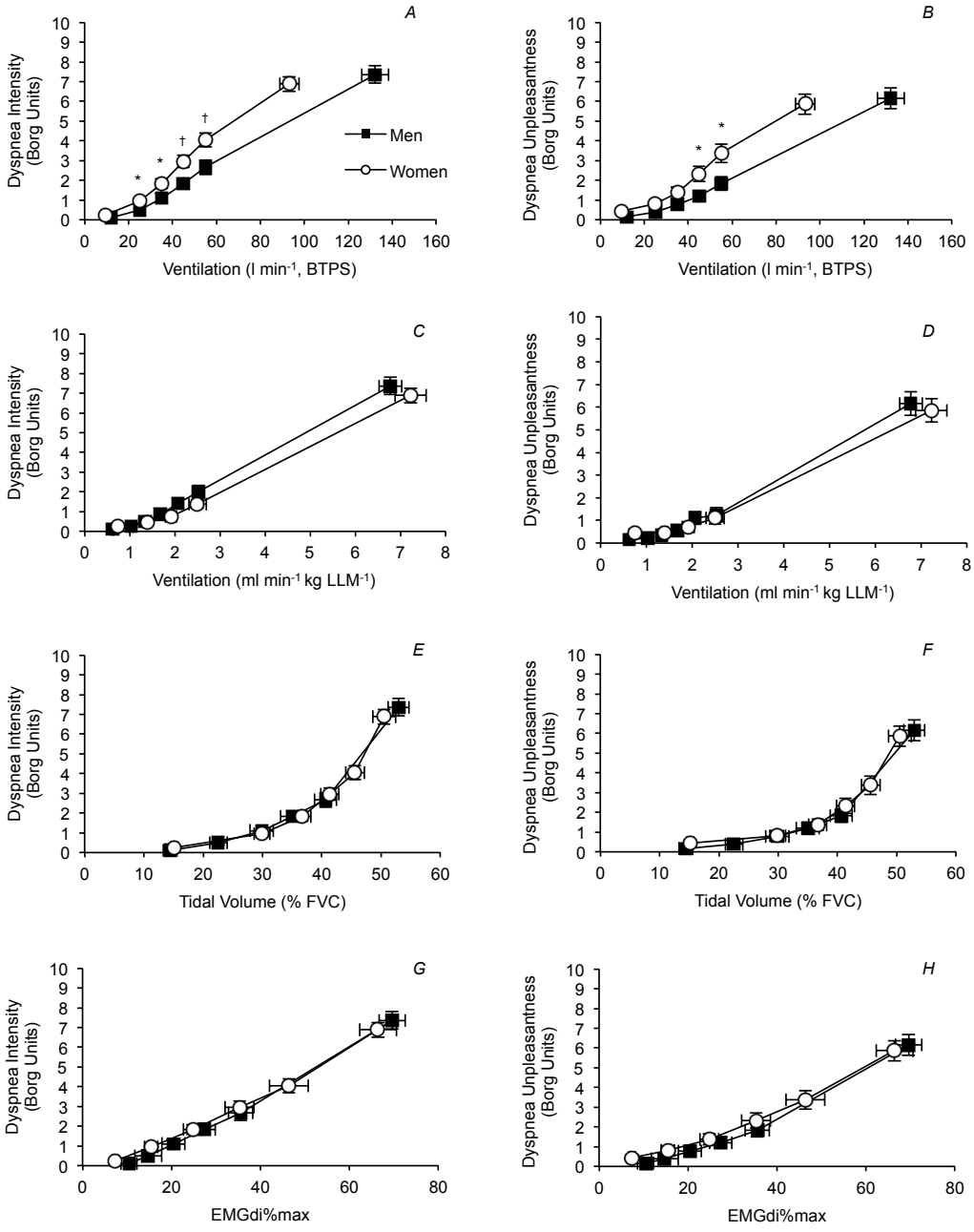

Figure 2.4. Sensory intensity and unpleasantness ratings of dyspnea during symptom-limited incremental cycle exercise in healthy, young men (n=25) and women (n=25). Data points are mean ± SEM for measurements at rest, standardized submaximal ventilations during exercise, and at peak exercise. FVC, forced vital capacity; EMGdi%max, root mean square of the diaphragm electromyogram (EMGdi,rms) expressed as a % of maximum EMGdi,rms; *, $p<0.05$ *vs.* men; †, $p<0.01$ *vs.* men.

