Sex-differences in respiratory mechanics during exercise in healthy aging

by

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Abstract

Purpose: Three studies were performed in order to comprehensively examine the combined effects of healthy aging and biological sex on respiratory mechanics and the perception of dyspnea during exercise in healthy adults.

Methods: Study #1 (Chapter 2) investigated the mechanical ventilatory and sensory responses to incremental exercise in a group of younger men and women (20-30 years old), and older men and women (60-80 years old). Study #2 (Chapter 3) examined inspiratory muscle recruitment patterns during incremental exercise in a group of younger men and women (20-30 years old), and older men and women (60-80 years old). Study #3 (Chapter 4) assessed whether experimentally manipulating the magnitude of mechanical ventilatory constraint during moderate-intensity exercise would alter the perception of dyspnea in a group of older men and women.

Conclusions: Healthy aging and biological sex independently increase the magnitude of ventilatory constraint during exercise in healthy adults. Specifically, older individuals and women have a higher work of breathing for a given minute ventilation, and a higher propensity towards expiratory flow limitation during exercise than men and younger individuals, respectively. Additionally, older women have a higher perception of dyspnea during exercise than older men, which could be explained by the combined effects of age and sex on mechanical ventilatory constraint during exercise (Study #1). Healthy aging and biological sex also independently affect the pattern of inspiratory muscle recruitment during exercise, where older individuals and women rely on extra-diaphragmatic inspiratory muscles to a greater extent than older individuals and women, respectively (Study #2). Despite these differences in respiratory mechanics, acutely manipulating the magnitude of mechanical ventilatory constraint during

moderate-intensity exercise did not have an effect of the perception of dyspnea (Study #3). Collectively, the results of this thesis suggest that sex-differences in respiratory mechanics during exercise persist throughout the healthy aging process, but do not contribute to the increased sensations of dyspnea observed in healthy older women relative to healthy older men.

Lay Summary

Healthy older women report breathlessness during exercise more frequently than older men, but it is not known why. This thesis was aimed at evaluating how the respiratory system responds to exercise in healthy older men and women, and identify the potential causes of sexdifferences in breathlessness in healthy older individuals. The results of the first two studies suggest that to breathe during exercise, older women's respiratory muscles must work harder than older men's, which could result in older women feeling more breathlessness. However, the third study in this thesis shows that even if you make it easier or more difficult for older men and older women to breathe during moderate intensity exercise, the perception of breathlessness is unaffected. I conclude that important differences in the way men and women breathe during exercise are present in older individuals, but these differences are not related to the sexdifferences in exertional breathlessness.

Preface

This thesis contains the work of the candidate, Yannick Molgat-Seon, under the supervision of Dr. A. William Sheel. Experimental design and conception were a joint effort between Dr. A. William Sheel and Yannick Molgat-Seon. Data collection, analysis, interpretation and document preparation are primarily the work of the candidate, Yannick Molgat-Seon. All data were collected at the Cardiopulmonary Exercise Physiology Laboratory in the Centre for Heart and Lung Innovation at St. Paul's Hospital, Vancouver, British Columbia.

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The experiments presented in Chapters 2 and 3 received ethical approval from the Providence Health Care Research Ethics Board (UBC-PHC REB #: H13-01062).

The experiment presented in Chapter 4 received ethical approval from the Providence Health Care Research Ethics Board (UBC-PHC REB #: H16-01732).

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List of Abbreviations

A-aDO ₂	alveolar-arterial oxygen gradient
BMI	body mass index
Cl	static lung compliance
COPD	chronic obstructive pulmonary disease
DL _{CO}	diffusion capacity of the lung for carbon monoxide
EELV	end-expiratory lung volume
EFL	expiratory flow limitation
EIAH	exercise-induced arterial hypoxemia
EILV	end-inspiratory lung volume
EMG	electromyography
EMG _{di}	electromyogram of the crural diaphragm
EMG _{di,max}	maximum EMG _{di} activity
EMG _{sca}	electromyogram of the scalene
EMG _{scm}	electromyogram of the sternocleidomastoid
F _B	breathing frequency
FEF ₂₅₋₇₅	forced expired flow between 25-75% volume expired
FEV_1	forced expired volume in 1 second
FEV ₁ /FVC	the quotient of FEV_1 and FVC
FiO ₂	Fraction of inspired oxygen
FRC	functional residual capacity
FVC	forced vital capacity
HR	heart rate
IC	inspiratory capacity
MEFV	maximal expiratory flow volume
MEP	maximal expiratory pressure
MIP	maximal inspiratory pressure
NEP	negative expiratory pressure
NMU	neuromechanical uncoupling
PEF	peak expiratory flow
$P_{ET}CO_2$	end-tidal carbon dioxide
Peso	esophageal pressure
P _{di}	transdiaphragmatic pressure
P _{ga}	gastric pressure
P _{mo}	mouth pressure
Pst 100%TLC	static recoil pressure of the lung at 100% total lung capacity
PTP	pressure-time product
PTP _{di}	trandiaphragmatic pressure-time product
PTP _{di}	esophageal pressure-time product
PTP _{di} /PTP _{eso}	diaphragmatic contribution to total inspiratory pressure generation
RER	respiratory exchange ratio
RMS	root mean square
RV	residual volume
S_pO_2	arterial oxygen saturation by pulse oximetry

TLC	total lung capacity
Ϋ́ _A	alveolar ventilation
VC	vital capacity
VCO ₂	carbon dioxide production
\dot{V}_{E}	minute ventilation
\dot{V}_{ECAP}	ventilatory capacity
$\dot{V}_E/\dot{V}CO_2$	ventilatory equivalent for carbon dioxide
$\dot{V}_E / \dot{V}_{E \ CAP}$	fractional utilization of ventilatory capacity
$\dot{V}_E/\dot{V}O_2$	ventilatory equivalent for oxygen
[.] VO ₂	oxygen uptake
^{VO} 2rm	oxygen cost of breathing
VT	tidal volume
V _{Th}	first ventilatory threshold
W _b	work of breathing

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Chapter 1: Introduction

1.1 Operational definitions of 'sex' and 'age'

The current thesis is focused on determining the effects of sex and healthy aging on the ventilatory and sensory responses to exercise in humans. While the concepts of both sex and healthy aging are familiar to most, the precise definitions can vary substantially based on context, discipline, and personal beliefs. Thus, it is essential to establish operational definitions of 'sex' and 'healthy aging' at the onset in order to ensure clarity.

Within the context of exercise physiology research, the term 'sex' is sometimes used interchangeably with 'gender', which is incorrect. Herein, I use the terms 'sex' and 'gender' according to the definitions provided by the Institute of Gender and Health from the Canadian Institutes of Health Research:

"Sex refers to a set of biological attributes in humans and animals. It is primarily associated with physical and physiological features including chromosomes, gene expression, hormone levels and function, and reproductive/sexual anatomy. Sex is usually categorized as female or male but there is variation in the biological attributes that comprise sex and how those attributes are expressed." (1)

"Gender refers to the socially constructed roles, behaviours, expressions and identities of girls, women, boys, men, and gender diverse people. It influences how people perceive themselves and each other, how they act and interact, and the distribution of power and resources in society. Gender is usually conceptualized as a binary (girl/woman and boy/man) yet there is considerable diversity in how individuals and groups understand, experience, and express it." (1)

The definition of 'healthy aging' presents a unique problem. Healthy aging is multidimensional, involving a variety of physical, cognitive, and social factors. Thus, while there are several well-established definitions for the term 'aging', the concept of 'healthy aging' is more ambiguous. Moreover, 'healthy aging' obligatorily includes the term 'aging' and therefore serves to describe a more specific form of 'aging' itself. Within the context of this thesis, I will use the term 'aging' according to the following definition:

"the progressive deterioration of function that occurs in the post-maturity phase, at the individual, physiological systems and cellular levels." (2)

Building on this definition, the term 'healthy aging' generally refers to the concept of aging in a healthy manner (i.e. in the absence of disease), but can be more precisely defined as follows:

"Healthy biological ageing includes survival to old age, delay in the onset of noncommunicable diseases and optimal functioning for the maximal period at individual levels (physical and cognitive capability), body systems and cells." (2)

Finally, an important question surrounds how best to study aging in a sample of human subjects. By definition, "aging" and "healthy aging" occur progressively over the course of one's lifespan in the post-maturity phase. Therefore, aging is best treated as a continuous variable, however; this is not always feasible. For the purposes of my research, I have chosen to study aging as a dichotomous variable (i.e. 'older' vs. 'younger') rather than a continuous variable due

to the constraints on subject recruitment for invasive physiological experiments, and the magnitude of interindividual variability in the physiological parameters that affect the ventilatory and sensory responses to exercise. The next question then becomes, what ages constitute 'older' and 'younger' individuals?

It is difficult to define specific chronological milestones which mark life stages. The chronological definition of 'older' people varies substantially based on geography, context, and social convention, but in most developed countries the age of 60 or 65 years (roughly equivalent to retirement ages) is said to be the beginning of 'old age' (3). Thus, in this thesis 'older' individuals will be defined as those between the ages of 60 and 80 years. The upper limit of this age range (80 years) for 'older' individuals was chosen to reflect the ages of participants in previous studies concerning the effects of aging on the mechanical ventilatory response to exercise (4,5). The 'younger' individuals included in this thesis were defined as those between the ages of 20 and 30 years, which corresponds to the phase of peak respiratory system maturation (6-9).

1.2 Background

The human respiratory system is ideally structured and regulated to respond to the homeostatic demands of dynamic exercise in healthy young adults. In fact, the respiratory system is generally regarded as being 'overbuilt' in that it possesses a remarkable capacity to respond to large increases in ventilatory demand commensurate with high-intensity exercise (10). Though this is certainly the case in most healthy young individuals, the respiratory system may contribute to limiting exercise capacity in certain populations, notably; highly-trained athletes (11,12), and individuals with respiratory diseases such as chronic obstructive pulmonary disease

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(COPD) (13). The former is an example of a physiological increase in ventilatory demand, whereas the latter is an example of a pathophysiological reduction in ventilatory capacity. In each case, the ventilatory demands of exercise meet or even exceed the finite capacity of the respiratory system. This unique physiological situation results in a blunted capacity of the respiratory system to provide oxygen to the working tissues, thereby contributing to decreasing exercise performance or reducing exercise tolerance. Traditionally it has been thought that healthy individuals of normative fitness are not capable of increasing ventilatory demand to the extent where they reach the mechanical limits of their respiratory system; however, it has been suggested that this may not be the case in healthy older individuals (14). The age-related decline in respiratory system function reduces ventilatory capacity and increases ventilatory demand for a given exercise intensity, which may contribute to reducing exercise performance.

Within the context of analyzing the ventilatory response to exercise, it is important to distinguish between the terms ventilatory *constraint* and ventilatory *limitation*. Ventilatory limitation refers to "factors that actually prevent a particular function from increasing in the face of an increased requirement for ventilation" (15). By contrast, ventilatory constraint refers to "the influence of some mechanism that opposes the requirement for ventilation and therefore induces a reduction in the achieved response but that does not in itself limit the system from further response" (15). It is generally agreed upon that healthy older individuals, like their younger counterparts, do not experience a ventilatory limitation during exercise (14), but do show evidence of a progressive increase in the degree of ventilatory constraint over the course of the healthy aging process (4).

Along with healthy aging, biological sex is also an important factor when considering the ventilatory response to exercise (16-18). It has been shown that relative to men, women have

smaller lungs, and even when matched for lung size, women have smaller large conducting airways (19). This inherent difference in the anatomy of the respiratory system predisposes women to a higher degree of ventilatory constraint during exercise than men (11,20). The ageinduced effects on respiratory system function coupled with sex-based differences in lung structure and function suggests that ventilatory constraint could be more pronounced in older women relative to older men during exercise (18). However, only a small number of studies pertaining to the ventilatory response to exercise have explicitly compared healthy men and women and even fewer studies have specifically investigated sex-differences in respiratory mechanics in healthy older individuals. As such, the primary focus of the proposed thesis will be to assess sex-differences in the mechanical ventilatory response to exercise in healthy older men and women.

1.2.1 The aging respiratory system

Aging is characterized by "a progressive, generalized impairment of function, resulting in an increasing vulnerability to environmental challenge and a growing risk of disease and death" (21). Virtually every aspect of an organism's phenotype undergoes modification (or rather, deterioration) with aging. One of the most prominent declines occurs in the respiratory system. The growth and development the respiratory system reaches its peak by the third decade of life, after which it undergoes a progressive age-dependent decline in function (22). This decline is characterized by a series of structural changes to the lungs, airways, chest wall, and respiratory muscles.

Several age-related changes are known to occur at the level of the lung parenchyma and the peripheral airways. The structure of the alveoli, the primary sites of gas exchange in the lung, are adversely affected by the aging process. Early radiographic studies comparing photomicrographs of the lungs of healthy humans have shown that the alveoli become progressively wider and shallower, the alveolar walls thinner, and the alveolar ducts more dilated with advancing age (23). This morphological deterioration of the lung has been has been termed "senile emphysema" due to its similarity to the emphysematous destruction of the alveoli observed in COPD (24). However, it is important to note that in contrast to the heterogeneous destruction of the lung parenchyma in COPD, the changes to the alveoli observed in healthy older individuals appears to be homogenous throughout the lung and doesn't lead to destruction of the alveolar walls per se (25). While it is difficult to relate the morphological and mechanical changes that occur within the lungs to simple variations in extra-cellular matrix composition, the age-related changes to the alveoli are thought to involve alterations to the elastin-collagen fiber network within the lung (26). Over the course of the healthy aging process, the elastin content of the lung decreases while the collagen content remains unchanged, thereby remodeling the connective and supporting tissues and causing a decline in lung elastic recoil (27). Overall, the age-associated alterations of the lung parenchyma decrease the surface area available for gas exchange (28), reduce in the overall elasticity of the lung, and modifies it's pressure-volume relationship (29). Another major change associated with aging is a reduction in airway size due to alterations in the supporting connective tissues. Morphological assessments of airway size in otherwise healthy individuals post-mortem revealed that mean bronchiolar diameter decreases progressively beyond the age of 40 years, and is associated with an increased peripheral airway resistance (30).

It is well known that advancing age is linked to a progressive reduction in skeletal muscle mass and strength, termed sarcopenia (31). The primary features of sarcopenia in older adults are

a reduction in the cross-sectional area of muscle fibres, loss of fast-twitch motor units, reductions in the number of type II muscles fibers, and disruptions in muscle coordination (32). Given that respiratory muscles are themselves skeletal muscles, it follows that they too likely exhibit a similar decline in mass and strength (9,32). However, there is a dearth of information on the contractile properties of respiratory muscles and how they change with aging, particularly in humans. Large population-based studies have reported significant age-dependent reductions in maximal inspiratory (MIP) and expiratory (MEP) pressures (9,33). However, maximal static airway pressures are volitional measures that reflect the integrated actions of several respiratory muscles rather than an objective assessment of the strength of one specific muscle. Detailed evaluations of diaphragm function using cervical magnetic stimulation revealed that diaphragm strength is 13-25% lower in 65-81 year-olds than that of 19-41 year-olds (34,35). Though direct evidence of a morphological basis for this apparent age-related diaphragm sarcopenia in humans is sparse, work in mouse models of aging indicates that reductions in diaphragm force occur in conjunction with a selective atrophy of type II muscle fibers (36,37). Data from other rodent models also suggests that the aging diaphragm is characterized by a reduction in capillary density (32), an alteration of the contractile properties and an increased propensity towards fatigue (38), but no change in oxidative capacity (39). While it is difficult to draw definitive conclusions regarding the cause of the age-associated reduction in respiratory muscle strength from animal work, it seems clear that the respiratory muscles experience sarcopenia, albeit to a lesser extent (at least for the diaphragm) than is typically observed in limb muscles (40). However, even a modest decline in respiratory muscle strength has the potential to detrimentally affect the capacity of the respiratory system to respond to a given ventilatory demand (41).

The shape and structure of the chest wall also changes with age, which in turn affects its interaction with the lung. Older individuals commonly exhibit a reduction in stature related to the adoption of a kyphotic posture (42). In many cases, this altered posture is caused by spinal kyphosis, which may develop as a result of a loss of vertebral height and even from vertebral body collapse (28). Consequently, older individuals show an increased anteroposterior diameter of the chest and a reduction in the size of intervertebral spaces. Lastly, the chest wall becomes progressively stiffer with age (43) due in part to a calcification of the costal cartilages and chondrosternal junctions (23). Accordingly, the aforementioned remodeling of the chest wall significantly alters breathing mechanics in older individuals (44).

Along with its effects on the lung, respiratory muscles, and chest wall, aging also alters the pulmonary vasculature (45). It has been shown that aging leads to a gradual increase in the stiffness of the pulmonary artery and the pulmonary vein, primarily due to an increase in muscle content and thickness (46). This progressive structural remodeling of the pulmonary vasculature modifies resting pulmonary pressures in older individuals. Indeed, studies have shown that older individuals exhibit markedly greater pulmonary artery pressures and pulmonary artery wedge pressures than younger individuals (47). Furthermore, morphometric studies have shown that aging causes a reduction in the number and volume of pulmonary capillaries (48). Together, these age-induced changes to the pulmonary vasculature significantly reduce the gas exchange capacity of the lungs (45).

The aforementioned age-related changes in the respiratory system can be summarized as five primary physiologic events: alveolar widening, airway narrowing, reduction in respiratory muscles strength, distortion and stiffening of the chest wall, and remodeling of the pulmonary vasculature. Collectively, these changes result in a marked decrease in respiratory system function as evidenced by: i) a reduction in pulmonary gas exchange efficiency, ii) a reduction in the capacity to generate flow, iii) alteration of static lung volumes, and iv) an alteration in respiratory system mechanics.