DISCUSSION

The main findings of this study were as follows: (1) relatively greater dynamic mechanical constraints on V_T expansion were evident at any given $\dot{V}_E$ during submaximal exercise in women *vs.* men; (2) multipair esophageal electrode catheter-derived measures of EMGdi%max were consistently higher at any given $\dot{V}_E$ during submaximal exercise in women *vs.* men; (3) sex differences in the EMGdi%max to $\dot{V}_E$ relationship during exercise were associated with relatively greater dynamic mechanical ventilatory constraints in women; (4) sensory intensity and unpleasantness ratings of dyspnea were significantly higher at any given $\dot{V}_E$ during submaximal exercise in women *vs.* men; (5) the higher sensory intensity and unpleasantness ratings of exertional dyspnea in women could not be readily explained by greater neuromechanical uncoupling of the respiratory system, but primarily reflected the awareness of a relatively higher EMGdi%max as manifest by the presence of greater dynamic mechanical constraints on V_T expansion; and (6) physiological and perceptual responses to incremental cycle exercise were similar in men and women when examined in relation to $\dot{V}_{E.LLM}$.

We studied a relatively large group of 50 healthy, young, non-smoking, non-obese men and women with normal lung function and cardiorespiratory fitness (Tables 1 and 2). Men and women were well matched for age, BMI, % predicted spirometric pulmonary function test parameters and aerobic working capacity (based on peak $\dot{V}O_2$ and work rate adjusted for LBM and LLM), thus permitting examination into the effects of biological sex on detailed physiological and perceptual responses to exercise.

Sex differences in dynamic ventilatory mechanics and neural respiratory motor drive. In keeping with the results of previous studies [3, 21, 38, 42] relatively greater dynamic mechanical ventilatory constraints with attendant increases in contractile respiratory muscle effort were evident during exercise in women compared to men: V_T%FVC, f_R, Pes,tidal%peak, Pga,tidal%peak, and Pdi,tidal%peak were significantly higher, while V_T, IC, and IRV were significantly lower at any given $\dot{V}_E$ during submaximal exercise in women (Table 2.2, Figs. 2.1, and 2.3). These differences primarily reflected women's naturally smaller lungs, narrower airways, and weaker respiratory musculature (e.g., sex differences in dynamic operating lung volumes for any given submaximal $\dot{V}_E$ during exercise disappeared when IC and IRV were adjusted to each subject's FVC (Fig. 2.1*F*)); and support the widely held view of a relatively greater ventilatory demand-to-capacity ratio at any given $\dot{V}_E$ during exercise in women.

The relatively lower dynamic IRV (Fig. 2.1*E*) and higher V_T%FVC (Fig. 2.1*C*) at any given $\dot{V}_E$ during submaximal exercise in women indicates that V_T is positioned on the upper alinear (non-compliant) portion of their respiratory system's sigmoid pressure-volume relation where there is increased elastic loading and functional weakening of the inspiratory pump muscles, including the diaphragm. It is reasonable to assume that, under these circumstances, a relatively greater neural respiratory motor drive is needed to achieve any given $\dot{V}_E$ during exercise in women. Indeed, the present study is the first to demonstrate that multipair esophageal electrode catheter-derived measures of EMGdi%max – an index of neural respiratory motor drive – were significantly higher during submaximal exercise at iso-$\dot{V}_E$ in women compared to men (46.4±4.3 *vs.* 35.6±2.7

%EMGdi,max; p=0.038) and that these sex differences became more prominent near the limits of tolerance (Table 2.2, Fig. 2.2*A*). By contrast, sex had no demonstrable effect on the relationship between increasing EMGdi%max and increasing V_T%FVC during exercise (Fig. 2.2*C*), indicating that sex differences in the EMGdi%max to $\dot{V}_E$ relationship (Fig. 2.2*A*) likely reflected concomitant sex differences in the V_T%FVC to $\dot{V}_E$ relationship (Fig. 2.1*C*). Indeed, the EMGdi%max:V_T%FVC ratio – an index of neuromechanical coupling of the respiratory system – was not significantly different at any given $\dot{V}_E$ during submaximal exercise in women compared to men (Table 2.2, Fig. 2.2*D*). Collectively, these findings support our contention that sex differences in the EMGdi%max to $\dot{V}_E$ relationship during exercise (Fig. 2.2*A*) reflected the presence of relatively greater dynamic mechanical constraints on V_T expansion in women due to their naturally smaller lungs, narrower airways and weaker respiratory musculature.

Mechanisms of sex differences in exertional dyspnea: role of neural respiratory motor drive. Consistent with the results of Killian et al. [2] and Ofir et al. [3], we found that sensory intensity ratings of dyspnea were consistently higher at any given $\dot{V}_E$ during submaximal exercise in women compared with men (e.g., by 1.3 Borg 0-10 scale units at iso-$\dot{V}_E$; p=0.009) (Fig. 2.4*A*). We are the first to demonstrate, however, that Borg 0-10 scale ratings of the unpleasantness of perceived dyspnea were uniformly higher at any given $\dot{V}_E$ during submaximal exercise in women (e.g., by 1.6 Borg 0-10 scale units at iso-$\dot{V}_E$; p=0.011) (Fig. 2.4*B*). Our *a priori* hypothesis was that sex differences in the intensity and unpleasantness of exertional dyspnea might reflect relatively greater

neuromechanical uncoupling of the respiratory system during exercise in women. However, this was not borne out: sensory intensity (Fig. 2.4*A*) and unpleasantness (Fig. 2.4*B*) ratings of dyspnea were significantly higher at any given submaximal $\dot{V}_E$ during exercise in women, even in the absence of concomitant sex differences in the EMGdi%max:V_T%FVC ratio (Fig. 2.2*D*).

As illustrated in Figs. 4*B-H*, sex differences in sensory intensity and unpleasantness ratings of exertional dyspnea disappeared when these data were examined in relation to $\dot{V}_{E,LLM}$, V_T%FVC, and EMGdi%max. Based on these observations as well as our current understanding of the neurophysiology of activity-related dyspnea [1, 13, 16, 20, 26], we contend that the higher sensory intensity and unpleasantness ratings of dyspnea for a given $\dot{V}_E$ during submaximal exercise in healthy, young women compared to men reflected the awareness of a relatively higher neural respiratory motor drive (as sensed by increased central corollary discharge to the somatosensory cortex) needed to achieve any given $\dot{V}_E$ during exercise in the setting of relatively greater dynamic mechanical ventilatory constraints in women. Indeed, in this study, sensory intensity ratings of dyspnea at iso-$\dot{V}_E$ were associated with concurrent measurements of EMGdi%max (Pearson R=0.373, p=0.008), which in turn were associated with simultaneous measurements of V_T%FVC (Pearson R=0.634, p<0.001) and dynamic IRV (Pearson R=-0.427, p=0.002) within the pooled data (n=50). Our interpretation is further supported by the results of Jensen et al. [27] who recently demonstrated that increasing ventilatory requirements via dead space loading increased (compared with the unloaded control condition) dyspnea intensity ratings in direct proportion to concurrent increases in

EMGdi%max during symptom-limited incremental cycle exercise testing in healthy, older adults; however, neuromechanical coupling of the respiratory system in these healthy subjects remained relatively preserved throughout exercise in the presence of an added dead space.

Methodological considerations. The mechanisms of activity-related dyspnea are multifactorial and include the integration of not only the physiological/biological factors uniquely examined in this study, but also psychological, behavioral, sociocultural, and environmental factors [1, 13, 34], which may have differed between our men and women.

Criticisms of using a multipair esophageal electrode catheter positioned at the crus of the diaphragm to assess neural respiratory motor drive in humans have been described in detail elsewhere [27, 51, 67-69]. Briefly, the use of EMGdi as a representative measure of neural respiratory motor drive is based on the fact that the phrenic nerve is the only motor nerve of the diaphragm and that there is a strong positive correlation (R^2=0.90) between simultaneously measured changes in phrenic nerve EMG and EMGdi [70-72]. In addition, both animal and human studies have indicated uniform changes in the level of crural and costal diaphragm EMG activity during various respiratory maneuvers [73-77].

It could be argued that sex differences in the behavior of dynamic operating lung volumes, the velocity of shortening of the diaphragm and/or spatial recruitment of accessory inspiratory muscles at any given $\dot{V}_E$ during exercise may have influenced our measurement and interpretation of EMGdi. In the current study, the esophageal electrode catheter was carefully positioned based on the strength of EMGdi recorded simultaneously from the 5 electrode pairs during tidal breathing at rest prior to exercise in

each subject, thus accounting for the potential influence of sex differences in resting (pre-exercise) IC and IRV (Fig. 1*E*) on EMGdi. Exercise-induced changes in dynamic IC and IRV (expressed as a % of FVC) were broadly similar in men and women (Fig. 1*F*), while EMGdi was normalized to each subjects' voluntary EMGdi,max, which is advocated for between-subject comparisons [78]. In the present study, mean tidal inspiratory flow rates (V_T/T_I) were not significantly different at any given $\dot{V}_E$ during submaximal exercise in women *vs.* men (e.g., 1.97±0.02 *vs.* 1.98±0.02 l s^{-1} at iso-$\dot{V}_E$; p=0.554) (Table 2.2). Furthermore, Beck et al. [79] provided no evidence for an influence of increasing inspiratory flow rates on multipair esophageal electrode catheter-derived measures of EMGdi in healthy, young volunteers. In light of these observations, it is unlikely that sex differences in the behavior of dynamic operating lung volumes and/or the velocity of diaphragm shortening influenced of our measurement and interpretation of EMGdi. By contrast, we cannot rule out the possibility that relatively greater spatial recruitment of the accessory inspiratory muscles may have helped to "unload" the diaphragm at any given $\dot{V}_E$ during submaximal exercise in women compared to men [80]. Nevertheless, such sex differences in respiratory muscle recruitment patterns would not influence the interpretation of our results inasmuch as they would they would only serve to minimize the magnitude of the observed difference in the EMGdi%max to $\dot{V}_E$ relationship during exercise in women *vs.* men (Fig. 2.2*A*).