1.2.1.1 Pulmonary gas exchange efficiency

The efficiency of pulmonary gas exchange is critically dependent on alveolar ventilation (\dot{V}_A) , alveolar perfusion, and diffusion through the alveolar-capillary membrane (49). The normative aging process affects each of these components, thereby decreasing pulmonary gas exchange efficiency. Alterations to the alveoli cause a decrease in the surface area available for gas exchange per unit of lung volume (23), which reduces the interface available for diffusion of oxygen into, and carbon dioxide out of, the blood. Cross-sectional studies indicate that there exists an age-associated increase in dead-space ventilation (50), and a larger physiologic shunt due to an elevated closing volume (51,52). By increasing both dead-space ventilation and shunt perfusion, the aging lung exhibits increased ventilation-perfusion mismatching (53). Lastly, several studies have shown that the diffusion capacity of the lungs for carbon monoxide (DL_{CO}), a reliable indicator of global lung diffusion capacity through the alveolar-capillary membrane, declines progressively with age (54,55). This age-related decrease in DL_{CO} may indicate that oxygen transport could be diffusion-limited in elderly subjects (56). Consequently, the aforementioned age-related changes to the lung and pulmonary vasculature greatly reduce pulmonary gas exchange efficiency, as evidenced by a lower arterial partial pressure of oxygen (P_aO_2) (57), and a wider alveolar-arterial oxygen gradient (A-aDO₂) (58) in older subjects relative to their younger counterparts.

1.2.1.2 Capacity to generate flow

Arguably the most significant age-related change in the respiratory system is a decrease in lung elastic recoil pressure. The majority of the expiratory limb of the maximum expiratory flow-volume envelope (MEFV) is considered "effort independent" and the maximum expiratory flow at any given volume over this portion of the MEFV curve is determined by the elastic recoil pressure (59). Thus, any reduction in the elastic recoil has a direct influence on the capacity to generate expired airflow at a given lung volume (29,60,61). The rate of decline in elastic recoil pressure over the course of the healthy aging process is approximately 0.1-0.2 cmH₂O·yr⁻¹, with changes being the most apparent at higher lung volumes (29,30). The resultant effect is a progressive reduction in the capacity to generate flow, which is evidenced by size and shape of the MEFV curve (62).

1.2.1.3 Static lung volumes

The reduction in static recoil of the lung not only affects the capacity to generate expired flow, but also the static lung volumes of older individuals. The loss in the elasticity of the lung is partially due to a reduction in the supporting connective tissues, which decreases tethering of the small airways thereby leading to premature small airways collapse and a corresponding increase in closing volume (51,52). The increase in closing volume leads to air trapping, therefore aging is associated with an increase in residual volume (RV) (63). This leads to an upward shift in the equal pressure point of the lung; also known as functional residual capacity (FRC). Since total lung capacity (TLC) does not increase proportionately to the increase in RV, vital capacity (VC) is reduced (64). The net result is a significant reduction in the volumetric capacity to expand tidal volume (V_T) in comparison to younger individuals.

1.2.1.4 Respiratory mechanics

The pressure-volume relationship of the respiratory system, or compliance, is divided into two separate components, the lung and the chest wall. As previously mentioned, the lung undergoes age-related alterations of the alveoli, which has been shown to increase lung compliance (29). Conversely, the chest wall undergoes significant structural remodeling, which causes it to become stiffer, or have a lower compliance, than in youth (65). When one considers the alteration of the pressure-volume relationship of the respiratory system (i.e., the lung and chest wall combined), the compliance exhibits a linear decrease with advancing age (65), indicating that the increase in lung compliance is overridden by the greater decrease in chest wall compliance. Overall, the reduction in the compliance of the respiratory system implies that the pressure required for a given change in volume is increased, a burden which is placed on weakened respiratory muscles. Lastly, despite the age-related reduction in bronchial diameter and the associated increase in peripheral airway resistance (30), the total resistance of the respiratory system does not increase with advancing age (66).

1.2.1.5 Mechanical ventilatory response to exercise in healthy older individuals

During incremental exercise, minute ventilation (\dot{V}_E) increases in order to meet the body's rising metabolic demands. The pattern of response by which older individuals increase \dot{V}_E during exercise occurs in a similar fashion to that observed in younger individuals (67). At low to moderate exercise intensities, older individuals initially increase \dot{V}_E primarily by expanding V_T before it reaches a plateau equivalent to approximately 60% of VC (67). The expansion of V_T occurs via a reduction in end-expiratory lung volume (EELV) and an increase in end-inspiratory lung volume (EILV) (68). At higher intensities, further increases in \dot{V}_E are achieved through increases in breathing frequency (F_b) (67). Throughout exercise, the ventilatory timing variables (inspiratory and expiratory time) are also similar to those observed in younger individuals (14). However, healthy older individuals have a higher \dot{V}_E for a given absolute work rate than younger individuals (69,70), resulting from the age-related reduction in pulmonary gas exchange efficiency (56) and the relative maldistribution of ventilation within the lungs (71). The increased ventilatory response to exercise occurs in the face of a reduced capacity to generate expired flow, and by association, older individuals are susceptible to experiencing expiratory flow limitation (EFL), which occurs when expired flow ceases to increase despite increasing expiratory effort (72). Several studies have shown that EFL occurs more frequently and at lower exercise intensities in older individuals relative to younger individuals (73,74), and has even been found to occur at rest in some older individuals (75). In order to further increase \dot{V}_E in the presence of EFL or to avoid EFL altogether, breathing must take place at a higher lung volume, which permits access to higher expired flows. Accordingly, the onset of EFL during exercise in older individuals has also been shown to alter the regulation of operating lung volumes, as evidenced by an increase EELV and/or EILV (73). This relative hyperinflation places the respiratory muscles, particularly the diaphragm, at a suboptimal length for the generation of tension (40). Furthermore, if the increase in EILV is large enough, breathing may encroach on the less compliant portion of the pressure-volume relationship of the respiratory system, which is already less compliant as a result of alterations to the chest wall in older individuals (65). Overall, the work of breathing (W_b) during exercise is higher in older individuals than in younger individuals (76) due to the reduced respiratory system compliance, the excessive expiratory pressure generation associated with EFL (73), and the increases in operating lung volumes (77).

Since work and oxygen uptake ($\dot{V}O_2$) are linearly related, it follows that the increased W_b during exercise in older individuals is accompanied by an increased $\dot{V}O_2$ of the respiratory muscles ($\dot{V}O_{2rm}$) (78). It has been speculated that the higher mechanical and metabolic cost of breathing during exercise in the face of weakened respiratory muscles may precipitate an increased frequency and severity of respiratory muscle fatigue in older individuals (35).

Overall, the ventilatory response to exercise in older individuals can be viewed from the perspective of demand *versus* capacity, where the ventilatory demand of exercise encroaches on the finite capacity of the respiratory system (14). Due to the reduction in VC and the decreased ability to generate flow, older individuals have a smaller ventilatory reserve to accommodate increases in \dot{V}_E during exercise relative to younger individuals (62). Since aging increases the ventilatory response to exercise, older individuals incur a relatively greater ventilatory demand on their respiratory system for a given absolute exercise intensity compared to their younger counterparts. Thus, the margin between ventilatory capacity and ventilatory demand during exercise is progressively reduced over the course of the healthy aging process (4), as evidenced by the propensity towards EFL, increased operating lung volumes, and the high mechanical (i.e. W_b) and metabolic cost of breathing (i.e. $\dot{V}O_{2rm}$). The question then becomes, does the respiratory system limit exercise performance in the healthy older adult? Despite the increased mechanical ventilatory constraint to exercise hyperphoea in healthy older individuals, it is important to note that the respiratory system in healthy older individuals is generally capable of meeting the ventilatory requirements of exercise (14). This idea is based on three primary lines of evidence. First, when the elevated ventilatory equivalent for carbon dioxide ($\dot{V}_E/\dot{V}CO_2$) observed during exercise in older individuals relative to younger individuals is normalized to \dot{V}_A , the effect of age on the ventilatory response to exercise is no longer present (70). The absence of an effect of age on $\dot{V}_A/\dot{V}CO_2$ implies that older individuals increase \dot{V}_E appropriately for a given carbon dioxide output ($\dot{V}CO_2$). Second, in the majority of reported cases, healthy older individuals appear capable of maintaining blood gas homeostasis throughout exercise, as evidenced by measures of P_aO_2 and A-aDO₂ that are within values observed in young individuals (12,79). Lastly, when experimentally increasing the chemical drive to breathe (using normoxic hypercapnic gas) during incremental exercise, highly-trained older individuals are capable of increasing ventilation, even at physiological maxima (80).

1.2.2 Sex-differences in the human respiratory system

Though largely similar, it is well established that women exhibit several anatomic and physiologic differences when compared to men. These differences are of particular importance when studying the physiological response to exercise. For example, it is known that there are important sex-based differences with regard to thermoregulation (81,82), cardiovascular function (83) as well as substrate metabolism (84). Recently, important sex-based differences in the respiratory system, and their corresponding effects on the ventilatory response to exercise, have been identified (85).

1.2.2.1 Sex-differences in the structure of the respiratory system

It was initially observed that healthy individuals of similar age and lung size exhibited widely different maximum expiratory flow rates. It is thought that the majority of this variability can be explained by differences in airway size. Thus, individuals with large lungs do not necessarily have proportionally large airways. The concept that airway size is not directly linked to lung size, later termed 'dysanapsis', was first proposed by Green *et al.* in 1974 (86). Further

study using measures sensitive to lung size and measures sensitive to airway size led to the observation that for a given lung size, men appeared to have larger indices of airway size than women (87). Other methods have yielded similar findings; for example, measurements of acoustic reflectance have shown that the tracheal cross-sectional area is 29% smaller in women compared with men matched for TLC (88). More recently, airway luminal area has been quantified using computed tomography scans in older former smokers and it has been shown that women have ~14-31% smaller central airways than men when matched for lung size (19). In addition to smaller airways relative to lung size, women also have smaller absolute lung volumes than height- and age-matched men (89). The smaller absolute lung volumes in women are thought to be related to women having a smaller total number of alveoli, as demonstrated by post-mortem examination of lungs obtained from boys and girls aged 6 weeks to 14 years old (90). Collectively, these findings highlight important sex-differences in the anatomy of the human respiratory system that have the potential to profoundly affect the ventilatory response to exercise.

1.2.2.2 Functional consequences of sex-differences in respiratory system anatomy

Due to their smaller lung volumes and maximal flow rates, women exhibit significant mechanical ventilatory constraints to exercise hyperphoea (91). Recent studies have highlighted that; i) women have a higher propensity towards EFL, ii) are prone to breathing at higher operating lung volumes, and iii) have higher mechanical and metabolic cost of breathing (85).

It has been shown that endurance-trained women exhibit EFL more frequently during maximal exercise than endurance-trained males (11). The notion that women are more likely to reach the limits of their capacity to generate expired flow during exercise likely relates to their

smaller airways relative to their lung size (92), but could also be attributed to differences in fitness. Recently, we've shown that the propensity towards EFL is higher in women than in men when considering highly-trained young individuals, but that this sex-difference is absent in young untrained individuals (93).

During exercise, EELV decreases below resting values and remains as such throughout exercise, whereas EILV progressively increases up to approximately 90% of TLC (94). The fact that EFL is more prevalent in women than men, at least in highly-trained individuals, suggests that sex-differences in operating lung volumes during exercise may occur. The effect of sex on operating lung volumes has been assessed in several studies; however, the results are conflicting. In young individuals, previous studies have found that women have a higher EILV during submaximal exercise (94), and a higher EELV but a similar EILV during maximal exercise (11,93), while others found no sex-differences in operating lung volumes at any point during incremental exercise (95). Some of the variability in the literature is likely attributable to the notion that although EFL has been shown to increase operating lung volumes under experimental conditions (96), it does not guarantee that operating lung volumes will increase. For example, it is possible that in the presence of EFL, some individuals preserve relatively low lung volumes to avoid breathing on the flat portion of the pressure volume relationship of the respiratory system. Collectively, the majority of the available data relating the effect of sex on the regulation of operating lung volumes suggests that the constraints on ventilation during exercise increase the likelihood of observing a higher EELV and/or EILV during exercise in women relative to men.

Since lung volume and airways size greatly affect airflow resistance (97), it would be expected that women would have a higher W_b for any given \dot{V}_E . In their study of endurance-trained athletes, Guenette *et al.* showed that women have a higher W_b at any \dot{V}_E above 50-60

 $1 \cdot \min^{-1}$ (11). In a later study, Guenette *et al.* showed that the higher W_b in women could be attributed to the flow-resistive component of W_b (20), a finding that we have since corroborated in a large group of men and women with a wide range of fitness levels (93). It follows that women also have a higher oxygen cost of breathing ($\dot{V}O_{2rm}$) than men (98). The high W_b and $\dot{V}O_{2rm}$ associated with exercise hyperphoea in women has the potential to precipitate significant respiratory muscle fatigue. However, it has been shown that women exhibit less diaphragm fatigue than men following intense exercise to exhaustion (99). Indeed, it has been consistently shown that women exhibit significantly less skeletal muscle fatigue than men in response to fatiguing isometric and dynamic contractions of similar intensity (100). One proposed mechanism for the apparent resistance to diaphragm fatigue in women is that during exercise, women recruit their extra-diaphragmatic inspiratory muscles to a greater extent than do men, thereby avoiding excessive diaphragmatic work that would otherwise lead to fatigue. Support for this hypothesis comes from an electromyographical study performed in healthy young men and women during constant load exercise (101); however, it is unclear whether sex-differences in respiratory muscle recruitment patterns are present at lower exercise intensities, or in the absence of potential diaphragm fatigue.

Women have smaller lungs (91), smaller airways (19), and lower resting lung diffusion capacity (102) than men. It has therefore been suggested that women may be more likely to have gas exchange disturbances during exercise than men (103). Previous studies have shown that both trained and untrained women frequently develop exercise-induced arterial hypoxemia (EIAH) (104,105), owing to their increased mechanical ventilatory constraints which limit effective ventilation and result in relative alveolar hypoventilation (91). Though EIAH has been

documented in highly trained men (106), there are no published reports of healthy untrained men developing EIAH.

Overall, it has become increasingly apparent that sex is an important factor when considering the mechanical ventilatory response to exercise. As outlined above, recent studies have identified fundamental sex-based differences in pulmonary structure and function that have the potential to greatly influence the respiratory system's response to exercise. Indeed, research suggests that young women, free of respiratory disease, may be especially susceptible to mechanical ventilatory constraint during exercise (107).

1.2.3 Dyspnea

Dyspnea can be defined as "a subjective experience of breathing discomfort that consists of qualitatively distinct sensations that vary in intensity" (108), and is a cardinal symptom of patients with respiratory and cardiovascular disease, particularly during physical exertional. However, dyspnea is also a common sensory consequence of exercise in healthy individuals (109). While dyspnea is not the primary exercise-limiting symptom in healthy adults (110), the magnitude of dyspnea (usually measured using a standardized scale (111)) has been shown to increase in proportion to exercise intensity, \dot{V}_E , and respiratory muscle force output (109,112,113). Although the causes of exertional dyspnea are considered complex and multifactorial, a unifying hypothesis of the putative mechanisms of dyspnea during exercise has been proposed (114).

During exercise, \dot{V}_A increases in proportion to metabolic requirements of active muscles in order to ensure adequate delivery of oxygen and elimination of carbon dioxide. The resulting effect is a precise maintenance of arterial blood gases (PaO₂ and PaCO₂) and pH within a relatively narrow range, even during high intensity exercise (115). The regulation of exercise hyperphoea, although not fully understood, is the product of several highly coordinated and neurally-mediated adjustments, which are aimed at ensuring an appropriate ventilatory response for a given metabolic requirement (116). This process involves a great deal of sensory information that originates from a variety of sensory inputs, including the chemoreceptors (both central and peripheral), respiratory muscles, as well as lung and airway receptors. When stimulated, these afferent receptors send sensory information to the primary sensorimotor cortex, which is in turn transmitted to the sensory cortex in the form of a 'corollary discharge'. The sensory information provided to the sensory cortex is then thought to result in the sensation of dyspnea (117). In the context of an appropriate ventilatory response to exercise, the sensation of dyspnea is likely to reflect the awareness of the central respiratory motor output. However, in some instances, the feed-forward drive to breathe may not cause an appropriate ventilatory response. The resulting series of sensory, neural, and physiological events results in a mismatch between respiratory motor output and the mechanical response of the respiratory system, also known as neuromechanical uncoupling (NMU) (112). In this circumstance, the magnitude of dyspnea will be a reflection of the degree of NMU.

According the above summary, it is reasonable to speculate that an increased respiratory motor output in combination with a constraint on the mechanical output of the respiratory system would cause an increase in dyspnea. As summarized in sections 1.1 and 1.2, healthy aging and biological sex increase the magnitude of mechanical ventilatory constraint. Therefore, it possible, that older individuals and women, would experience higher levels of dyspnea during exercise due to differences in mechanical constraints relative to younger individuals and men respectively.

1.2.3.1 The effect of aging on dyspnea

Dyspnea is a common symptom in healthy older individuals, especially during physical exertion (118). The prevalence of activity-related dyspnea, measured using the Medical Research Council dyspnea questionnaire, has been estimated to be in the range of 23-32% in healthy individuals above the age of 70 (119,120). Moreover, a large cohort study involving 2792 subjects aged 65-85 years showed that the prevalence of dyspnea during activities of daily living was linearly related to age beyond 65 years (121). Although compelling, these findings are based on self-reported measures of dyspnea and do not account for differences in the degree of physical exertion that provokes the perception of activity-related dyspnea. Carefully conducted laboratory experiments, where the perception of dyspnea is evaluated using the Borg 0-10 scale (111) and exercise intensity was standardized, have indicated that aging causes a progressive increase in dyspnea for a given absolute work rate (109,122). Similar findings were observed during treadmill exercise, where healthy older individuals (60-80 years) reported higher levels of dyspnea at a standardized metabolic work load than younger individuals (40-59 years) (74). However, despite the wealth of observational data, our understanding of the mechanisms that cause the age-related increase in exertional dyspnea is incomplete. Nevertheless, some preliminary conclusions can be drawn from a limited set of recent studies.

Based on the summary provided in section 1.1, older individuals have an increased ventilatory response to a given absolute exercise intensity than younger individuals, and this increased ventilatory response encroaches on a significantly reduced ventilatory capacity resulting in a greater degree of mechanical ventilatory constraint. Additionally, the mechanical and metabolic cost of exercise hyperpnoea is increased in older individuals relative to younger

individuals, and must be accomplished by weakened respiratory muscles. Thus, based on our current understanding of the overall mechanisms of dyspnea, one can infer that the increased perception of dyspnea during exercise in older relative to younger individuals likely reflects; i) the perception of increased ventilation and respiratory muscle effort, and/or ii) the magnitude of mechanical ventilatory constraint (114). Support for this hypothesis is based on two important findings derived from studies comparing the perception of dyspnea during exercise in groups of healthy older individuals and younger individuals (74,109,122,123). First, when comparisons of the perception of exertional dyspnea between older and younger individuals are normalized for exercise intensity or maximal ventilation, the effect of age on dyspnea disappears (74,109,123). The notion that the slope of the dyspnea- \dot{V}_E relationship is preserved in older individuals implies that the increased perception of dyspnea is related to the age-related increase in \dot{V}_E for a given absolute exercise intensity rather than derangements in respiratory mechanics per se. Second, exertional dyspnea during exercise is significantly associated with indices of mechanical ventilatory constraint in healthy older and younger individuals (74). Although this second postulate is partially at odds with the first, experimental data in healthy young individuals supports the notion that increasing the magnitude of ventilatory constraint during exercise has a corresponding effect on the perception of dyspnea (124,125). However, precisely controlled experimental studies in older and younger individuals are required in order to confirm the hypothesis that the perception of exertional dyspnea is caused by an awareness of the age-related increase in ventilation and respiratory muscle work, alterations in respiratory mechanics, or both.
1.2.3.2 Sex-differences in dyspnea

In the general population, women have a higher prevalence and severity of activityrelated breathlessness than men (126,127). This observation is evident even when accounting for factors such as age, body mass index (BMI), smoking history, socioeconomic status, or the presence of respiratory disease (126,127). Laboratory-based studies in healthy young individuals (94,95), and healthy older individuals (74,109) have shown that women report higher levels of dyspnea for a given absolute exercise intensity than do men. The cause of this apparent sexdifference in the perception of breathlessness during exercise is thought to be related to sexdifferences in the structure and function of the human respiratory system. As outlined in section 1.2, women have smaller lungs, smaller-diameter airways, weaker respiratory muscles, and a lower surface area for pulmonary gas exchange when compared to height- and/or lung sizematched men. Consequently, women have a smaller ventilatory capacity available to respond to a given ventilatory demand and have a greater degree of mechanical ventilatory constraint than do men. Thus, for a given absolute exercise intensity, women utilize a higher fraction of their available ventilatory capacity, have a greater mechanical and metabolic cost of breathing, are more likely to experience EFL and potentially breathe at higher fractions of TLC. It follows that when comparisons of dyspnea between the sexes are made at a given absolute \dot{V}_E , women report higher levels of dyspnea than do men. However, if comparison between men and women are made at discrete fractions of maximal ventilation, the sex-differences in dyspnea during exercise disappear (74,94,95). Therefore, the increased exertional dyspnea in women relative to men likely reflects the increased perception of respiratory muscle work (*i.e.* W_b), required to achieve a given \dot{V}_E and the magnitude of mechanical ventilatory constraint. Nevertheless, our understanding of sex-based differences in the perception of dyspnea is still lacking. To date, only

a few observational studies have investigated sex-differences in dyspnea during exercise in healthy younger and older individuals (74,94,95,109). Clearly, additional work in required in both health and disease in order to determine the specific mechanisms that lead to the observed sex-differences in the perception of breathlessness.

1.3 Summary and rationale

Healthy aging causes a progressive reduction in pulmonary function that has an adverse effect on the mechanics of respiration at rest and during exercise (see section 1.1). Additionally, important sex-based differences in the structure of the human respiratory system that impact the ventilatory response to exercise have been identified in healthy young men and women (see section 1.2). Unfortunately, careful comparisons between men and women have been relatively few and by association our understanding of the respiratory system's response to exercise in women remains incomplete (128,129). This fact is even more evident when we consider the combined effects of biological sex and healthy aging on the ventilatory and sensory response to exercise, where most studies have been performed in older men. Given the detrimental effect of aging and the inherent effect of sex on the structure and function of the respiratory system, it stands to reason that healthy older women may be at particular risk of mechanical ventilatory constraints to exercise hyperphoea (18,107). One line of evidence that supports this hypothesis relates to the perception of dyspnea (see section 1.3). It been recently reported that dyspnea ratings are higher in women aged 60-80 years relative to age-matched men (74). Although the physiological underpinning for such differences remains unclear, one possibility is that combined effects of biological sex and healthy aging on the mechanics of respiration influence the perception of dyspnea during exercise. As previously described, older individuals are

predisposed to mechanical ventilatory constraints during exercise (13,77), as evidenced by a greater propensity towards EFL (4,73,75), increases in operating lung volumes, and a high W_b (14). Based on experimental data in humans (124,125,130,131), the above-mentioned factors all have the potential to be dyspneogenic (132). In light of the effect of sex on the mechanical ventilatory response to exercise, it is plausible that these differences in the sensory response to exercise are in fact linked to the previously observed sex-based differences in the structure and function of the respiratory system that have been exacerbated by the healthy aging process.

1.4 Purpose

The primary purposes of this thesis were twofold; i) to comprehensively examine the combined effects of healthy aging and biological sex on respiratory mechanics and the perception of dyspnea during exercise in healthy adults, and ii) to determine if sex-differences in mechanical ventilatory constraint influence the perception of dyspnea in healthy older men and women. In order to address these purposes, two separate studies (outlined in Chapters 2, 3, and 4) were performed. Chapters 2 and 3 outline the results from the first study, while Chapter 4 outlines the results of the second study.

1.5 Research questions

The three studies contained within this thesis were designed to address three distinct but interrelated research questions:

1. Do older women experience a greater magnitude of mechanical ventilatory constraint and higher levels of dyspnea than older men during incremental exercise?

- 2. Do healthy aging and biological sex have an effect on inspiratory muscle recruitment patterns during incremental exercise?
- 3. Is the increased level of dyspnea in older women relative to older men caused by sexdifferences in the magnitude of mechanical ventilatory constraint during exercise?

The first study of this thesis was designed to address research question #1 (outlined in Chapter 2) and research question #2 (outlined in Chapter 3). The second study of this thesis was designed to address research question #3 (outlined in Chapter 4).

1.6 Hypotheses

For each of the three research questions above (see section 1.6), our corresponding hypotheses were as follows:

- 1. We hypothesized that regardless of age, women would have a higher W_b for a given \dot{V}_E , breathe at higher operating lung volumes, and have a higher propensity towards EFL during exercise than men. We also hypothesized that older women would have a higher perception of dyspnea during exercise at a given workload than older men.
- 2. We hypothesized that regardless of age, women would rely on extra-diaphragmatic inspiratory muscles to a greater extent during exercise than men.
- 3. We hypothesized that acutely increasing or decreasing the degree of mechanical ventilatory constraint during moderate-intensity exercise in older individuals would lead to a corresponding change in the perception of dyspnea. We further hypothesized that the effect of manipulating mechanical ventilatory constraint on dyspnea during exercise would be more pronounced in older women than in older men.

Chapter 2: Independent and combined effects of age and sex on mechanical ventilatory constraint and dyspnea during exercise in healthy humans

2.1 Introduction

The normative aging of the respiratory system involves significant structural changes to the lungs, airways, chest wall, and respiratory muscles (14), leading to a progressive decline in pulmonary function (61). Consequently, when compared to individuals 20-30 years of age, those above the age of 60 have a reduced ventilatory capacity, as reflected by the size and shape of their MEFV curves (62). It follows that older individuals have a reduced reserve for accommodating increases in ventilatory demand during dynamic exercise (133). Moreover, the ventilatory response to exercise at a given absolute work rate is higher in older individuals relative to their younger counterparts (69). Thus, in older individuals it is possible that the ventilatory demand of exercise meets or even exceeds the maximum ventilatory capacity of the respiratory system, resulting in mechanical ventilatory constraint. Several indices can be used to determine the presence and magnitude of mechanical ventilatory constraint during exercise such as W_b, changes in operating lung volumes, and the presence of EFL (134). Healthy aging of the respiratory system is associated with a progressive increase in mechanical ventilatory constraint to exercise hyperphoea (4), as evidenced by a higher W_b for a given \dot{V}_E , an increase in EELV, and a higher propensity towards EFL (73,77).

Along with aging, biological sex is an important factor when considering the mechanical ventilatory response to exercise. When matched for height, women have smaller lungs and lower maximum expiratory flows than men (6). Even when matched for lung size, women have smaller large conducting airways than men - a concept known as dysanapsis (19). Given the

aforementioned sex-differences in lung size, airway size, and expiratory flow rates, healthy young women appear to be predisposed to greater mechanical ventilatory constraint during exercise compared to men. We recently demonstrated that during exercise, young women have a higher W_b for a given \dot{V}_E (11,93) and a higher oxygen-cost of breathing for a given \dot{V}_E than young men (98). It has also been shown that there are sex-differences in the regulation of operating lung volumes, where young women tend to breathe at a higher relative EILV for a given submaximal work rate and \dot{V}_E (94), and a higher relative EELV at maximal exercise than young men (93). Furthermore, EFL appears to be more common in young endurance-trained women than in their male counterparts (11).

There is growing evidence suggesting that the magnitude of exertional dyspnea increases during the healthy aging process (114). For example, during cycle exercise, older men and women report a higher intensity of dyspnea for a given absolute work rate than younger men and women (118). Additionally, older women report a higher intensity of dyspnea during exercise at a standardized $\dot{V}O_2$ than older men; this is thought to be related to the interaction between the effects of age and sex on ventilatory constraint (74). Indeed, when the magnitude of ventilatory constraint is experimentally increased during exercise, the perception of dyspnea is increased concomitantly (124). Given the effects of age and sex on the mechanical ventilatory response to exercise, it can be surmised that the sex-differences in exertional dyspnea noted in older individuals may be explained, at least in part, by mechanical ventilatory constraint. However, it is unknown if sex-based differences in the mechanical ventilatory response to exercise observed in young individuals persists in older individuals.

Several studies have investigated the independent effects of age (73,77,118) and sex (11,93,94) on the mechanical ventilatory and perceptual responses to exercise. However, to our

knowledge, no study has assessed the combined and potentially interactive effects of age and sex on W_b , operating lung volumes, and EFL during exercise, nor how they relate to dyspnea. Accordingly, the primary aim of the present study was to determine if sex-based differences in the mechanical ventilatory response to exercise in younger adults are present in older individuals. A secondary aim was to determine if indices of mechanical ventilatory constraint are related to sex-differences in dyspnea during exercise in older individuals. Based on the above summary, our hypotheses were threefold: i) that older women have a higher W_b for a given \dot{V}_E than older men, ii) that older women would exhibit a greater increase in relative EELV during exercise than older men, and iii) that older women would exhibit EFL more frequently than older men. We also hypothesized that, across all subjects, indices of mechanical ventilatory constraint would be significantly correlated with dyspnea intensity during exercise.

2.2 Methods

Subjects. After providing written informed consent, 22 older men and women (60-80 y, n=12 women) and 22 younger men and women (20-30 y, n=11 women) participated in the study. All subjects had normal pulmonary function based on predicted values (6-9). Additional inclusion criteria were: a BMI of 18-30 kg·m⁻², peak aerobic power \geq 80% predicted, and no evidence of respiratory disease. Subjects were excluded if they were current smokers or had previously smoked >5 pack-years, had a history or current symptoms of cardiorespiratory disease, or any contraindications to exercise testing. Eight of twenty-two older subjects (n=4 men, n=4 women) had previously smoked <5 pack/years, all of whom had quit smoking >25 y prior to their participation in the current study. All healthy younger subjects had never smoked. Subjects were divided into 4 groups based on sex and age: younger women (20-30 y), younger men (20-30 y),

older women (60-80 y), older men (60-80 y). All study procedures were approved by the University of British Columbia Providence Health Care Research Ethics Board, which adheres to the *Declaration of Helsinki*.

Experimental overview. Subjects completed two days of testing separated by a minimum of 48 h. On Day 1, anthropometric measurements were taken, followed by detailed pulmonary function testing and a symptom-limited incremental cycle exercise test. The incremental exercise test performed on Day 1 was intended to familiarize subjects with the exercise protocol. On Day 2, subjects were instrumented with a balloon catheter that was passed through the naris following the application of a topical anesthetic (Lidocan® endotracheal spray, Odan Laboratories, Montreal, QC, Canada) in order to measure esophageal pressure. Following instrumentation, lung static recoil pressure at TLC (P_{st 100%TLC}) and static lung compliance were assessed using standardized techniques as previously described (29,135). Subjects then performed a maximal incremental cycle exercise test using the same protocol as on Day 1. During the incremental exercise test, EFL was assessed using the negative expiratory pressure (NEP) technique (see *Expiratory Flow Limitation*). On Day 2, subjects performed a series of forced vital capacity (FVC) maneuvers at different efforts before and after exercise in order to construct MEFV curves by taking into account exercise-induced bronchodilation and thoracic gas compression (136). All reported resting pulmonary function data, apart from P_{st 100%TLC} and lung compliance were obtained on Day 1, whereas all reported exercise data were obtained on Day 2.

Pulmonary function testing. Spirometry, whole-body plethysmography, single breath diffusing capacity of the lungs for carbon monoxide, maximum voluntary ventilation, and maximum

inspiratory and expiratory pressures were assessed using a commercially available system (Vmax Encore 229, V62J Autobox; CareFusion, Yorba Linda, CA) according to standard recommendations (137-140). Pulmonary function measurements were expressed as absolute values and as percentages of predicted (6-9).

Exercise protocol. Exercise testing was conducted on an electronically braked cycle ergometer (Ergoselect 200P; Ergoline, Bitz, Germany). Each test began with a 6 min rest period followed by 1 min of unloaded pedaling then 20 W step-wise increases in workload (starting at 20 W) every 2 min until volitional exhaustion. The exercise protocol was selected in order to allow for comparisons between groups at discrete work rates. Peak work rate was defined as the highest work rate sustained for at least 30 s.

Flow, volume and pressure. During the incremental cycle exercise test on Day 2, subjects breathed through a low resistance (0.3-0.7 cmH₂O $1^{-1} \cdot s^{-1}$ at 0.5-8 $1 \cdot s^{-1}$) circuit with minimal dead-space (130 ml). Bi-directional flow was measured using a heated, calibrated pneumotachograph (model 3813, Hans Rudolph, Kansas City, MO, USA). Volume was obtained by numerical integration of the flow signal. Mouth pressure was sampled through a port in the mouthpiece while esophageal pressure was measured using an esophageal balloon catheter (Guangzhou Yinghui Medical Equipment Ltd, Guangzhou, China). Placement of the catheter was performed as previously described (141), with 0.5 ml of air placed into the esophageal balloon. Validity of the esophageal balloon pressure was verified by performing an occlusion test, as previously described (142). Mouth pressure (P_{mo}) and esophageal pressure (P_{eso}) were measured using independent, calibrated differential pressure transducers (DP15-34, Validyne Engineering,

Northridge, CA, USA). Flow, volume, and pressures were composite averaged by selecting breaths within a 30 s epoch during rest and at the end of each exercise stage.

Cardiorespiratory responses. Standard cardiorespiratory measures were recorded on a breath-bybreath basis and averaged over 30 s periods at rest and during exercise. In the younger subjects, heart rate was measured using a heart rate monitor (Polar T34; Polar Electro, Kempele, Finland). In the older subjects, heart rate and electrocardiogram changes were monitored continuously using a 12-lead electrocardiogram (Cardiosoft Diagnostics System v6.71, GE Healthcare, Canada) and arterial oxygen saturation was measured using a pulse oximeter (Radical-7, Massimo Corporation, Irvine, CA, USA). Inspiratory capacity maneuvers were performed at rest and at the end each exercise stage. Operating lung volumes (EELV and EILV) were derived from the inspiratory capacity maneuvers (143). Theoretical maximum ventilation (\dot{V}_{ECAP}) was calculated at rest and for each exercise stage based on the maximum expiratory airflow throughout a composite averaged tidal breath at a given lung volume as previously described (144). Fractional utilization of available ventilatory capacity (\dot{V}_E/\dot{V}_{ECAP}) was determined as the quotient of \dot{V}_E and \dot{V}_{ECAP} .

Work of breathing. W_b was determined by integrating the area within an composite averaged tidal esophageal pressure–volume loop (145). For each subject, W_b data were plotted as a function of \dot{V}_E . To compare the independent effects of age, sex, and their interaction on W_b for a given \dot{V}_E , curves were fit to each individual subject's data according to the following equation (11):

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

where, for a given \dot{V}_E , $a\dot{V}_E^3$ represents the resistive component of W_b and $b\dot{V}_E^2$ represents the viscoelastic component of W_b . To determine if *a* and *b* are affected by sex and/or age, we used a similar model as previously described (93), but with the addition of a term for age:

$$W_b^{(i,j)} = (a + \alpha_i + tS + qA)\dot{V}_E^3 + (b + \beta_i + uS + rA)\dot{V}_E^2 + \varepsilon \qquad (eq. 2)$$

where $W_b^{(i,j)}$ is the jth observation taken on the ith individual; α_i and β_i are subject level random effects; S=0 for men and 1 for women; *t* and *u* are the sex effects influencing W_b ; A=0 for younger subject and 1 for older subjects; *q* and *r* are the age effects influencing W_b ; and ε denotes the independent, normally distributed measurement error. By using this statistical framework, the subject level effects (i.e., α_i and β_i) allow each individual to have different coefficients for \dot{V}_E^3 and \dot{V}_E^2 , respectively, and simultaneously permit population level estimates of the population level parameters *a*, *b*, *t*, *u*, *q*, and *r*. The model in *eq. 2* was fit to the data using commercially available software (JMP, Version 11.2, SAS Institute, Cary, NC, USA) via restricted maximum likelihood.