Conclusions. The novel results of this study suggest that the higher sensory intensity and unpleasantness ratings of exertional dyspnea in healthy, young women compared to men ultimately reflects the awareness of a higher neural respiratory drive needed to achieve

any given ventilation during exercise in the setting of relatively greater dynamic mechanical constraints on tidal volume expansion in women. These findings may have implications for our understanding of the physiological mechanisms of sex differences in activity-related dyspnea in healthy elderly adults [3] and in patients with chronic lung disease [10, 31-33, 44, 81].

CHAPTER III. Conclusion

Summary and Implications. The general aim of this thesis was to better understand the physiological mechanisms of sex differences in activity-related dyspnea. More specifically, we sought to scrutinize the contribution of NRMD (EMGdi%max), dynamic respiratory mechanical/muscular function and their interaction to sex differences in sensory intensity and unpleasantness ratings of dyspnea during symptom-limited incremental cycle exercise in a relatively large group of healthy, young men (n=25) and women (n=25). The results demonstrated relatively greater dynamic mechanical constraints on V_T expansion at any given $\dot{V}_E$ during submaximal exercise in women *vs.* men, with concomitant increases in EMGdi%max and sensory intensity and unpleasantness ratings of dyspnea in the former. In contrast to our *a priori* hypothesis, the higher sensory intensity and unpleasantness ratings of exertional dyspnea in women could not be readily explained by greater neuromechanical uncoupling of the respiratory system. The novel results of this thesis strongly suggest that sex-related differences in the intensity and unpleasantness of exertional dyspnea in health most likely reflects the awareness of a relatively higher NRMD (as manifest by greater dynamic mechanical constraints on V_T expansion) needed to achieve any given $\dot{V}_E$ during exercise in women.

In addition to improving our fundamental understanding of sex-based differences in integrative respiratory, exercise, and sensory physiology, we anticipate that the results of this study may help to explain why, in various cardiopulmonary disease states (e.g. COPD, asthma, restrictive lung disease, pulmonary vascular disease, congestive heart failure), women report greater levels of activity-related dyspnea and exercise intolerance than men matched for age and disease severity. For example, Guenette et al. [31] recently demonstrated that, even after matching for age and the extent of airway

obstruction, women with mild COPD were far more dyspneic for a given $\dot{V}_E$ compared to their male counterparts (Fig. 1.6). Guenette et al. speculated (but did not formally demonstrate) that this difference likely reflected the awareness of a higher NRMD needed to achieve a given $\dot{V}_E$ in the setting of greater dynamic respiratory mechanical constraints in women. Our findings support this interpretation.

Our results in healthy subjects demonstrate that, during exercise and independent of sex, dyspnea intensity ratings increase in direct proportion to increasing levels of NRMD (Fig. 2.5), which in turn increased in direct proportion to the magnitude of dynamic mechanical constraints on V_T expansion (Fig. 2.3). These data suggest that the higher than normal sensory intensity and unpleasantness ratings of exertional dyspnea commonly reported by patients with chronic obstructive and restrictive pulmonary disorders may not necessarily reflect the presence of relatively greater neuromechanical uncoupling of the respiratory system as generally believed [16], but rather the awareness of a higher than normal NRMD needed to achieve any given $\dot{V}_E$ during exercise in the presence of relatively greater dynamic respiratory mechanical constraints. This hypothesis requires further detailed investigation.

Our findings may also help to explain sex-related differences in symptom perception in an aged population [3], where sex differences in EMGdi%max and dynamic respiratory mechanical/muscular function reported here may be further magnified. In this regard, the established effects of age-related deterioration of pulmonary structure and function may translate into greater magnitude increases in EMGdi%max and, by extension, perceived dyspnea during exercise in elderly women compared to men, particularly when considered in the context of women's naturally reduced maximal

ventilatory capacity. This requires further investigation.

Overall, by improving our understanding of the physiological mechanisms of sex differences in activity-related dyspnea, the results of my research could inform the development and implementation of interventions to relieve dyspnea (with attendant improvement in health-related quality-of-life) in health, variants of health and in patients with chronic cardiorespiratory disease. More specifically, my data suggest that future dyspnea relieving interventions should target NRMD alone or in combination with dynamic respiratory mechanical/muscular function.

Future research. An interesting observation in the present study was that at any given $\dot{V}_E$ during submaximal exercise, indices of (i) global respiratory, (ii) diaphragmatic, and (iii) expiratory muscle pressure development were higher in women vs. men. A logical extension of this study would be to determine if there were concomitant sex differences in respiratory muscle blood flow. For example, sex differences in the work and oxygen cost of breathing may have implications for the competition of blood flow, and therefore oxygen supply, between respiratory and peripheral locomotor muscles during exercise. Furthermore, much of the data presented in this study was normalized to sex differences in baseline forced vital capacity (FVC), $\dot{V}_{E,peak}$, and DXA-derived estimates of exercising muscle mass (kg lean leg mass). It would be interesting to perform a similar experiment in men and women matched for lung volume (e.g., by studying taller than average women and shorter than average men) to more closely examine the roles and inter-relationships of lung size, airway diameter, and accessory muscle recruitment during exercise on ventilatory responses (e.g., breathing pattern, operating lung volumes,

EMGdi, Pes, Pdi, Pga, *etc.*), exercise tolerance, and perceptual ratings (e.g., dyspnea intensity and unpleasantness).