Expiratory flow limitation. At rest and for each stage of exercise on Day 2, EFL was determined using the NEP technique (11,146). Briefly, the NEP technique involves the generation of a negative pressure at the mouth during the expired portion of a breath. Negative pressure was achieved using a Venturi device (207A, Raytech Instruments, Vancouver, BC, Canada) attached to the distal portion of the pneumotachograph. The Venturi device was connected to a tank of compressed air and controlled by an electronically triggered solenoid valve connected to a computer. Bespoke software was used to detect the onset of expiration then rapidly trigger negative mouth pressure, and immediately terminate it at the end of the expiration. The desired negative pressure was set at approximately -5 to -10 cmH₂O, and developed within 50 ms of the 32

onset of the expired breath. A control flow-volume loop was created by composite averaging the 3 tidal breaths immediately prior to each NEP breath to represent spontaneous patterns of flow and volume at a given stage during the exercise test. The raw expired flow-volume loop of the NEP breath was then overlaid on the corresponding control flow-volume loop based on the assumption that end-inspiratory volumes of the control breath and the NEP breath are identical (146). Expiratory flow limitation was considered present when the NEP breath overlapped with the expired portion of the control breath.

The most common method of detecting EFL during exercise involves the placement of tidal flow–volume loops within a maximum flow–volume loop (144). However, we chose to use the NEP technique for two reasons: First, the NEP technique does not require forced expiratory efforts or the correction for the effects of thoracic gas compression and bronchodilation. Second, the volume history of the control expiration and the subsequent expiration with the negative expiratory pressure is identical.

Perceptual responses. At rest and during the last 30 s of each 2 min exercise stage, subjects rated the intensity of "breathing discomfort" (dyspnea) and "leg discomfort" using the modified category-ratio 0–10 Borg scale (111). Dyspnea was defined as "the sensation of labored or difficult breathing" and leg discomfort was defined as the "sensation of leg muscle fatigue". The endpoints of the scale were anchored such that 0 represented "no breathing/leg discomfort" and 10 represented "the most severe breathing/leg discomfort ever experienced or imagined".

Data processing. All data (see *Flow, Pressure and Volume*, and *Cardiorespiratory Responses*) were collected using a 16-channel analogue-to-digital data acquisition system (PowerLab 16/35,

ADInstruments, Colorado Springs, CO, USA), sampled at 2000 Hz, and recorded using LabChart 7.3.7 software.

Statistical analysis. Descriptive characteristics, pulmonary function data, and maximal exercise data were compared using a 2x2 analysis of variance for age and sex differences between the four groups. In the case of a significant interaction between age and sex, four pairwise comparisons were performed with Bonferroni corrections where appropriate (older men vs. older women, younger men vs. younger women, older men vs. younger men, older women vs. younger women). To determine the effect of age and sex as well as their interactions on W_b for a given absolute \dot{V}_E , each subject's W_b equation (eq. 1) was solved for successive independent variables in 5 1 min^{-1} increments up to the highest \dot{V}_E achieved by any single subject (200 1 min^{-1}), and compared using a mixed-effect model analysis of variance. In the case of a significant two-way interaction between \dot{V}_E and age, \dot{V}_E and sex, or a significant three-way interaction between \dot{V}_E , age and sex, pairwise comparisons with Bonferroni corrections were performed where appropriate. The same analysis was performed in order to determine the effect of age and sex as well as their interactions on W_b at relative fractions of the maximal \dot{V}_E achieved during exercise for each subject in 5% increments from 5% to 100%. We performed a mixed-effect model repeated-measures analysis using generalized estimating equations to evaluate the main effects of age and sex as well as their interaction between groups and work rate (rest, 20 W, 40 W, 60 W, 80 W, and maximal exercise) on EFL at rest and during exercise. Cardiorespiratory and perceptual variables were compared at rest and at absolute submaximal work rates up to the highest equivalent work rate achieved by all subjects using a mixed model analysis of variance. In the case of a significant two-way interaction between work rate and age, work rate and sex, or

a significant three-way interaction between work rate, age and sex, Bonferroni-adjusted post-hoc comparisons were conducted where appropriate. This analysis was repeated in order to determine the effect of age and sex as well as their interactions on dyspnea and operating lung volumes at relative work rates (i.e. at rest, 20%, 40%, 60%, 80%, and 100% of peak work rate). Relationships between dyspnea and possible physiological contributors were determined using the Pearson product moment correlation analysis was used to determine the relationship between dyspnea and possible physiological contributors. For all analyses, the level of statistical significance was set at p<0.05. All data are presented as means±SE unless otherwise noted.

2.3 Results

Subjects. Subject characteristics and pulmonary function data are shown Table 2-1. Resting pulmonary function was within the normal predicted range for all groups. As expected, there were significant age-related differences in maximum expiratory flows, lung volumes (with the exception of TLC, p=0.97), diffusing capacity, and respiratory muscle strength (all p<0.05). Furthermore, when expressed in absolute terms, the majority of pulmonary function measures were greater in men than women (all p<0.05), with the exception of the ratio of forced expired volume in 1 s and forced vital capacity (FEV₁/FVC) (p=0.81), forced expired flow between 25% and 75% of forced vital capacity (FEF_{25-75%}) (p=0.23), and residual volume (p=0.14). However, when measures of pulmonary function were expressed as a percentage of predicted normal values, there were no significant differences between groups (all p>0.05).

Peak exercise data are shown in Table 2-2. At peak exercise, there was a significant effect of age and sex on absolute $\dot{V}O_2$, work rate, \dot{V}_E , the ventilatory equivalent for carbon

dioxide, \dot{V}_{ECAP} , and W_b (all p<0.05). When $\dot{V}O_2$ at peak exercise was expressed as a percent of predicted values, there was no significant effect of age or sex, indicating that subjects had statistically similar levels of relative fitness. Independent of sex, there was a significant effect of age on heart rate, breathing frequency, the ventilatory equivalent for oxygen, the ventilatory equivalent for carbon dioxide, and EELV (all p<0.05). Independent of age, there was a significant effect of sex on V_T (p<0.05). There were no significant interaction effects between age and sex at peak exercise. On average, subjects in each group achieved respiratory exchange ratios >1.10 and near maximum heart rates based on predicted normal values, indicating that maximal effort was exerted across groups. There were no significant differences in the $\dot{V}O_2$ -work rate slopes between groups on the basis of age or sex (Table 2-2; both p>0.05).

Maximum ventilatory capacity. Figure 2-1 shows composite average resting tidal flow-volume loops and MEFV curves for all older and younger women (panel A), as well as all older and younger men (panel B). While there was no significant effect of age on TLC (Table 2-1), older subjects had a reduced capacity to generate expired flow within the "effort-independent" range, as evidenced by a significantly lower FEF_{25-75%} (Table 2-1, *p*<0.05). Independent of sex, older subjects had lower $P_{st 100\%TLC}$ (*p*<0.001) (Figure 2-1, panels C and D), and there was a significant linear correlation between FEF_{25-75%} and $P_{st 100\%TLC}$ (*r*²=0.34, *p*<0.001).

Ventilatory response to exercise. Ventilatory responses to exercise are shown in Figure 2-2. Independent of age, women had a significantly lower V_T and a higher breathing frequency than men at a given submaximal absolute work rate (all p < 0.05). Regardless of sex, older subjects had a higher \dot{V}_E than younger subjects for given submaximal work rate (all p < 0.05). Accordingly, the

ventilatory equivalents for carbon dioxide and oxygen were consistently elevated in older subjects at rest and throughout exercise (all p < 0.05). For all variables shown in Figure 2-2, there were no significant interaction effects between age and sex (all p > 0.05).

Absolute and fractional utilization of \dot{V}_{ECAP} at rest and during exercise are shown in Figure 2-3. Given that older subjects had a reduced capacity to generate expired flow (Figure 2-1), their available \dot{V}_{ECAP} was significantly lower at rest and throughout exercise (all *p*<0.05, Figure 2-3, panel A). Furthermore, given their increased ventilatory response for a given workload (Table 2-2), older subjects had a higher \dot{V}_E/\dot{V}_{ECAP} at rest and across exercise intensities than younger individuals (all *p*<0.05, Figure 2-3, panel B). Additionally, there was a significant effect of sex on \dot{V}_{ECAP} , with women having a lower \dot{V}_{ECAP} than men at rest and across exercise intensities intensities (all *p*<0.05). However, there was no significant effect of sex on \dot{V}_E/\dot{V}_{ECAP} at any time point (all *p*>0.05).

Operating lung volumes at rest and during exercise are shown in Figure 2-4. There was a significant effect of sex (p<0.05) but not age (p=0.36) on absolute EELV and EILV, whereby women had lower EELV and EILV at rest and throughout exercise (Figure 2-4, panels A and B). However, when normalized for TLC, EELV and EILV were not significantly different on the basis of sex (p=0.29), but were significantly higher in older relative to younger subjects at rest and throughout exercise (Figure 2-4, panels C and D, p<0.05). The effect of sex on absolute EELV and EILV, as well as the effect of age on relative EELV and EILV were present at rest and throughout exercise when comparisons were made at relative exercise intensities (all p<0.05).

Work of breathing. Individual subject values for the W_b as a function of \dot{V}_E are shown in Figure 2-5 (panels A and B). Each subject's W_b- \dot{V}_E curve was fitted to *eq. 1*, and without exception there was excellent fit (mean r²: 0.99±0.01). Then, by pooling each individual's constant *a* and constant *b* from *eq. 1*, a mean curve was constructed for each group (Figure 2-5, panel C). There were significant main effects of \dot{V}_E , sex, and age on W_b (all *p*<0.001). There was a significant interaction between \dot{V}_E and sex, as well as \dot{V}_E and age (both *p*<0.001), but no significant effect of the interaction between \dot{V}_E , sex, and age (*p*>0.05). W_b was significantly higher in women at and above a \dot{V}_E of 65 1·min⁻¹ (*p*<0.001), and significantly higher in older subjects at and above a \dot{V}_E of 60 1·min⁻¹ (*p*<0.001). When W_b was compared at relative fractions of peak exercise \dot{V}_E , there was no significant effect of age or sex at any submaximal \dot{V}_E (both *p*>0.05). However, at peak exercise men had a significantly higher W_b than women, and older individuals had a significantly lower W_b than younger individuals (both *p*<0.05, Table 2-2).

The W_b- \dot{V}_E data were also fit to the model outlined in *eq. 2*, the results of which are summarized in Table 2-3, where marginal *p*-values are reported for the fixed effects. The overall model fit had an adjusted r²>0.97. To identify the significant effects within our model, we used the same approach as previously described (93). Briefly, we used both forward and backwards selection inferential procedures. For the forward selection procedure, we started with the base model (*eq. 1*), but with the addition of individual level random effects (α_i and β_i). We then successively added individual parameters to the model, and kept only the parameter with the smallest marginal *p*-value. This process was repeated in a step-wise fashion until either all parameters had been added or there were no significant parameters to add. For the backward selection procedure, we started with the model outlined in *eq. 2*, and removed the individual parameter with the highest marginal *p*-value above *p*=0.05. This process was repeated

successively until either all the parameters were removed or there were no parameters with a marginal *p*-value above p=0.05 to remove. The model selected by the forward and backward selection procedures was:

$$W_b^{(i,j)} = (\alpha_i + tS)\dot{V}_E^3 + (\beta_i + rA)\dot{V}_E^2 + \varepsilon \qquad (eq. 3)$$

The forward selection and backward selection procedures arrived at the same final models, which demonstrates that *t*, and *r* were significant effects (*p*<0.001 at each stage of the forward and backward selection procedures). The results of our analysis indicate that there was a significant effect of sex (S) on the resistive component of W_b, represented by \dot{V}_{E}^{3} , but no significant effect of age (A). Also, age (A) but not sex had a significant effect on the viscoelastic component of W_b, represented by \dot{V}_{E}^{2} .

Expiratory flow limitation. Examples of flow-volume loops in four individuals (one representative sample from each group) used to determine the presence of EFL using the NEP technique are shown in Figure 2-6. Successful NEP maneuvers were obtained at rest and at each exercise stage in all but two subjects (n=1 older woman, and n=1 younger woman) whose data were excluded from the analysis since the application of the NEP caused a sustained decrease in expiratory flow relative to the control breath. The frequency of EFL in each group at rest and throughout exercise is shown in Figure 2-7. No subjects had EFL at rest, but as exercise intensity increased the fraction of subjects who had EFL increase progressively. Based on our model, there was a significant effect of age, sex, and their interaction on EFL (all p<0.05). When the analysis was repeated at relative exercise intensities, there were significant effects of age and sex (both p<0.05); however, there was no significant interaction effect between age and sex (p=0.39).

Dyspnea. Figure 2-8 shows dyspnea intensity ratings in all groups at rest and during exercise. There was a significant effect of age, sex, and their interaction on dyspnea during exercise (all p < 0.05). At 80 W, the difference in dyspnea between younger men and younger women were subtle, albeit significant (0.1 \pm 0.1 versus 0.7 \pm 0.2, p<0.001). By contrast, older women reported significantly higher dyspnea at 80 W than older men by 1.3 Borg units $(0.6\pm0.2 \text{ versus } 1.9\pm0.4,$ p < 0.001). Moreover, Dyspnea/ \dot{V}_E slopes showed a significant effect of age (0.093 vs. 0.065 Borg units l^{-1} min⁻¹, p < 0.05) and sex (0.092±0.006 vs. 0.064±0.010 Borg units l^{-1} min⁻¹, p < 0.05), but not their interaction (p=0.52). Similarly, dyspnea/ \dot{VO}_2 slopes showed a significant effect of age $(5.4\pm0.7 \text{ vs. } 3.2\pm0.3 \text{ Borg units} \cdot 1^{-1} \cdot \text{min}^{-1}, p < 0.05)$ and sex $(5.3\pm0.7 \text{ vs. } 3.1\pm0.3 \text{ Borg units} \cdot 1^{-1} \cdot \text{min}^{-1}, p < 0.05)$ ¹·min⁻¹, p < 0.05), but not their interaction (p=0.41). Correlates of dyspnea intensity at a standardized absolute work rate of 80 W are shown in Table 2-4. The four strongest correlates of dyspnea intensity at 80 W were W_b , \dot{V}_E/\dot{V}_{ECAP} , breathing frequency, and \dot{V}_E . There was excellent agreement ($r^2=0.85$, p<0.001) between dyspnea at any given work rate during the incremental exercise test on Day 1 and the incremental exercise test on Day 2, indicating the esophageal balloon catheter had a negligible impact on the perception of breathing discomfort. Finally, when dyspnea was compared between groups at relative exercise intensities, there was no significant effect of sex or age (both p < 0.05).

2.4 Discussion

Major findings. We assessed the effects of age and sex on the mechanical ventilatory and perceptual responses to exercise in healthy individuals. Our study is the first to determine whether sex-differences in the mechanical ventilatory response to exercise are influenced by the

healthy aging process. The major findings are four-fold. First, women have a higher W_b for a given \dot{V}_E during exercise compared men, regardless of age. Second, regardless of sex, older individuals breathe at higher relative lung volumes during exercise at a given submaximal absolute work rate. Third, EFL occurs more frequently during exercise in older women than in older men. Finally, dyspnea during submaximal exercise is associated with indices of mechanical ventilatory constraint. Collectively, our findings suggest that sex-based differences in the mechanical ventilatory response to exercise previously observed in healthy young individuals are also present in healthy older individuals. Our data also provide support for the notion that mechanical ventilatory constraint may explain, at least in part, the sex-differences in dyspnea during exercise in older individuals.

Maximum ventilatory capacity and ventilatory response to exercise. The primary age-related change to the respiratory system that contributes to decreasing pulmonary function is thought to be the progressive decrease in elastic recoil pressure of the lung (147). As the elastic recoil pressure of the lung decreases, so too does the ability to generate expired flow, thereby reducing maximum ventilatory capacity (148). As expected, older individuals had a significantly lower P_{st} 100%TLC than younger individuals (Figure 2-1, panels C and D). It follows that despite similar TLC, older individuals had a reduced capacity to generate expiratory flow (Figure 2-1, panels A and B), as evidenced by significantly lower mid-expiratory flows than younger individuals (Table 2-1). Accordingly, we observed a significant linear correlation between $P_{st 100\%TLC}$ and FEF_{25-75%}. However, it should be noted that we did not detect statistical differences in $P_{st 100\%TLC}$ or FEF_{25-75%} on the basis of sex. While the effect of aging on the static recoil pressure of the lung is well characterized (29), evidence of sex-differences in static recoil pressure of the lung

remains equivocal. Indeed, some authors have reported that older and younger women have lower static recoil pressure at any given fraction of TLC than men (147), while others report no sex-differences in older individuals or younger individuals (149). Overall, the age-related decline in ventilatory capacity observed in our study resulted in a reduction in the available reserve for accommodating increases in ventilatory demand, as evidenced by a significantly lower \dot{V}_{ECAP} at rest and throughout exercise in the older relative to the younger individuals (Figure 2-3). Moreover, due to their relatively smaller lungs, women had a lower \dot{V}_{ECAP} than men at rest and throughout exercise.

During exercise, older individuals had a higher \dot{V}_E for a given absolute work rate above 20 W than younger individuals (Figure 2-2, Panels E and F), a finding that is in agreement with previous work (69). In keeping with previous studies in older (150) and younger individuals (94), we demonstrate that women have a tendency to adopt a relatively more rapid and shallow breathing pattern than men for a given absolute work rate above 20 W (Figure 2-2). Sexdifferences in breathing pattern were not affected by age, and are likely related to inherent differences in lung size and the associated constraints on V_T expansion (91). When coupled with the age-related decline in \dot{V}_{ECAP} , the higher ventilatory response to exercise in older individuals increases the likelihood of reaching the mechanical limits of the respiratory system at a given absolute work rate. Indeed, older individuals utilized a greater fraction of their available ventilatory capacity (\dot{V}_E/\dot{V}_{ECAP}) at rest and during exercise than younger individuals (Figure 2-4).

Operating lung volumes. Several studies have described the changes in operating lung volumes during exercise in healthy young adults (94,151) and healthy older adults (4,73). In most young adults, EELV decreases below resting FRC and remains as such throughout exercise, whereas

EILV progressively increases up to approximately 90% of TLC (94). These changes in operating lung volumes serve to optimize the ventilatory response to exercise: the decrease in EELV increases the length of the diaphragm, while the increase in EILV allows access to higher expiratory flows at higher lung volumes. In our study, we found that younger individuals followed the pattern of reductions in EELV and increases in EILV up to 90% of TLC (Figure 2-4, panel D). In older individuals, the age-related reduction in vital capacity and expiratory flows results in operating lung volumes that are shifted to higher fractions of TLC (74). We found that older individuals had a higher EELV throughout exercise and a higher EILV during submaximal exercise than the younger individuals (Figure 2-4, panel C). We also observed that EELV decreased during exercise and remained below resting EELV. Like their younger counterparts, most older individuals reduce EELV during exercise until they approach EFL, at which point EELV may begin to increase back towards resting EELV in order to avoid excessive mechanical constraint (73). In some cases, EELV continues to increase to the extent where it exceeds resting EELV, a phenomenon known as dynamic hyperinflation (152). We did observe that 7 of 22 (n=3 men, n=4 women) older individuals showed evidence of dynamic hyperinflation at maximal exercise, which we defined as an increase in EELV >0.15 l above resting EELV. None of the younger individuals hyperinflated during exercise. Although EILV was higher in the older individuals than the younger individuals during submaximal exercise, EILV was similar between age groups at maximal exercise. The fact that, regardless of age, the highest EILV reached during exercise was approximately 90% is likely due to the sigmoidal shape of the pressure-volume relationship of the respiratory system, whereby any further increase in EILV would substantially increase W_b. Overall, it appears that on average older individuals regulate their operating lungs volumes during exercise in a similar manner to

younger individuals, but at a higher fraction of TLC. However, the increase in EILV is limited due to the age-related reduction in inspiratory reserve volume.

It can be argued that because women have smaller lungs and lower maximum expired flows than men, they have the tendency to breathe at a higher EELV and EILV during exercise. The effect of sex on operating lung volumes has been assessed in several studies, but the results are conflicting. In young individuals, previous studies have found that women have a higher EILV during submaximal exercise (94), and a higher EELV but a similar EILV during maximal exercise (11,93), while others found no sex-differences in operating lung volumes at any point during incremental exercise (95). In older individuals, the available evidence is equally variable. Delorey & Babb (4) found that EELV was consistently higher at rest and during exercise in women than in men. Conversely, another study found that older women had lower EELV and EILV than older men during exercise (150). In the current study, we did not observe a systematic effect of sex on operating lung volumes when EELV and EILV were expressed as a fraction of TLC. While it is tempting to hypothesize that women are more likely to increase EELV and/or EILV during exercise to avoid EFL, we believe that this is an oversimplification. Although EFL has been shown to increase operating lung volumes under experimental conditions (96), the fact that an individual exhibits EFL does not guarantee that operating lung volumes will increase. For example, it is possible that in the presence of EFL, some individuals preserve relatively low lung volumes to avoid breathing on the flat portion of the pressure volume relationship of the respiratory system.

Work of breathing. The mechanical and metabolic cost of maintaining adequate \dot{V}_A during exercise can be substantial and increases exponentially as a function of \dot{V}_E (98). Since healthy

aging causes a decrease in the compliance of the chest wall (65), and a reduction in airway diameter (30), it would be expected that the W_b for a given \dot{V}_E would be higher in older relative to younger individuals. We demonstrated that for a given \dot{V}_E above 60 l min⁻¹, older individuals have a significantly higher W_b than younger individuals (Figure 2-5). The relationship between W_b and \dot{V}_E during exercise has previously been assessed in highly trained older men (77), and highly trained younger men (12). When comparing the data from these two studies, it appears that older men have a higher W_b for a given \dot{V}_E than younger men. However, combining the data from these two separate studies does not permit direct statistical comparisons between older and younger individuals. Only one study has investigated the effect of age on W_b during exercise within the same study (76). They found that older individuals had a higher W_b than younger individuals during exercise at an absolute $\dot{V}O_2$ of 1.5 l·min⁻¹ as well as at 40% and 60% of cardiac reserve. However, they did not normalize W_b for \dot{V}_E , and like previous studies have only included men. Thus, our study is the first to show that W_b is higher for a given \dot{V}_E in older men and women by directly comparing them to younger men and women. Given the linear relationship between external work and VO2, it is reasonable to hypothesize that older individuals also have a higher $\dot{V}O_2$ of the respiratory muscles than younger individuals. Indeed, the respiratory muscle $\dot{V}O_2$ required to generate a given \dot{V}_E appears to increase as a function of age (78). Moreover, the fact that older individuals had a statistically similar W_b for a given relative \dot{V}_E below peak exercise than younger individuals would suggest that older individuals have to dedicate a greater fraction of whole-body $\dot{V}O_2$ to their respiratory muscles in order to maintain adequate \dot{V}_A .

We have previously shown that for a given \dot{V}_E above ~55-65 l·min⁻¹, W_b (11,93) and respiratory muscle $\dot{V}O_2$ (98) are higher in young women relative to young men. In the present

study, we also demonstrate that for a given \dot{V}_E above 65 l·min⁻¹, young women have a significantly higher W_b than young men (Figure 2-5). Importantly, the effect of sex on W_b appears to be maintained in older individuals such that older women have a higher W_b for a given \dot{V}_E than do older men. However, we found no interaction effect between age and sex on W_b , which we interpret to mean that sex differences in W_b during exercise are maintained rather than exacerbated or mitigated as a function of the healthy aging process. This finding is in keeping with previous work showing that older women have a higher $\dot{V}O_2$ of the respiratory muscles during exercise than older men (153).

Based on the results of our model (eq. 3, Table 2-3), we found that the age-related increase in W_b is primarily related to changes in the viscoelastic component of W_b, while sexdifferences in W_b are principally related to the resistive component of W_b. Our interpretation of these findings is as follows. First, regardless of age, women likely have a higher W_b due to inherent sex-differences in the size of their airways (19), which results in a higher resistance to airflow for a given V_E (93). In addition, the fact that there was no significant effect of sex on the viscoelastic component of W_b indicates that regardless of age, men and women have similar lung tissue elasticity, a finding which is in keeping with our previous work (93) and that of others (149). Second, regardless of sex, older individuals have a higher W_b due to the age-related changes to the mechanical properties of the respiratory system that increase the amount of mechanical work required to overcome the viscous resistance of the lung tissues to deformation and of the respiratory tract to the laminar flow of air. The absence of a significant effect of aging on the resistive component of W_b was an unexpected finding. It is well known that healthy aging increases airway resistance, due in large part to age-related reductions in airway diameter (30). However, since resistance is flow-dependent, it is also important to consider that older

individuals have reduced capacity to generate flow. Therefore, it is possible that the resistive component of W_b would only become evident at relatively high flow rates, once flow becomes turbulent. Collectively, our findings suggest that during exercise, the W_b for a given \dot{V}_E is independently affected by sex and age; the healthy aging process increases the viscoelastic component of W_b , while innate sex-differences in the human respiratory system contribute to differences in the resistive component of W_b .

Expiratory Flow Limitation. It is well known that older individuals are predisposed to EFL during exercise due to their reduced ventilatory capacity and increased ventilatory response to exercise (4,14). In the present study, older individuals exhibited EFL during exercise more frequently than younger individuals (Figure 2-7). We also demonstrated that older women have a higher propensity towards EFL than older men, an observation that we attribute to sexdifferences in the structure of the respiratory system. When ventilatory demand approaches maximum ventilatory capacity, small sex-differences in airway anatomy may play a crucial role in determining the extent of mechanical ventilatory limitation. A corollary to this finding can be drawn from previous work in young endurance-trained athletes, where the maximum capacity of the respiratory system is high, but so too is the ventilatory demand associated with the intensity of exercise they are capable of achieving (11). In the context of high ventilatory capacity and high ventilatory demand, the relatively small sex-differences in the structure of the respiratory system become important and likely predispose women to EFL. Our research group has shown that young endurance-trained women have a higher propensity towards EFL at maximal exercise than young endurance-trained men (11). While we found a main effect of sex on EFL in the present study, the differences between men and women were far more apparent in the older

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relative to the younger group (Figure 2-7), a finding that is in keeping with our previous work in younger recreationally active subjects of slightly above-average fitness (93). Based on previous studies (11,91), it can be argued that young women may be more susceptible to EFL during exercise than young men. However, the factors that determine EFL are complex and multifactorial (92). While differences in lung and airway anatomy play an important contributory role, other factors may supersede these relatively small differences. For example, young individuals of average fitness have a similarly high ventilatory capacity as their endurance-trained counterparts, but often lack the fitness necessary to generate the ventilatory demand that would be required to cause EFL. Therefore, in most young individuals, fitness is likely the primary determinant of EFL rather than sex-differences in the structure of the respiratory system. By contrast, given the reduced ventilatory capacity and their increased ventilatory response to exercise, sex-differences in respiratory system anatomy are likely an important determinant of EFL in older individuals.

Though the older individuals in our study had a greater frequency of EFL during exercise than the younger individuals, their \dot{V}_E/\dot{V}_{ECAP} was above 70% at peak exercise, which is indicative of a mild degree of ventilatory constraint (144). By contrast, younger individuals had a \dot{V}_E/\dot{V}_{ECAP} that was significantly lower than older individuals and consistently below 70%, even at maximal exercise. Together, these findings suggest that while mechanical ventilatory constraint are more prominent in healthy older individuals, they still possess a reserve for increasing \dot{V}_E even at maximal exercise. Though the current study was not designed to assess the presence of ventilatory limitations to exercise in older individuals, we believe this point merits brief comment. Given the apparent ventilatory reserve, our data seem to support the notion that the respiratory system does not pose a primary limitation to maximum oxygen uptake. Nevertheless, based on our work (98), we believe it is likely that further increases in \dot{V}_E would come at a substantial, and potentially unsustainable, mechanical and metabolic cost.

Dyspnea. We found that age, sex, and their interaction had an effect on dyspnea at fixed work rates during exercise. Older individuals reported higher dyspnea during submaximal exercise than their younger counterparts (Figure 2-8), and women reported significantly higher dyspnea during submaximal exercise than men. The difference in dyspnea between men and women at 80 W was more pronounced in the older subjects. Ofir et al. (74) found that older women reported a higher intensity of dyspnea at a standardized $\dot{V}O_2$ of 20 ml·kg⁻¹·min⁻¹ than older men by approximately 1 Borg unit. We observed a remarkably similar finding with older women reporting dyspnea ratings that were 1.3 Borg units higher $(1.9\pm0.4 \text{ vs. } 0.6\pm0.2)$ than older men at 80 W, which corresponded to a relative $\dot{V}O_2$ of 20.5±1.0 and 21.3±0.9 ml·kg⁻¹·min⁻¹ in older men and older women, respectively. Although the perception of dyspnea in older individuals was significantly higher for any given absolute work rate above 40 W than in younger individuals, the degree of dyspnea in older individuals was relatively modest throughout exercise and only reached an average of 5.0±0.5 Borg units at maximal exercise. The relatively low dyspnea ratings observed in the healthy older individuals in our study is in close agreement with previous investigations using incremental cycle exercise (74,154), and further supports the notion that the respiratory system is unlikely to be the primary locus of exercise limitation in healthy older individuals. In the younger individuals, women also reported a significantly higher intensity of dyspnea than men at 80 W; however, the difference was only to the order of 0.6 Borg units $(0.7\pm0.2 \text{ vs. } 0.1\pm0.1)$. At maximal exercise, dyspnea ratings were slightly but significantly lower

in the older subjects than in the younger subjects, which we attribute to differences in absolute \dot{V}_E (Table 2-2).

In light of the observed differences in the mechanical ventilatory response to exercise described herein, it stands to reason that respiratory mechanics may explain, at least in part, the age- and sex-differences in the perception of respiratory sensation. Across all subjects, W_b , breathing frequency, \dot{V}_E/\dot{V}_{ECAP} and \dot{V}_E were the strongest correlates of dyspnea at 80 W, each explaining >50% of the variance in dyspnea (Table 2-4). We speculate that at 80 W, those with the highest indices of mechanical constraint (W_b and \dot{V}_E/\dot{V}_{ECAP}) had the highest sensations of dyspnea. In addition, those who had the highest \dot{V}_E response and dead-space ventilation (due to the high breathing frequency) also had higher sensations of dyspnea. We emphasize that we are cognizant of the limits of correlative evidence and of the multifactorial causes of dyspnea. In the absence of experimental manipulations, we hesitate to overstate the link between mechanical ventilatory constraint and dyspnea, nor argue the primacy of mechanical ventilatory constraint over other factor that cause dyspnea within the context of the present study. Instead, our findings present a hypothesis that awaits experimental testing. Specifically, that mechanical ventilatory constraint plays a contributory role in the genesis of dyspnea during exercise in healthy humans.

Perspectives on sex-based comparisons. A consistent problem when conducting studies to investigate age- or sex-differences in the pulmonary physiology of exercise concerns how to most appropriately compare groups. The principal issue revolves around whether to make comparisons in absolute or relative terms. On one hand, making comparisons in absolute terms allows for the assessment of the effects of sex and age, but ignores the potential confounding effects of body size and functional capacity. On the other hand, making comparisons in relative

terms accounts for differences in body size and functional capacity, but potentially obscures important sex-differences and overlooks the physical and metabolic requirements of a given task. In the present study, we compared our primary outcome variables (i.e. W_b, operating lung volumes, EFL, and dyspnea) at both absolute and relative work rates or ventilations since both are required in order to truly determine the influences of age and sex. However, our approach results in a large number of permutations and contributes to interpretive complexities. Thus, we offer the following perspectives when interpreting our findings. First, we emphasize that the results of absolute and relative comparisons each have inherent caveats, and one should not be favored at the expense the other. Second, in some instances we found some differences between groups when comparisons were made in absolute terms that were absent in relative terms. It follows that the generalizability of our findings will depend on context. For example, if one considers our findings relating to the effects of age and sex on the perception of dyspnea, the confounding influence exercise intensity is of critical importance.

Limitations. While our study reveals novel findings regarding the mechanical ventilatory and perceptual responses to exercise in older and younger men and women, some limitations must be considered. First, our measure of W_b (integrated esophageal pressure-volume loops) does not take into account other components of ventilatory work, such as chest-wall distortion and abdominal stabilization (155). Given the age-related changes to the mechanics of the respiratory system, it is possible that age-related differences in chest wall distortion exist, and could impact total ventilatory work. However, without measures of respiratory system kinematics or estimates of respiratory muscle $\dot{V}O_2$, this limitation cannot be overcome. Second, EFL can be assessed using a variety of different methods. We chose to use the NEP technique given its numerous

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advantages (146). However, it should be noted that the NEP technique provides an assessment of EFL at a single point in time rather than a continuous measure, or one that is averaged over a longer period of time. It follows that our measures of the frequency of EFL do not represent the entirety of each exercise stage or the dynamic nature of EFL during exercise (152). However, given that the technique was applied consistently within each subject and each group, it is unlikely that this limitation affected the overall results of our study. Third, we acknowledge that our study did not account for psychological and/or sociocultural differences between subjects that are known to influence the perception of breathlessness (156).

Conclusions. We found that during exercise, sex-differences in W_b for a given \dot{V}_E appear to be maintained in older individuals, older women have a greater propensity towards EFL than older men, and that sex does not have a significant effect on the regulation of operating lung volumes. Our data suggest that sex-differences in the mechanical ventilatory response to exercise observed in younger individuals are present in healthy older individuals. We also found that older women report a higher intensity of dyspnea during exercise than older men. Although the mechanisms of dyspnea are complex and multifactorial, mechanical ventilatory constraint seems to play an important contributory role. However, experimental manipulations of respiratory mechanics are required in order to directly test this hypothesis. Overall, superimposing the normal age-related changes in respiratory structure and function on innate sex-differences in airway anatomy appears to have a significant effect on the mechanical ventilatory and perceptual responses to exercise in older women.





Static recoil pressure of the lung at 100% TLC for older and younger women (panel C) as well as older and younger men (panel D). On panels C and D, individual values for each subject are shown along with group mean values. All data are presented as mean \pm SE. P_{st 100%TLC}; static recoil pressure of the lung at 100% TLC. * *p*<0.05 main effect of age, comparisons made between all older and all younger subjects, regardless of sex.



Figure 2-2. Ventilatory response to incremental cycle exercise in older men and women, as well as younger men and women

Ventilatory response to incremental cycle exercise in older men and women (Panels A, C, and E) as well as younger men and women (Panels B, D, and F). The highest equivalent work rate achieved by all subjects was 80 W. Dashed lines within each group connect the 80W data point to the peak exercise data point. All data are presented as mean \pm SE. V_T, tidal volume; F_b, breathing frequency; \dot{V}_E , minute ventilation. * *p*<0.05, main effect of age, comparisons made between all older and all younger subjects, regardless of sex. † *p*<0.05, comparisons made between all men and all women, regardless of age. No significant interaction effect was observed.



Figure 2-3. Absolute ventilatory capacity and fractional utilization of ventilatory capacity during incremental cycle exercise in older men and women, as well as younger men and women

The highest equivalent work rate achieved by all subjects was 80 W. Dashed lines within each group connect the 80W data point to the peak exercise data point. All data are presented as mean±SE. \dot{V}_{ECAP} , ventilatory capacity; \dot{V}_E/\dot{V}_{ECAP} , fractional utilization of ventilatory capacity. * p<0.05, main effect of age, comparisons made between all older and all younger subjects, regardless of sex. † p<0.05, comparisons made between all men and all women, regardless of age. No significant interaction effect was observed.