It would also be interesting to superimpose the effects of obesity onto the already increased NRMD associated with the female sex. Based on the results of the present study, it is reasonable to contend that the increased metabolic and ventilatory requirements of exercise in obesity combined with the restrictive ventilatory deficits associated with this condition [20, 82] may precipitate a disproportionately greater rise in the intensity and unpleasantness of dyspnea during exercise in obese women compared to men; however, this has not yet been examined.

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APPENDIX I: Informed consent form

Sex differences in the diaphragm EMG (EMGdi) response to incremental cycle exercise in healthy, young volunteers: implications for respiratory sensation

SUBJECT INFORMATION AND CONSENT FORM

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PURPOSE OF THE SUBJECT INFORMATION AND CONSENT FORM

The purpose of this form is to give you information about this research study and if you sign it, it means that you will have agreed to take part in the study. The form describes the purpose, procedures, benefits and risks of the research study. It may contain words and/or phrases you do not understand. Please do not hesitate to ask the principal investigator or study personnel to explain any words, phrases and/or procedures you do not clearly understand: it is very important that you read and understand the following patient information. You may refuse to take part or withdraw from this study at any time without any reason. Before you agree to take part in this study, please feel free to take this information home and discuss it with a family member, friend and/or your primary care provider. This study has been reviewed for ethical compliance by the Institutional Review Board, Faculty of Medicine, McGill University.

INTRODUCTION AND PURPOSE OF THE PROPOSED STUDY:

You are being invited to take part in this research study because you are a young (20-40 yrs), non-smoking, non-obese man or woman with no history of heart or lung disease. We aim to include 32 people in this study: 16 men and 16 women.

Population studies indicate that breathing discomfort (breathlessness) is more frequently reported during activities of daily living (e.g., stair climbing) in healthy women than men. There is also evidence that, in patients with various heart and lung diseases, women report greater activity related breathlessness, activity-limitation and poorer quality-of-life than men with similar disease severity. The main cause(s) of these sex differences in activity-related breathlessness are poorly understood and represent the focus of this study. Women have naturally smaller lungs and airways, and weaker breathing muscles, which may contribute to their awareness of increased breathlessness, particularly during exercise. To evaluate the possible role of these factors, we will compare detailed breathing responses to exercise in healthy, young men and women. We believe that sex differences in activity-related breathlessness may reflect differences in the activity of the breathing muscles during exercise.

PROCEDURES AND MEASUREMENTS INVOLVED IN THE STUDY:

After informed consent and screening of your medical history, you will be asked to visit the *Clinical Exercise & Respiratory Physiology Laboratory* (located in the Currie Memorial Gymnasium) on 2 separate occasions. Each visit will last approximately 1.5 hours and will be separated by at least 24 hours. Study *Visit 2* for female volunteers will be scheduled 4-7 days into the inactive or no pill phase of their normal oral contraceptive routine.

Visits 1: At this visit, you will be asked questions about your general health and medical history. Your height and weight will be measured. A dual-emission x-ray absorptiometry (iDXA) scan will be performed to estimate your body composition. You will perform a group of simple tests to check the health of your lungs and breathing muscles. Finally, you will exercise on a stationary bicycle for as long as you can while breathing through a rubber mouthpiece (similar to a snorkel) with your nose gently clipped.

Visits 2: At this visit, you will perform a group of simple tests to check the health of your lungs and breathing muscles. You will also exercise on a stationary bicycle for as long as you can. Before exercise, you will be asked to swallow a long, thin tube that will be inserted through your nose and positioned in the food tube (esophagus) leading to your stomach and in the top part of your stomach.

THE FOLLOWING PROCEDURES ARE INCLUDED AS PART OF THIS STUDY:

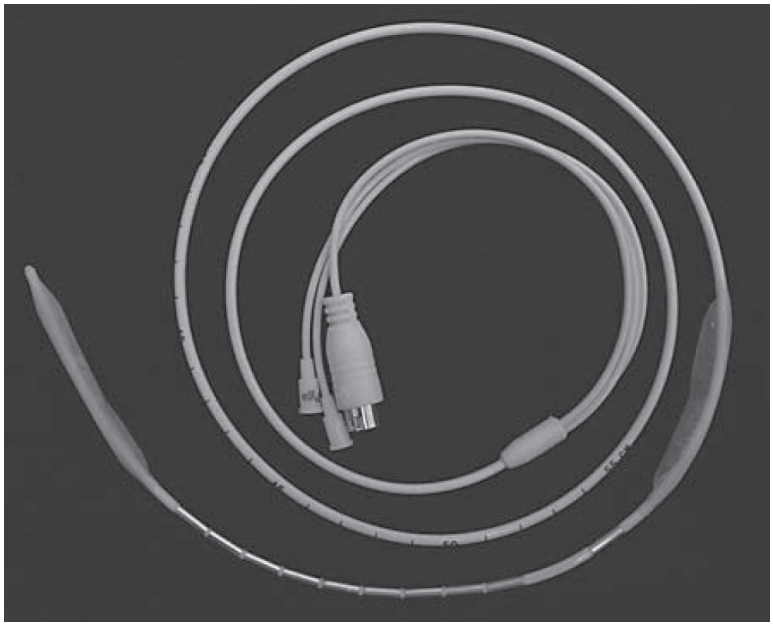
Lung Function Tests: During each visit, you will perform a series of simple tests to determine the health of your lungs, airways and breathing muscles. These tests are safe and each test will be repeated at least 3 times. For all tests, you will be asked to breathe through a rubber mouthpiece while wearing a pair of noseclips. During one test, you will be asked to take a big breath all the way in until your lungs are completely full before blowing the air out of your lungs as hard as you can, as fast as you can and for as long as you can. In another test, you will be asked to breathe as deep and as fast as you possibly can for 15 seconds.

Body Composition (iDXA) Scan: During *Visit 1*, an iDXA scan will be performed to estimate your body composition. You will be asked to lie down on a flat surface for 10-12 minutes while a computerized and automated scanning device, which hovers above and below you, moves slowly from your head to your feet.

Exercise Tests: During each visit, you will perform an *incremental* exercise test on a stationary bicycle, which means that the resistance on the pedals will be very low to start and will get a little bit harder every 2 minutes until you feel the need to stop. This exercise test will make you feel like you are bicycling up a hill that gets a little bit steeper every 2 minutes. You will be verbally encouraged by the experimenters to exercise as long and as hard as you can.

Before and during exercise tests, we will closely monitor your vital signs, including your heart rate, blood pressure and the oxygen content in your blood. Several times at rest and during exercise, you will be asked to take a big breath all the way in until your lungs are completely full. You will also be asked to rate the intensity and unpleasantness of your breathing as well as the intensity of your leg discomfort using a special scale at rest, every 2 minutes during exercise and at the very end of exercise.

Measurement of the activity of your breathing muscles: Before exercise testing at study *Visit 2*, you will be asked to swallow a long, thin tube (catheter) that will be inserted through your nose and positioned in the food tube leading to your stomach (esophagus) and in the top part of your stomach. This tube will be used to measure the activity of your breathing muscles as you breathe in and out at rest and during exercise. As shown in the picture below, the tube consists of ten 1-cm silver coils with two small latex balloons. Dr. Dennis Jensen, a trained exercise scientist with approximately 10 years of experience performing this procedure, will insert and position the tube. Once the tube has been positioned, it will be taped to your nose so that it will not move as you breathe.



POTENTIAL RISKS AND DISCOMFORTS ASSOCIATED WITH THE STUDY:

As described in more detail below, the risks and discomforts associated with this study are related to the measurements being performed. The study investigators will take every means to minimize the occurrence of such risks. The following measures will be in place to minimize the occurrence of such risks and discomforts:

- Screening and examination of your physical health prior to study participation
- Careful and continuous monitoring of vital signs (e.g., heart rate, blood pressure, blood oxygen saturation)
- Direct access to emergency medical equipment (e.g., automated external defibrillator)
- Close access to trained medical doctors of the McGill Sports Medicine Clinic, which is located in the same building as the *Clinical Exercise & Respiratory Physiology Laboratory*.

All exercise tests will be performed by experienced exercise scientists (Dr. Jensen and his trainees) who have up to date CPR (cardiopulmonary resuscitation) certification, automated external defibrillator training and are, therefore, trained to deal with problems that may arise. At any time during the study, it is very important that you tell the experimenters if you feel unwell or experience any problems or side-effects.

Lung Function Tests: Major discomfort is unusual during lung function tests. However, you may experience mild light-headedness and/or shortness of breath. You may also cough at the end of some of the breathing tests. These discomforts go away as soon as the test is stopped. People who have had recent eye, chest or stomach surgery, or any history of coughing up significant amounts of blood in the previous 6 months will not be asked to perform these tests.

Body Composition (iDXA) Scan: You will be exposed to a very small amount of radiation (approximately 3 mrem) from the body composition scan. This measure or dose of radiation absorbed by the body during scanning procedures is not significant. The naturally occurring radiation that our bodies absorb from our environment (e.g., from soil, food, air) can range from 100 to 400 mrem per year.

Exercise Tests: As with any type of strenuous exercise, there is a very small risk that the exercise test(s) may cause heart rate abnormalities, chest discomfort, light-headedness, fainting, breathing discomfort, leg muscle fatigue and/or temporary abnormal changes in blood pressure. The risk of heart rate abnormalities (including heart attack) during laboratory exercise testing in healthy, young men and women is reported to be very low (less than 0.1%) and no different than if such exercise were performed at home or in a local gymnasium. In fact, exercising in the research laboratory is probably safer than exercising at home or in a local gymnasium because your vital signs (e.g., heart rate, blood pressure, blood oxygen saturation, symptoms) will be carefully monitored by trained exercise scientists and because emergency medical equipment and medical doctors will always be available in case problems arise.