Figure 2-4. Operating lung volumes during exercise incremental cycle exercise in older men and women, as well as younger men and women

The highest equivalent work rate achieved by all subjects was 80 W. Dashed lines within each group connect the 80W data point to the peak exercise data point. On panels A and B, EELV and EILV are presented in absolute terms including residual volume, while on panels C and D EELV and EILV are presented in relative terms normalized for total lung capacity. All data are presented as mean±SE. EELV, end-expiratory lung volume; EILV, end-inspiratory lung volume. * p<0.05, main effect of age, comparisons made between all older and all younger subjects, regardless of sex. † p<0.05, comparisons made between all men and all women, regardless of age. No significant interaction effect was observed.



Figure 2-5. The relationship between work of breathing and minute ventilation during incremental cycle exercise

Individual curves of the work of breathing versus minute ventilation in older men and women (panel A) and younger men and women (panel B). Mean curves relating work of breathing to minute ventilation in all groups are shown in panel C. All mean curves are based on mean values of constants *a* and *b* from *eq. 1*, and each curve has been extrapolated to the average peak minute ventilation within each group. The group mean curves were extrapolated to the average maximum \dot{V}_E achieved for each group. Data for older men are displayed as thick grey lines, while data for older women are displayed as thick black lines. Data for younger men are displayed as thin grey lines, while data for older women are displayed as thin black lines. W_b , work of breathing; \dot{V}_E , minute ventilation. * *p*<0.05, main effect of age, comparisons made between all older and all younger subjects, regardless of sex. † *p*<0.05, comparisons made between all men and all women, regardless of age. No significant interaction effect was observed.


Figure 2-6. Tidal flow–volume loops at a fixed work rate of 100 W in 4 individual subjects closely matched for height

Thin black lines represent the control breath and thick black lines represent the negative expiratory pressure breath. All data are raw traces.



Figure 2-7. Frequency of EFL at rest and during exercise

Data are shown for each group at rest, at discrete absolute work rates achieved by all subjects, and at maximal exercise during the exercise test on Day 2; older men are shown in filled grey bars, older women in filled black bars, younger men open black bars, and younger women open grey bars. The highest equivalent work rate achieved by all subjects was 80 W.



Figure 2-8. Dyspnea intensity responses to incremental cycle exercise in older men and women, as well as younger men and women

The highest equivalent work rate achieved by all subjects was 80 W. Dashed lines within each group connect the 80 W data point to the peak exercise data point. All data are presented as mean \pm SE. * p<0.05, main effect of age, comparisons made between all older and all younger subjects, regardless of sex. † p<0.05, comparisons made between all men and all women, regardless of age. $\ddagger p<0.05$, interaction effect between age and sex, men vs. women within each age group.

	Older (n=22)		Younger (n=22)		
	Men $(n=10)$	Women (n=12)	Men $(n=11)$	Women (n=11)	
Age, y	70±6	66±5	26±4	24±2	*
Height, cm	173±7	163±8	176±8	166±6	Ť
Body Mass, kg	76±13	65±10	73±8	58±7	Ť
BMI, $kg \cdot m^{-2}$	25±3	25±4	23±2	21±2	*
FVC, 1	4.30±0.37	3.46±0.67	5.46±0.68	4.26±0.59	*†
FVC, % predicted	102±8	108±10	101±10	104±8	
$FEV_1, 1$	3.06±0.41	2.49±0.56	4.27±0.63	3.55±0.59	*†
FEV ₁ , % predicted	102±12	104±13	99±10	106±8	
FEV ₁ /FVC	71±8	71±6	79±6	82±5	*
FEV ₁ /FVC, %predicted	99±11	96±8	94±7	95±5	
PEF, $1 \cdot s^{-1}$	8.63±1.32	6.82±1.25	10.79±1.25	7.69±0.50	*†
FEF ₂₅₋₇₅ , 1·s ⁻¹	2.10±0.91	1.90±0.61	4.18±1.09	3.67±0.74	*
FEF ₂₅₋₇₅ , %predicted	82±36	77±12	91±15	97±19	
TLC, 1	6.84±0.97	5.57±0.86	6.89±0.74	5.42±0.68	Ť
TLC, % predicted	102±9	108±11	102±10	103±8	
VC,1	4.45±0.43	3.57±0.61	5.56 ± 0.65	4.32±0.56	*†
VC, % predicted	101±9	109±10	103±11	102 ± 8	
IC, 1	3.03±0.89	2.39±0.69	3.48±0.54	2.77±0.51	*†
IC, % predicted	100±26	100 ± 28	99±15	115±17	
FRC, 1	3.90±0.76	3.18±0.52	3.41±0.54	2.64±0.41	*†
FRC, % predicted	97±14	108±12	104±18	92±13	
RV, 1	2.39±0.76	1.96±0.36	1.33±0.38	1.07 ± 0.24	*
RV, % predicted	95±26	100±15	90±34	81±15	
DL_{co} , ml·min ⁻¹ ·mmHg ⁻¹	27±6	23±4	33±5	25±4	*†
DL _{co} , % predicted	107±19	105±13	111±12	105±12	
MIP, cmH_2O	-103±27	-76±27	-138±31	-106±24	*†
MIP, % predicted	98±25	108±29	107±24	115±28	
MEP, cmH_2O	151±49	109±27	178±34	142 ± 30	*†
MEP, % predicted	78±8	81±6	74±5	90±6	
C_l , l , $l \cdot cmH_2O^{-1}$	0.29 ± 0.03	0.28 ± 0.09	0.31±0.06	0.26 ± 0.05	
$P_{st 100\% TLC}$, cm H_2O	19±7	20±6	28±4	31±6	*

Table 2-1. Baseline subject characteristics and pulmonary function data

Abbreviations: BMI, body mass index; FVC, forced vital capacity; FEV₁, forced expired volume in 1 s; PEF, peak expiratory flow; FEF₂₅₋₇₅, forced expired flow between 25 and 75% of FVC; TLC, total lung capacity; VC, vital capacity; IC, inspiratory capacity; FRC, functional residual capacity; RV, residual volume; DLco, diffusion capacity of the lungs for carbon monoxide; MIP, maximal inspiratory pressure; MEP, maximal expiratory pressure; Cl, lung compliance; P_{st} $_{100\%TLC}$, static recoil pressure of the lungs at 100% total lung capacity. All data are presented as mean±SE. * *p*<0.05, main effect of age, comparisons made between all older and all younger subjects, regardless of sex. † *p*<0.05, comparisons made between all men and all women, regardless of age. No significant interaction effect was observed.

	Older (n=22)		Younger (n=22)		
	Men (n=10)	Women (n=12)	Men (n=11)	Women (n=11)	
$\dot{V}O_2$, $1 \cdot min^{-1}$	2.63±0.20	1.89±0.12	3.91±0.29	3.01±0.15	*†
\dot{VO}_2 , ml·kg ⁻¹ ·min ⁻¹	34.7±2.0	30.6±2.6	53.4±3.1	51.7±2.8	*†
VO ₂ , % predicted	119±5	122±6	123±6	140±7	
$\dot{V}CO_2$, $1 \cdot min^{-1}$	2.92 ± 0.20	2.23±0.14	4.33±0.27	3.34±0.17	*†
RER	1.12 ± 0.20	1.18 ± 0.02	1.12 ± 0.03	1.11±0.02	
HR, beat min^{-1}	150±5	153±5	186±3	185±3	*
HR, % predicted	100 ± 2.9	98±3.2	96±1.6	95±1.8	
S _p O ₂ , %	98±1	98±1	97±1	98±1	
V _T , 1	2.84 ± 0.18	1.95 ± 0.14	2.96±0.17	2.19±0.08	Ť
F_b , breaths min ⁻¹	39.5±4.1	45.7±3.2	51.5±2.5	55.1±3.0	*
$\dot{\mathbf{V}}_{\mathrm{E}}, \mathbf{l} \cdot \mathbf{min}^{-1}$	108±8	85±4	151±10	119±5	*†
$\dot{V}_{E}/\dot{V}O_{2}$	42.4±3.4	46.3±2.8	39.7±2.2	39.8±1.2	*
$\dot{V}_E / \dot{V}CO_2$	37.6±2.4	39.1±2.1	35.4±1.7	35.9±1.1	*†
P _{ET} CO ₂ , mmHg	30.8±0.9	32.2±1.4	33.3±3.8	31.6±1.4	
Work rate, W	172 ± 10	140±11	269±21	222±12	*†
^{VO} ₂:Work rate slope	11.7±0.7	10.8 ± 0.50	11.5±0.3	11.2±0.3	
EELV (% TLC)	56±3	56±2	50±2	51±2	*
EILV (% TLC)	91±1	92±1	91±2	91±1	
W_b , J·min ⁻¹	257±24	236±28	335±52	307±39	*†
Resistive W_b , J·min ⁻¹)	76±19	101±20	61±16	140±33	†
Viscoelastic W _b , J·min ⁻¹	181±32	135±23	274±42	166±22	*
\dot{V}_{ECAP} , $1 \cdot min^{-1}$	151.1±13.2	118.4±8.2	220.4±9.1	181.1±11.8	*†
$\dot{V}_{E}/\dot{V}_{ECAP}$, %	73.2±4.3	75.1±4.9	69.6±4.4	67.5±3.7	*
Dyspnea, Borg scale	4.6±0.7	5.3±0.6	6.0 ± 0.8	6.5±0.9	*
Leg Discomfort, Borg scale	6.2±0.9	6.3±0.8	9.2±0.8	8.8±0.5	*

Table 2-2. Peak exercise data

Abbreviations: \dot{VO}_2 , oxygen uptake; \dot{VCO}_2 ; carbon dioxide output; RER; respiratory exchange ratio; HR, heart rate; S_pO_2 , oxygen saturation by pulse oximetry; V_T , tidal volume; F_b , breathing frequency; \dot{V}_E , minute ventilation; \dot{V}_E/\dot{VO}_2 , ventilatory equivalent for oxygen; \dot{V}_E/\dot{VCO}_2 , ventilatory equivalent for carbon dioxide; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; EELV, end-expiratory lung volume; EILV, end-inspiratory lung volume; W_b , work of breathing; \dot{V}_{ECAP} , ventilatory capacity. All data are presented as mean±SE. * *p*<0.05, main effect of age, comparisons made between all older and all younger subjects, regardless of sex. † *p*<0.05, comparisons made between all men and all women, regardless of age. No significant interaction effect was observed.

Table 2-3. Marginal *p*-values for fixed effects specified in *eq.* 2

Te	rm	<i>t</i> -ratio	<i>p</i> -value
a	(resistive component of W _b)	-1.82	0.0568
t	(sex effect on <i>a</i>)	4.67	$3.95 \cdot 10^{-06}$
q	(age effect on <i>a</i>)	-0.51	0.609
b	(visco-elastic component of W _b)	0.85	0.398
и	(sex effect on <i>b</i>)	0.97	0.309
r	(age effect on b)	7.54	$2.80 \cdot 10^{-13}$

Abbreviations: W_b , work of breathing.

Variable	r^2	<i>n</i> -value	
	1	<i>p</i> -value	
V_E , l·min ⁻¹	0.54*	< 0.001	
$\dot{V}_{E}/\dot{V}_{ECAP}$, %	0.56*	< 0.001	
V _T , 1	-0.43	0.004	
V _T %IC	0.14	0.371	
V _T %VC	0.05	0.760	
$F_{\rm B}$, breaths min ⁻¹	0.69*	< 0.001	
S _p O ₂ , %	-0.34*	0.024	
P _{ET} CO ₂ , mmHg	-0.32*	< 0.001	
EELV, %TLC	0.33*	0.032	
IRV, %TLC	-0.23*	0.014	
IC, 1	-0.47*	< 0.001	
IC, %predicted	-0.01	0.792	
ΔIC (exercise – rest), l	-0.03	0.315	
Total W_b , J·min ⁻¹	0.76*	< 0.001	

Table 2-4. Correlates of dyspnea at a standardized absolute work rate during exercise

Abbreviations: \dot{V}_E , expired minute ventilation; \dot{V}_E/\dot{V}_{ECAP} , fractional utilization of ventilatory capacity; V_T , tidal volume; IC, inspiratory capacity; VC, vital capacity; F_b , breathing frequency; S_pO_2 , arterial oxygen saturation by pulse oximetry; $P_{ET}CO_2$, end-tidal partial pressure of carbon dioxide; EELV, end-expiratory lung volume; TLC, total lung capacity; IRV, inspiratory reserve volume; W_b , work of breathing. * p < 0.05.

Chapter 3: Sex-differences in inspiratory muscle recruitment patterns during exercise are maintained in healthy older adults

3.1 Introduction

During incremental exercise, \dot{V}_E increases progressively in order to meet rising metabolic demands. Generating the pressure required to achieve a given \dot{V}_E is accomplished via the coordinated action of the respiratory muscles (157). During inspiration at rest, the diaphragm performs the majority of respiratory muscle work with other obligatory inspiratory muscles, such as the scalenes, contributing less to overall pressure generation (158). As \dot{V}_E increases during incremental exercise, the relative contribution of the diaphragm to overall pressure generation decreases, while the contribution of other obligatory inspiratory muscles as well as accessory inspiratory muscles (e.g. the sternocleidomastoids) increase progressively (159,160). The progressive recruitment of extra-diaphragmatic inspiratory muscles effectively serves to distribute the work needed to support exercise hyperpnoea (161).

A number of sex-differences in the structure and function of the respiratory system have been reported (85). For example, on average, women have smaller absolute lung volumes and maximal flows than age- and height-matched men (6,7). Moreover, when men and women are matched for absolute lung size, women have smaller large conducting airways (19). During exercise, the smaller airways in women are thought to increase the mechanical and metabolic cost of generating a given \dot{V}_E above ~50-65 l·min⁻¹ (98). Previous work suggests that the higher W_b in women may be achieved by employing a different pattern of respiratory muscle recruitment than that observed in men (95,99). Indeed, during constant-load exercise to exhaustion, young women recruit scalene and sternocleidomastoid muscles to a greater extent for a given relative exercise intensity than young men (101). However, the aforementioned sexdifference in inspiratory muscle recruitment patterns was observed during high-intensity constant load exercise, where men have a greater ventilatory response and a higher propensity towards diaphragm fatigue than women (99). Both factors have the potential to influence the pattern of inspiratory muscle recruitment. Therefore, it is unclear whether the differences in inspiratory muscle recruitment patterns during exercise between men and women are the product of biological sex, or the exercise modality.

It is well known that healthy aging results in significant structural changes to the respiratory system which decrease pulmonary function (162). In particular, the respiratory muscles undergo a progressive loss in strength, as evidenced by reductions in maximal respiratory pressures (33). Age-associated reductions in respiratory muscle strength in humans are supported by work in rodent models that demonstrate a progressive age-related change in the morphology of the diaphragm which causes reductions in its force generating capacity (163). Overall, the effect of healthy aging on the human respiratory system drastically alters the mechanics of breathing during exercise (14). Older individuals have a higher W_b for a given \dot{V}_E during exercise than their younger counterparts (see Chapter 2), which must be accomplished by relatively weakened respiratory muscles. Therefore, it is likely that the pattern of respiratory muscle recruitment during exercise is also affected by the healthy aging on inspiratory muscle recruitment patterns during exercise.

Given that biological sex and age independently affect the load on the respiratory muscles during exercise (see Chapter 2), it stands to reason that when compared to men and younger individuals, women and older individuals may rely on extra-diaphragmatic inspiratory muscles to a greater extent throughout incremental exercise in order to minimize the load on the diaphragm. Accordingly, the aim of the present study was to characterize the independent and combined effects of age and sex on inspiratory muscle recruitment patterns during incremental exercise. We hypothesized that women and older individuals would exhibit a greater diaphragm, scalene and sternocleidomastoid activation for a given \dot{V}_E during exercise than men and younger individuals, respectively.

3.2 Methods

Subjects. After providing written informed consent, twenty healthy older men and women between the ages of 60 and 80, and twenty healthy younger men and women between the ages of 20 and 30 participated in the study. Both groups were evenly distributed between the sexes. All subjects had previously participated in a study designed to characterize mechanical ventilatory and perceptual responses to incremental exercise in older and younger men and women (see Chapter 2). The primary outcome measures in the present study did not overlap with any of the previous analyses. All subjects had normal pulmonary function parameters based on predicted values (6-8), a BMI between 18-30 kg·m⁻², and peak aerobic capacity \geq 80% predicted based on population-specific normative values (164,165). Subjects were excluded if they were current smokers or had previously smoked >5 pack-years, had a history or current symptoms of cardiorespiratory disease, or any contraindications to exercise testing. Subjects were divided into 4 groups based on sex and age; younger women (20-30 y), younger men (20-30 y), older women (60-80 y), and older men (60-80 y). All study procedures were approved by the University of British Columbia Providence Health Care Research Ethics Board, which adheres to the Declaration of Helsinki.

Experimental overview. Testing took place over two seperate days. On Day 1, anthropometric measurements were taken followed by detailed pulmonary function testing. On Day 2, subjects were instrumented with a dual-balloon catheter (Guangzhou Yinghui Medical Equipment Ltd, Guangzhou, China) to measure P_{eso} , gastric pressure (P_{ga}), and electromyography (EMG) of the crural diaphragm (EMG_{di}), as well as skin surface electrodes to measure the electromyography of scalene (EMG_{sca}) and sternocleidomastoid (EMG_{scm}). Subjects then performed an incremental cycle exercise test to exhaustion.

Pulmonary function testing. Spirometry, whole-body plethysmography, single breath diffusing capacity for carbon monoxide, maximum voluntary ventilation, as well as maximum inspiratory and expiratory pressures were assessed using a commercially available pulmonary function testing system (Vmax Encore 229, V62J Autobox; CareFusion, Yorba Linda, CA) with participants seated at rest according to standard recommendations (137-140). Pulmonary function measurements were expressed in absolute values and as a percentage of predicted normal values (6-8).

Exercise protocol. The incremental cycle exercise test was conducted on an electronically braked cycle ergometer (Ergoselect 200P; Ergoline, Bitz, Germany). Testing began with a rest period of 6 min followed by a 1-min warm-up consisting of unloaded pedaling, and then 20 W step-wise increases in workload (starting at 20 W) every 2 min until volitional exhaustion. The exercise protocol was selected to allow for comparisons between groups across a wide range of absolute and relative \dot{V}_E . Peak work rate was defined as the highest workload sustained for at least 30 s.

Inspiratory muscle electromyography. Diaphragm electromyography was measured using a multi-pair esophageal electrode catheter. The raw signal was processed using an amplifier (bioamplifier model RA-8, Yinghui Medical Technology Co. Ltd., Guangzhou, China) through a notch filter at 60 Hz, and band-pass filtered between 20 and 1000 Hz. EMG_{scm} and EMG_{sca} were assessed with bipolar skin surface electrodes over the medial scalenes, and the sternocleidomastoid muscles after carefully cleaning and abrading the skin. The positions of the surface electrodes were as follows: EMG_{sca} was placed within the posterior triangle of the neck at the level of the cricoid process, and EMG_{scm} was placed at the midpoint along the longitudinal axis of the sternocleidomastoid muscle between the mastoid process and the medial clavicle. EMG_{scm} and EMG_{sca} electrodes were placed unilaterally on the right side of the body. Raw EMG_{scm} and EMG_{sca} signals were recorded and low-pass filtered at 500Hz using wireless surface EMG system (TeleMyo DDTS, Noraxon USA, Inc., Scottsdale, AZ, USA). All EMG data were transformed into root mean square (RMS) with a time constant of 100 ms. For EMG normalization purposes, subjects performed inspiratory capacity maneuvers at rest and during each stage of exercise as previously described (143). For each individual, the peak RMS data in each respiratory muscle during any inspiratory capacity maneuver at rest or during exercise was defined as the maximal EMG activity of each respective respiratory muscle (166).

Flow, pressure and volume. During exercise, subjects breathed through a low resistance (0.3-0.7 $\text{cmH}_2\text{OT}^{-1}\text{s}^{-1}$ at 0.5-8 1 s⁻¹) circuit with minimal dead-space (130 ml). Bi-directional flow was measured using a heated, calibrated pneumotachograph (model 3813, Hans Rudolph, Kansas City, MO, USA). Volume was obtained by numerical integration of the flow signal. P_{eso} and P_{ga}

were measured by connecting the distal end of each respective balloon on the dual-balloon catheter to independent, calibrated differential pressure transducers (DP15-34, Validyne Engineering, Northridge, CA, USA). Transdiaphragmatic pressure (P_{di}) was then calculated as the difference between P_{ga} and P_{eso} .

Cardiorespiratory responses. Standard metabolic and ventilatory responses were measured on a breath-by-breath basis using a commercially available metabolic cart (Vmax Encore 229; CareFusion, Yorba Linda, CA, USA). In the younger subjects, heart rate was measured using a heart rate monitor (Polar T34; Polar Electro, Kempele, Finland). In the older subjects, heart rate was measured continuously using a 12-lead electrocardiogram (Cardiosoft Diagnostics System v6.71, GE Healthcare, Canada). Arterial oxygen saturation was estimated using a pulse oximeter (Radical-7 Pulse CO-Oximeter, Masimo, Irvine, CA, USA).

Data processing and analysis. At rest and during exercise, all data were collected using a 16channel analog-to-digital data acquisition system (PowerLab/ 16/35, ADInstruments, Colorado Springs, CO, USA), sampled at 2000 Hz, and recorded using LabChart 7.3.7 software. All EMG data was analyzed during the last 30 s of each 2 min exercise stage, and at rest. For each breath within a given 30 s epoch, peak RMS data for EMG_{di}, EMG_{sca} and EMG_{scm} were obtained by manually selecting RMS signals falling between zones of cardiac artefact (167). EMG data for each inspiratory muscle were then expressed as a percent of maximum EMG activity; defined as the highest level of EMG observed during and inspiratory capacity maneuvers at rest or during exercise (168). Flow, volume, and pressures were composite averaged by selecting breaths within the same 30 s epochs as the EMG data. Diaphragmatic pressure-time product (PTP_{di}) and

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esophageal pressure-time product (PTP_{eso}) were then determined by integrating P_{di} and P_{es} , respectively, over time during inspiration and then multiplying these values by breathing frequency. The quotient of PTP_{di} and PTP_{eso} was then calculated in order to determine the fraction of total inspiratory muscle pressure production performed by the diaphragm.

Statistical analysis. Descriptive characteristics, pulmonary function data, and peak exercise data were compared using a 2x2 analysis of variance for age and sex differences between the four groups. In the case of a significant interaction between age and sex, four pairwise comparisons with Bonferroni corrections were performed where appropriate (older men vs. older women, younger men vs. younger women, older men vs. younger men, older women vs. younger women). The relationships between EMG_{di} and PTP_{di}, EMG_{sca} and PTP_{di}/PTP_{eso}, as well EMG_{scm} and PTP_{di}/PTP_{eso} were assessed via random-coefficients regression (169). All EMG and PTP data were compared between groups at absolute \dot{V}_E (30, 50, and 70 l·min⁻¹) using a mixed model analysis of variance. These increments of absolute \dot{V}_E were selected since all subjects reached each level of \dot{V}_E at some point during the incremental exercise test, thereby enabling statistical comparisons at discrete levels of absolute \dot{V}_E . In the case of a significant two-way interaction between \dot{V}_E and age, \dot{V}_E and sex, or a significant three-way interaction between \dot{V}_E , age and sex, Bonferroni-adjusted post-hoc comparisons were conducted where appropriate. This analysis was repeated in order to determine the effect of age and sex as well as their interactions on EMG and PTP data at relative fractions of maximal \dot{V}_E (i.e. at 20%, 40%, 60%, 80%, and 100% of maximal \dot{V}_E).

3.3 Results

Subject characteristics. Subject characteristics, pulmonary function data, and peak exercise data have previously been reported elsewhere (see Chapter 2). When expressed as a percentage of predicted values, resting pulmonary function was within the normal range for all groups and did not differ on the basis sex or age. On average, subjects in each group achieved near maximum heart rates based on predicted normal values and respiratory exchange ratios >1.10, indicating that maximal effort was exerted in all groups. At peak exercise, there was a significant effect of sex and age on absolute and relative \dot{VO}_2 (p<0.05). When \dot{VO}_2 at peak exercise was expressed as a percentage of predicted values (164,165), there was no significant effect of sex or age, indicating that subjects had similar levels of relative fitness across groups. There were no significant interaction effects between age and sex on any variables at peak exercise.

Inspiratory muscle electromyography. Figure 3-1 shows a representative trace of the electrical activity of the diaphragm, scalene, and sternocleidomastoid, as well as the corresponding esophageal and transdiaphragmatic pressure, as well as flow in a single subject at three relative exercise intensities. Figure 3-2 shows electrical activity of the inspiratory muscles as a function of absolute \dot{V}_E during exercise. Women and older individuals had a higher EMG_{di} for a given submaximal absolute \dot{V}_E above rest and a higher EMG_{scm} for a given $\dot{V}_E \ge 50 \ 1 \ \text{min}^{-1}$ than men and younger individuals respectively (both p < 0.05). Furthermore, regardless of age, women had a higher EMG_{sca} for a given absolute \dot{V}_E above rest than men (p < 0.05). When the same comparisons were repeated as a function of relative \dot{V}_E (Figure 3-3), there were no significant effects of sex or age on EMG_{di} (p > 0.05). However, when compared to men, women had a higher EMG_{sca} for a given relative \dot{V}_E above rest and a higher EMG_{scm} for a given fraction of maximal

 $\dot{V}_E \ge 40\%$ (both *p*<0.05). Moreover, older individuals had a lower EMG_{sca} at 60% and 80% of maximal \dot{V}_E (*p*<0.05), and a higher EMG_{scm} at 40% of maximal \dot{V}_E . There were no significant interaction effects between sex and age.

Respiratory pressure generation. Figure 3-4 shows PTP_{di} and PTP_{eso} as well as their quotient as a function of absolute \dot{V}_E during exercise. Regardless of sex, older individuals had a higher PTP_{di} for a given absolute submaximal \dot{V}_E during exercise than younger individuals (p>0.05), and women and older individuals had a higher PTP_{eso} for a given absolute submaximal \dot{V}_E during exercise than men and younger individuals respectively (both p<0.05). There was a significant effect of sex and age on PTP_{di}/PTP_{eso} at a \dot{V}_E of 70 l·min⁻¹ (both p<0.05). There were no significant interaction effects between sex and age. Additionally, when the same comparisons were repeated with \dot{V}_E expressed in relative terms (Figure 3-5), there were no significant effects of sex or age on PTP_{di} , PTP_{eso} or PTP_{di}/PTP_{eso} (all p>0.05).

Association between inspiratory muscle activation and pressure generation. Assessing the link between electrical activity of the inspiratory muscles and measures of pressure generation is complicated by the fact that the diaphragm is the only respiratory muscle where the pressure resulting from its contraction can be measured directly (115). In order to confirm that an increase in EMG_{di} was in fact associated with an increase in P_{di} generation, we assessed the relationship between EMG_{di} and PTP_{di} across all subjects (Figure 3-6) and found that there was a strong, significant correlation (r²=0.84, p<0.001). While this same analysis cannot be performed for the scalene nor the sternocleidomastoid, it can be surmised that as the relative contribution of the diaphragm to total inspiratory pressure generation decreases, that the balance must be performed by the extra-diaphragmatic inspiratory muscles (159). We found that there was a significant negative correlation between EMG_{sca} and PTP_{di}/PTP_{eso} (r^2 =0.40, p<0.001), as well as EMG_{scm} and PTP_{di}/PTP_{eso} (r^2 =0.34, p<0.01). Lastly, there were no significant effects of sex or age on the slopes of the regressions lines for EMG_{di} and PTP_{di}, EMG_{sca} and PTP_{di}/PTP_{eso}, and EMG_{scm} and PTP_{di}/PTP_{eso} (all p>0.05).

3.4 Discussion

Major findings. We assessed the independent and combined effects of age and sex on the inspiratory muscle recruitment patterns during incremental exercise in healthy individuals. The major findings were three-fold. First, regardless of age, women rely on scalene and sternocleidomastoid muscles to a greater extent during exercise than do men. Second, regardless of sex, older individuals rely on sternocleidomastoid muscles to a greater extent during exercise than do younger individuals. Third, the effects of sex and age on inspiratory muscle recruitment patterns corresponded to measures of inspiratory pressure generation when comparisons were made as a function of absolute but not relative \dot{V}_E , where women and older individuals had a lower diaphragmatic contribution to total inspiratory pressure generation at a $\dot{V}_E \geq 70 \ 1 \cdot \min^{-1}$ than men and younger individuals, respectively. Collectively, our findings suggest that effects of biological sex and healthy aging on the mechanics of respiration during exercise have significant independent effects on inspiratory muscle recruitment patterns.

Sex-differences in inspiratory muscle recruitment patterns. Given that women have a higher mechanical and metabolic cost of breathing for a given absolute \dot{V}_E (98), we would expect that women's inspiratory muscles would also exhibit a correspondingly higher degree of relative

activation. When older and younger subjects were pooled, we found that for a given absolute \dot{V}_E , women had higher EMG_{di} than men (Figure 3-2, Panels A & B), a finding that is in keeping with a previous study in healthy young men and women (95). Although the increased activation of the diaphragm in women relative to men likely contributes to accomplishing the increased work required to breathe, it has been suggested that women may also have a greater recruitment of the extra-diaphragmatic inspiratory muscles than men (95,99). A recent study in healthy young individuals found that women activate their scalene and sternocleidomastoid muscles to a greater extent than men during constant-load cycle exercise at 85% peak work rate (101). However, these data may be affected by the ventilatory response to constant-load exercise or the potential presence of diaphragm fatigue, both of which have been shown to differ on the basis of biological sex (99). In the current study, we simultaneously assessed the electrical activity of the diaphragm, scalene, and sternocleidomastoid muscles during incremental exercise, which enables the comparison of inspiratory muscle recruitment patterns between groups across a wide range of absolute and relative of V_E. Additionally, diaphragm fatigue is unlikely to occur during incremental exercise to exhaustion (170). We observed that in addition to a higher EMG_{di} for a given absolute V_E, women had substantially greater EMG_{sca} and EMG_{scm} than men (Figure 3-2, Panels C-F). The important question then becomes; does the increased activation of the accessory muscles correspond to increased inspiratory pressure generation? We found that for a given absolute submaximal \dot{V}_E during exercise, women had a higher PTP_{eso} but a statistically similar PTP_{di} than men (Figure 3-4, Panels A-D). We also observe that the diaphragm contributed significantly less to total inspiratory pressure generation at a $\dot{V}_E \geq$ 70 $1\mbox{ min}^{-1}$ in women than in men (Figure 3-4, Panels E and F).

When comparisons were made as a function of relative \dot{V}_E , we observed that women still had a higher EMG_{sca} and EMG_{scm}, but a similar EMG_{di} (Figure 3-3). However, despite the higher EMG_{sca} and EMG_{scm}, there were no significant differences in PTP_{eso} or PTP_{di} for a given relative \dot{V}_E (Figure 3-5). Our findings that women have a higher EMG_{sca} and EMG_{scm} for a given relative \dot{V}_E could be due to three potential factors. First, we previously found that younger women have lower respiratory muscle efficiency than younger men at any fraction of maximal \dot{V}_E while precisely mimicking exercise hyperphoea (98). The increased activation of the scalene and sternocleidomastoid in the absence of increased measures of inspiratory pressure generation could be a reflection of the relative differences in efficiency between male and female respiratory muscles. Second, contraction of the scalene and sternocleidomastoid muscles during inspiration serves to increase V_T by expanding the volume of the rib cage (157). Therefore, if women rely on the scalene and sternocleidomastoid muscles during inspiration more than do men, one would expect to observe a corresponding sex-difference in rib cage and abdominal volumes during exercise. Using optoelectronic plethysmography, it has been shown the women expand V_T during incremental exercise by increasing chest wall volume to a greater extent and increasing abdominal volume to a similar extent than men (171). It is possible that some of the work done on the chest wall by the scalene and sternocleidomastoid is not accounted for by measures of Peso and Pdi (159). Third, the aforementioned sex-differences in the volume of the chest wall likely alters the length of the scalene and sternocleidomastoid, which would in turn affect the level of muscle activation required to generate a given pressure. Collectively, we interpret our findings to mean that regardless of age, women recruit their scalene and sternocleidomastoid muscles during exercise to a greater extent than do men. This apparent sex-difference in the pattern of inspiratory muscle recruitment during exercise likely reflects the higher W_b in women, as well as sex-differences respiratory muscle efficiency and/or chest wall kinematics.

The effect of aging on inspiratory muscle recruitment patterns. The healthy aging process is known to decrease the strength the respiratory muscles (33), and increase the mechanical and metabolic costs of breathing during exercise (78). It follows that the increased load on the respiratory muscles during exercise in older individuals may alter the pattern of inspiratory muscle recruitment. We found that independent of sex, aging was associated with a higher EMG_{di} for a given absolute submaximal \dot{V}_E during exercise and a higher EMG_{sem} for a given \dot{V}_E \geq 50 l·min⁻¹, but a similar EMG_{sea} for a given absolute \dot{V}_E (Figure 3-2). Additionally, PTP_{di} and PTP_{eso} were greater in the older individuals than the younger individuals for a given absolute submaximal \dot{V}_E during exercise (Figure 3-4). When comparisons were made as a function of relative \dot{V}_E , we found that older individuals had a similar EMG_{di}, a lower EMG_{sea}, and a higher EMG_{sem} a 40% of maximal \dot{V}_E than younger individuals, whereas there was no significant effect of age on PTP_{di} and PTP_{eso} (Figure 3-5).

We have previously shown that during incremental exercise, older individuals have a higher W_b for a given \dot{V}_E and breathe at a higher fraction of TLC than younger individuals (see Chapter 2). The higher W_b in older individuals requires increased inspiratory muscle activation, which is evidenced in our study by a higher diaphragm and sternocleidomastoid activation for a given absolute \dot{V}_E . Breathing at a higher fraction of TLC does not necessarily require a greater degree of inspiratory muscle work, unless breathing occurs along the relatively flat portion of the pressure-volume relationship of the respiratory system. However, lung volume is known to effect inspiratory muscle recruitment patterns; the sternocleidomastoid starts becoming active once V_T

encroaches on approximately 70% of inspiratory capacity (160), which is likely to occur at a lower absolute and relative \dot{V}_E in older individuals. Overall, it would seem that aging affects inspiratory muscle recruitment via its impact on total respiratory muscle work and the regulation of operating lung volumes during exercise.

Combined influences of sex and age on inspiratory muscle recruitment patterns. It should be noted that we did not observe a statistically significant interaction effect between biological sex and age on any measures within the current study. We interpret this to mean that during exercise, the effect of aging on the pattern of inspiratory muscle activation is similar in men and women, and that the effect of sex on the pattern of inspiratory muscle activation is present regardless of age. Thus, we believe that biological sex and the healthy aging process exert independent influences on the recruitment of inspiratory muscles during incremental exercise.