It is important that you let the experimenters know if you have ever been advised not to participate in strenuous exercise. The study personnel may also decide that you

should not perform the exercise tests, based on information in your medical history. If at any time during the exercise tests you do not wish to continue for any reason, you may stop exercising voluntarily without penalty.

Esophageal Catheter Insertion: Insertion of the esophageal catheter may result in temporary discomfort such as gagging, coughing or a sense of added pressure in your throat and nose. "*Numbing*" of your throat and nasal passages with a topical anesthetic (2% lidocaine) spray will help to minimize any discomfort you might have during this procedure. If at any time it becomes more uncomfortable than you like, this procedure will be stopped. There is a chance that you will notice some traces of blood if you blow your nose after we remove the tube following exercise tests - this rarely happens and is not serious.

BENEFITS ASSOCIATED WITH THE STUDY:

From participation in this study, you will be provided with information regarding your maximal exercise capacity (i.e., maximal oxygen uptake) - an established measure of a persons' health and fitness level. Several of the tests being conducted in this study are commonly used by medical doctors. Therefore, the study supervisor will (upon your request) forward a summary of these results to your primary care provider. Otherwise, you will not have a direct personal benefit from participation in this study.

CONFIDENTIALITY:

All information obtained during the course of this study is strictly confidential and your anonymity will be protected at all times. Paper records containing names and addresses will be filed securely in a locked cabinet in a locked room. When information is being stored on computer or on study documents, you will be identified by your initials and a research number. Computerized files will be securely stored on a password-protected computer. The principal investigator, his trainee(s) and your personal physician (at your specific request) will have access to your study-related records. Individuals who are not part of the research program will not have access to this information. Documentation will be securely stored for at least 15 years after the study ends; at this time, your records will be destroyed. The principal investigator will be responsible for data collection and confidentiality of your records. Monitoring and data analysis will be conducted by the principal investigator and his trainee(s). Results of this study may be presented at scientific meetings or in publications without disclosure of your identity. If required, auditors and ethics committees will be granted direct access to your records for verification of study procedures and data.

VOLUNTARY PARTICIPATION AND WITHDRAWAL:

It is entirely your choice whether or not you choose to participate in the study. In other words, your participation is 100% voluntary. You may refuse to participate or you may decide to withdraw from this study at any time during the study without penalty and without affecting your future medical care. You may also be removed from this study by the principal investigator and/or medical supervisor should it be decided that it is not in the best interest of your health or the study to continue.

COMPENSATION FOR RESEARCH INJURY:

Every effort to prevent injury that could result from this study will be taken by the investigator and study personnel. In the event of injury or illness suffered by participating in this study, you will receive appropriate medical care under the Quebec Medicare or private insurance plans.

STUDY/FINANCIAL COMPENSATION:

You will receive a total of $25 per visit (or a total of $50) for expenses incurred during your participation in this study (e.g., time, travel, parking, etc.).

PERSONS TO CONTACT:

If you have any further questions, problems or adverse events during the study, you can contact:

Dennis Jensen, Ph.D. (Principal Investigator): (514) 398-4184 ext. 0572

Jean Bourbeau, M.D., M.Sc., F.R.C.P.C. (Medical Supervisor): (514) 934-1934 ext. 32158

Theodore Milner, Ph.D. (Chair, Department of Kinesiology and Physical Education: (514) 398-4184 ext. 0477

If you have questions regarding your rights as a research subject, you may contact:

Ms. Ilde Lepore, Institutional Review Board Senior Ethics Administrator: (514) 398-8302

PARTICIPANT INFORMED CONSENT FORM

1. I am aware that this is a research study.
2. I have read all the pages of the consent form. The research personnel have explained the information and procedures involved in the study. I have had the opportunity to ask questions and my questions have been answered satisfactorily. I have been given time to consider the information carefully and to decide whether or not to participate in this study.
3. I have been informed that my participation in this study is entirely voluntary and that I may refuse to participate, or withdraw at any time, without any consequences to my ongoing medical care at this institution.
4. I authorize access to my records by study investigators, as well as the regulatory authorities and the ethics committee of this institution for purposes of this study only.
5. I am aware that I will be given a copy of this informed consent to keep for my own information, once it is signed.
6. I am aware that I do not give up any of my legal rights by signing this form nor am I freeing the investigator or the establishment where the study takes place from their legal and professional responsibilities.
7. My signature below indicates that I voluntarily agree to take part in this study.

_____	_____	
Subjects' signature	Subjects' name (please print)	Date
_____	_____	
Signature of Person Administering Informed Consent	Name (in block letters)	Date

STATEMENT OF STUDY INVESTIGATOR:

I, or one of my trainees, have carefully explained to the participant the nature of the above research study. I certify that, to the best of my knowledge, the participant understands clearly the nature of the study and demands, benefits, and risks involved to him/her in this study.

_____	
Signature of Principal Investigator (Dennis Jensen, Ph.D.)	Date

APPENDIX II. Candidate's list of awards, presentations, and publications

AWARDS

Graduate Research Enhancement and Travel Award ($500), May 2013
Department of Kinesiology and Physical Education, McGill University, Montreal, Québec, Canada.
Awarded for travel to present at the American Thoracic Society 2013 International Conference in Philadelphia, Pennsylvania, USA.

Graduate Excellence Award ($4,500), March 2013
Department of Kinesiology and Physical Education, McGill University, Montréal, Québec, Canada.

Une bourse d'étude au nom de la compagnie Boehringer Ingelheim ($500), February 2013
Institut Universitaire de Cardiologie et de Pneumologie de Québec
Awarded for an excellent presentation at the Recontre Provincial Annuelle du Regroupement Stratégique MPOC du RSR du FRQS, Québec City, Québec, Canada.

Respiratory Axis Research Day Award ($300), June 2012
Meakins-Christie Laboratories, McGill University, Montréal, Québec, Canada.
Awarded for best presentation in the M.Sc. category.

Graduate Research Enhancement and Travel Award ($500), May 2012
Department of Kinesiology and Physical Education, McGill University, Montreal, Québec, Canada.
Awarded for travel to present at the American Thoracic Society 2012 International Conference in San Francisco, California, USA.

PUBLICATIONS

Schaeffer MR, Mendonca CT, LeVangie MC, Andersen RE, Taivassalo T, Jensen D. Physiological mechanisms of sex differences in exertional dyspnea: role of neural respiratory motor drive. *Experimental Physiology* (under review; submitted June 28, 2013).

ABSTRACTS

Schaeffer MR, Mendonca CT, LeVangie MC, Andersen RE, Taivassalo T, Jensen D. (2013). Sex Differences In The Diaphragm EMG Response To Incremental Cycle Exercise In Healthy, Young Adults: Implications For Respiratory Sensation. *Am J Respir Crit Care Med*, *187*, A4870.

Mendonca CT, Schaeffer MR, Riley P, LeVangie CM, Jensen D. (2013). Physiological Mechanisms Of Dyspnea During Exercise In The Presence Of External Thoracic Restriction: Role Of Increased Neural Respiratory Motor Drive. *Am J Respir Crit Care Med*, *187*, A4871.

Jensen D, Glicksman R, Chan Chun Kong D, Schaeffer MR, Mendonca CT. (2013). Effects Of Abdominal Binding On Neuromechanical Coupling Of The Diaphragm During Exercise In Healthy, Young Men: Implications For Dyspnea And Exercise Tolerance. *Am J Respir Crit Care Med*, *187*, A4872.

Schaeffer M, Pearsall L, Jensen D. (2012). Variability Of The Diaphragm Electromyogram (EMGdi) Response To Strenuous Exercise In Healthy Humans. *Am J Respir Crit Care Med*, *185*, A6390.

CONFERENCE PRESENTATIONS

M Schaeffer, D Jensen. *Sex differences in the diaphragm EMG response to incremental cycle exercise in healthy, young adults: implications for respiratory sensation.* Poster presented at the ATS 2013 International Conference, Philadelphia, Pennsylvania, USA; May 2013.

M Schaeffer, D Jensen. *Sex differences in the diaphragm EMG (EMGdi) response to incremental cycle exercise in healthy, young volunteers: implications for respiratory sensation.* Research presented at the Recontre Provincial Annuelle du Regroupement Stratégique MPOC du RSR du FRQS, Québec City, Québec, Canada; February 2013.

M Schaeffer, D Jensen. *Sex differences in the diaphragm EMG (EMGdi) response to incremental cycle exercise in healthy, young volunteers: implications for respiratory sensation.* Research presented as part of the Department of Kinesiology and Physical Education Graduate Seminar Series, McGill University, Montréal, Québec, Canada; November 2012.

M Schaeffer, D Jensen. *Sex differences in the diaphragm EMG (EMGdi) response to incremental cycle exercise in healthy, young volunteers: implications for respiratory sensation.* Research presented at the MUHC-RI Respiratory Axis Research Day, Meakins-Christie Laboratories, McGill University Health Centre, Montréal, Québec, Canada; June 2012.

M Schaeffer, L Pearsall, D Jensen. *Variability of the diaphragm electromyogram (EMGdi) response to strenuous exercise in healthy humans.* Poster presented at the ATS 2012 International Conference, San Francisco, California, USA; May 2012.

M Schaeffer, D Jensen. *Sex differences in the diaphragm EMG (EMGdi) response to incremental cycle exercise in healthy, young volunteers: implications for respiratory sensation.* Research presented at the Recontre Provincial Annuelle du Regroupement Stratégique MPOC du RSR du FRQS, Montréal, Québec, Canada; February 2012.