Association between inspiratory muscle activation and pressure generation. We are cognizant that increased electromyographical activity does not directly equate to increased force production by a muscle. However, we did observe a significant linear relation between absolute EMG_{di} and PTP_{di} across all subjects, where 84% of the variability to PTP_{di} was explained by EMG_{di} (Figure 3-6). We also assessed the relationship between EMG_{sca} and PTP_{di}/PTP_{eso} as well as EMG_{scm} and PTP_{di}/PTP_{eso} based on the assumption that as the relative contribution of the diaphragm to overall inspiratory pressure generation decreased, that the activation of extra-diaphragmatic (such as the scalene and the sternocleidomastoid) would increase. Indeed, we observed that there was a significant negative correlation between EMG_{sca} and PTP_{di}/PTP_{eso} as well as EMG_{scm} and PTP_{di}/PTP_{eso}, with PTP_{di}/PTP_{eso} explaining 40% and 34% of the variability to in EMG_{sca} and

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EMG_{scm}, respectively. Nevertheless, there are some considerations with respect to our findings and interpretation that merit discussion. The fact that we observed sex- and age-related differences in EMG_{sca} and EMG_{scm} when \dot{V}_E was expressed in relative terms despite no significant differences in PTP_{eso} and PTP_{di} implies that; i) there is increased inspiratory muscle activation that does not result in increased muscular work, and/or ii) that there is work conducted on the structures of the respiratory system that are not being accounted for by measures of P_{eso} and P_{di}. Another caveat of this approach is the fact that we are not recording the electrical activity of all muscles involved in inspiration. Therefore, it is likely that a portion of the inspiratory pressure generation is not explained by the activation of the diaphragm, scalene, or sternocleidomastoid muscles. We propose that the discrepancy between electrical activity of the inspiratory muscles we assessed and the associated pressure generation is likely related to a combination of the aforementioned factors. Clearly, additional research is required in order to further characterize the link between sex-differences in respiratory mechanics and respiratory muscle recruitment patterns.

Limitations. There are important limitations of our study that merit discussion. First, we did not assess the activity of the expiratory muscles, which could also be affected by biological sex and the healthy aging process. We chose to focus on the inspiratory muscles due to the fact that during cycle exercise, the scalene, sternocleidomastoid, and diaphragm muscles perform functions that are almost exclusively related to respiration. By contrast, expiratory muscles (e.g. the rectus abdominis) are active independent of respiration in order to ensure trunk stability. The presence of non-respiratory muscle activity would have greatly impacted our ability to address our primary hypotheses. Second, the intricate anatomical arrangement of the respiratory muscles,

particularly the scalene and sternocleidomastoid muscles, presents a significant difficulty in accurately measuring the electrical activity of the respiratory muscles in humans. Due to the close proximity of the scalene and sternocleidomastoid muscles to one another, it is possible that our measures of the electrical activity of these two muscles were affected by "cross-talk" from the surrounding musculature. While there is no way of eliminating this possibility, we took great care placing the surface electrodes on the precise anatomical location of each muscle in addition to ensuring that the subjects limited unnecessary head or neck movement during analysis periods. Third, our recordings of the electrical activity of the respiratory muscles were made using skin surface electrodes and therefore were susceptible to artifact due to factors such as interindividual differences in subcutaneous adipose tissue. We attempted to minimize this source of error by normalizing EMG signals to the maximal level of activity achieved during a volitional inspiratory maneuver on a muscle-specific and subject-specific basis. Lastly, our measure of EMG_{di} only represents crural diaphragm activation, and does not represent costal diaphragm activation (172). By contrast, P_{di} represents overall transdiaphragmatic pressure generation. It is possible that some of the pressure generated by the diaphragm during inspiration was not captured by our esophageal electrode catheter.

Conclusions. We found that during incremental exercise, biological sex and the healthy aging process independently influence the pattern of inspiratory muscle recruitment in healthy humans. Specifically, we found that regardless of age, women had a greater activation of the scalene and sternocleidomastoid muscles during exercise than men, even when comparisons were made as a function of relative \dot{V}_E . We also found that regardless of sex, older individuals exhibit a greater degree of diaphragm activation to generate a given absolute \dot{V}_E above rest, and higher

sternocleidomastoid muscle activation to generate a given absolute $\dot{V}_E \ge 50 \ 1 \cdot min^{-1}$ during exercise. Overall, our results suggest that the increased pressure required to generate a given \dot{V}_E during exercise in women and older individuals is achieved by recruiting diaphragmatic and extra-diaphragmatic muscles such as the scalenes and/or the sternocleidomastoids to greater extent than in men and younger individuals, respectively.



Figure 3-1. Raw data traces in a single representative subject

Representative traces of the electrical activity of the diaphragm, scalene, and sternocleidomastoid, esophageal pressure, transdiaphragmatic pressure, and flow at three relative work rates (50%, 75%, and 100% of peak work rate) in a single young female subject. The gray shaded areas denotes periods of expiration. EMG_{di}, electromyogram of the diaphragm; EMG_{sca}, electromyogram of the scalene; EMG_{scm}, electromyogram of the sternocleidomastoid; P_{eso} , esophageal pressure; P_{di} , transdiaphragmatic pressure.



Figure 3-2. Electrical activity of the diaphragm, scalene, and sternocleidomastoid as a function of absolute minute ventilation during incremental cycle exercise in older men and women as well as younger men and women

Subjects were compared at rest, 30 l·min⁻¹, 50 l·min⁻¹, 70 l·min⁻¹, and at peak exercise. Dashed lines within each group connect the 70 l·min⁻¹ data point to the peak exercise data point. All data are presented as mean±SE. \dot{V}_E , minute ventilation; EMG_{di}, electromyogram of the diaphragm; EMG_{sca}, electromyogram of the scalene; electromyogram of the sternocleidomastoid. * p<0.05, main effect of age, comparisons made between all older and all younger subjects, regardless of sex. † p<0.05, comparisons made between all men and all women, regardless of age. No significant interaction effect was observed.



Figure 3-3. Electrical activity of the diaphragm, scalene, and sternocleidomastoid as a function of relative minute ventilation during incremental cycle exercise in older men and women as well as younger men and women.

Subjects were compared at rest, as well as at 20%, 40%, 60%, 80, and 100% of peak work rate. All data are presented as mean±SE. \dot{V}_E , minute ventilation; EMG_{di}, electromyogram of the diaphragm; EMG_{sca}, electromyogram of the scalene; electromyogram of the sternocleidomastoid. * *p*<0.05, main effect of age, comparisons made between all older and all younger subjects, regardless of sex. † *p*<0.05, comparisons made between all men and all women, regardless of age. No significant interaction effect was observed.



Figure 3-4. Transdiaphragmatic and esophageal pressure-time products, as well as their quotient as a function of absolute minute ventilation during incremental cycle exercise in older men and women as well as younger men and women

Subjects were compared at rest, 30 $1 \cdot \min^{-1}$, 50 $1 \cdot \min^{-1}$, 70 $1 \cdot \min^{-1}$, and at peak exercise. Dashed lines within each group connect the 70 $1 \cdot \min^{-1}$ data point to the peak exercise data point. All data are presented as mean±SE. \dot{V}_E , minute ventilation; PTP_{di}, transdiaphragmatic pressure-time product; PTP_{eso}, esophageal pressure-time product. * p < 0.05, main effect of age, comparisons made between all older and all younger subjects, regardless of sex. † p < 0.05, comparisons made between all men and all women, regardless of age. No significant interaction effect was observed.



Figure 3-5. Transdiaphragmatic and esophageal pressure-time products, as well as their quotient as a function of relative minute ventilation during incremental cycle exercise in older men and women as well as younger men and women

Subjects were compared at rest, as well as at 20%, 40%, 60%, 80, and 100% of peak work rate. All data are presented as mean \pm SE. \dot{V}_E , minute ventilation; EMG_{di}, electromyogram of the diaphragm; EMG_{sca}, electromyogram of the scalene; electromyogram of the sternocleidomastoid. * p<0.05, main effect of age, comparisons made between all older and all younger subjects, regardless of sex. † p<0.05, comparisons made between all men and all women, regardless of age. No significant interaction effect was observed.



Figure 3-6. Correlation between transdiaphragmatic pressure-time product and corresponding electrical activity of the diaphragm

Regression lines for individual subjects a depicted as thin grey lines and the average regression line for all subjects is depicted by a thick black line.

Chapter 4: Experimental manipulations of mechanical ventilatory constraint during exercise do not influence dyspnea in healthy older men and women

4.1 Introduction

Dyspnea, briefly defined as "a subjective experience of breathing discomfort" (108), is a common sensory consequence of physical exertion. The magnitude of dyspnea during exercise has been shown to increase over the course of the healthy aging process, where older individuals report higher levels of dyspnea for a given absolute exercise intensity than their younger counterparts (109,118). While our understanding of the mechanisms of dyspnea is incomplete, exertional dyspnea in older individuals is generally thought to occur due to the perception of increased respiratory effort required to meet the ventilatory demands of exercise (130). Healthy aging is associated with significant changes to the structures of the respiratory system that lead to a progressive decline in pulmonary function (42), and an increased ventilatory response to exercise (69). Consequently, older individuals are more susceptible to mechanical ventilatory constraint during exercise than younger individuals (4), which is indicated by a greater $W_{\rm b}$, increased operating lung volumes, and a higher propensity for developing EFL (see Chapter 2). The magnitude of mechanical ventilatory constraint directly affects the degree of respiratory effort required to exercise at a given absolute intensity, and therefore is likely to influence the perception of dyspnea.

It is becoming increasingly apparent that biological sex also affects dyspnea during exercise in healthy older individuals. Activity-related dyspnea is twice as common in females than in males in the general population between the ages of 38-67 (127). Moreover, we (see Chapter 2) and others (74) have shown that older women report higher levels of dyspnea during

exercise at a standardized rate of oxygen consumption ($\dot{V}O_2$) and absolute work load than older men. However, sex-differences in the perception of dyspnea disappear when comparisons are made at discrete fractions of maximal ventilatory capacity (74,94). Although the precise causes of sex-differences in the perception of breathing discomfort during exercise are largely unknown, it has been proposed that inherent sex-differences in the structure and function of the respiratory system may play a contributory role (74,94). Specifically, the smaller lungs and airways in women relative to men are thought to predispose women to mechanical ventilatory constraint during exercise (173), which could result in a greater perception of breathing discomfort. We base this hypothesis on three primary lines of evidence. First, older women have a higher W_b for a given \dot{V}_E and are more likely to experience EFL during exercise than older men (see Chapter 2). Second, indices of mechanical ventilatory constraint and respiratory effort during submaximal exercise have been shown to correlate with ratings of dyspnea in older men and women (see Chapter 2). Third, when the degree of mechanical ventilatory constraint is experimentally increased during exercise using resistive loading or dead-space loading, the sensation of dyspnea is increased concomitantly (124,125,130). It follows that acutely manipulating mechanical ventilatory constraint during exercise in healthy older men and women would result in a corresponding change in the perception of dyspnea. Furthermore, reducing mechanical ventilatory constraint in older women may eliminate the sex-differences in exertional dyspnea observed in older individuals.

The aim of the present study was to determine the effect of acute alterations of the magnitude of mechanical ventilatory constraint during submaximal exercise on the perception of dyspnea in healthy older men and women. We hypothesized that during submaximal exercise; i) reducing mechanical ventilatory constraint would decrease the perception of dyspnea, and ii) that

increasing mechanical ventilatory constraint would increase the perception of dyspnea. We further hypothesized that the effect of manipulating mechanical ventilatory constraint would have a significantly greater effect on the perception of dyspnea in women than in men.

4.2 Methods

Subjects. Twelve healthy older individuals (n=6 men, n=6 women) between the ages of 60 and 80 participated in this study. All subjects provided written, informed consent, and study procedures were approved by the Providence Health Care Research Ethics Board at the University of British Columbia, which adheres to the *Declaration of Helsinki*. All subjects had normal pulmonary function based on predicted values (6-9). Additional inclusion criteria were as follows: BMI of 18-30 kg·m⁻², and peak aerobic power \geq 80% predicted. Subjects were excluded if they were current smokers or had previously smoked >5 pack-years, had a history or current symptoms of cardiorespiratory disease, or any contraindications to exercise testing.

Experimental overview. Participants reported to the laboratory for two visits separated by a minimum of 48 hours. During visit 1, anthropometric measurements were taken, followed by detailed pulmonary function testing and an incremental cycle exercise test to exhaustion. During visit 2, subjects performed a series of constant load cycle exercise tests under 4 experimental conditions (see *Experimental Conditions*) separated by periods of rest.

Pulmonary function testing. Spirometry, whole-body plethysmography, DL_{CO} , maximum voluntary ventilation, as well as maximum inspiratory and expiratory pressures were assessed using a commercially available pulmonary function testing system (Vmax Encore 229, V62J

Autobox; CareFusion, Yorba Linda, CA) according to standard recommendations (137-140). Pulmonary function measurements were expressed in absolute values and as a percentage of predicted normal values (6-8).

Exercise protocol. During both visits, exercise testing was conducted on an electronically braked cycle ergometer (Ergoselect 200P; Ergoline, Bitz, Germany). During visit 1, exercise testing began with a 6 min rest period followed by 1 min of unloaded pedaling then 20 W step-wise increases in workload (starting at 20 W) every 2 min until volitional exhaustion. Peak work rate was defined as the highest work rate sustained for at least 30 s. Visit 2 included 4 identical exercise protocols, each performed under 1 of 4 randomly selected experimental conditions. Each constant-load cycle exercise test was preceded by a 6 min rest period and a 1 min warmup consisting of unloaded pedaling. After the warm up, power output progressively increased in a ramp fashion over 1 min up to each individual's predetermined constant load, which was then held for 5 min. The exercise intensity for the constant load exercise tests was set at each individual's first ventilatory threshold (V_{Th}), which was determined using a combination of methods based upon the gas exchange data obtained during the incremental exercise test performed during visit 1 as previously described (174,175). The V_{Th} for each individual was defined as the work rate that was most congruent among the different threshold-determination methods. Subjects maintained similar cycling cadence across all constant-load cycle exercise tests, and 15-30 min of rest was allowed between trials.

Experimental conditions. During visit 2, subjects performed a series of constant load cycle exercise tests under 4 experimental conditions with the primary goal of manipulating mechanical

ventilatory constraint during exercise. In order to reduce the magnitude of mechanical ventilatory constraint, subjects breathed a normoxic helium-oxygen inspirate (HEL). Replacing nitrogen with helium as the backing gas reduces resistance to flow, and increases the ability to generate flow (176). Thus, helium reduces the potential of experiencing EFL by increasing $\dot{V}_{E CAP}$ at a given lung volume (133), and could theoretically result in a reduction in the resistive component of W_b (176). To increase mechanical constraint, subjects breathed room air through a resistor placed in the inspiratory limb of the breathing apparatus (RES). The aperture of the resistor was tailored to each subject in order to increase inspiratory resistance to approximately 5 cmH₂O·1·s⁻¹ (average: 5.7 ± 0.3 cmH₂O·1·s⁻¹, range: 4.2-6.8 cmH₂O·1·s⁻¹) thereby increasing W_b for a given \dot{V}_E . As a control condition, subjects breathed room air through an unobstructed breathing circuit (CON). Lastly, we also had subjects perform a trial where they breathed a mildly hyperoxic gas mixture (HOX) in order to resolve any attendant EIAH (if present), which could theoretically affect the perception of dyspnea (177). The level of hyperoxia ($FiO_2=26\%$) was selected in order to slightly increase oxygen delivery to the exercising muscle without significantly reducing the drive to breathe.

During all constant load cycle exercise trials, inspired gas was delivered by connecting a non-diffusing 100 l reservoir bag (Vacumed model 1196-100, Ventura, CA) to the inspired limb of the breathing circuit. The reservoir bag was connected to a series of compressed gas tanks that delivered gas through a humidifier. The order of the experimental conditions was randomized and performed in a counterbalanced fashion with subjects blinded. We took several measures to ensure that subjects were blinded to the experimental condition. First, the breathing apparatus remained identical in appearance for each trial. Second, for all trials, compressed air was delivered through reservoir connected to the inspired limb of the breathing circuit. Finally, all

calibration and setup procedures between trials were performed in an identical fashion. We confirmed that subjects were blinded by asking them to guess which experimental condition they had completed after each trial. On average, subject guessed correctly on $27\pm8\%$ of occasions.

Flow, respiratory pressures, and diaphragm electromyography. During rest and exercise on both visits, subjects breathed through a mouthpiece connected to a two-way non-rebreathing valve (Hans Rudolph 2700B, Hans Rudolph, Kansas City, MO, USA). Inspired and expired flow were measured using individual heated, calibrated pneumotachographs (model 3813, Hans Rudolph, Kansas City, MO, USA), and volume was obtained by numerical integration of the flow signals. P_{mo} was sampled through a port in the mouthpiece connected to a calibrated differential pressure transducer (DP15-34, Validyne Engineering, Northridge, CA, USA). During visit 2, subjects were instrumented with a multi-pair esophageal electrode catheter equipped with two balloons, which was used to measure P_{eso}, P_{ga}, and EMG_{di}. P_{eso} and P_{ga} were measured by connecting the distal end of each respective balloon on the dual-balloon catheter to independent, calibrated differential pressure (Pdi) was then calculated as the difference between Pga and Peso. EMG_{di} was measured by connecting the catheter to a grounded bio-amplifier (model RA-8, Yinghui Medical Technology Co. Ltd., Guangzhou, China).

Cardiorespiratory responses. Standard cardiorespiratory measures were recorded at rest and during exercise using a commercially available metabolic cart (True One 2400, Parvomedics, Sandy, UT, USA). Heart rate and electrocardiogram changes were monitored continuously using a 12-lead electrocardiogram (Cardiosoft Diagnostics System v6.71, GE Healthcare, Canada) and
arterial oxygen saturation was measured using a pulse oximeter (Radical-7, Massimo Corporation, Irvine, CA, USA). End-tidal CO₂ ($P_{ET}CO_2$) was sampled through a port in the mouthpiece connected to a calibrated CO₂ analyzer (Vacumed model 17630, Ventura, CA). Since helium interferes with the infrared signal used by the CO₂ analyzers to determine CO₂ concentration, the CO₂ analyzers were calibrated using two different calibration gases. One calibration gas had nitrogen as a backing gas (for CON, RES, and HOX trials), whereas the other contained helium as the backing gas (for the HEL trial). The CO₂ analyzers were calibrated to the appropriate gases before each trial.

Maximal ventilatory capacity and operational lung volumes. Prior to and following exercise during visit 1, subjects performed a series of FVC maneuvers at different efforts in order to construct MEFV curves by taking into account exercise-induced bronchodilation and thoracic gas compression (136). In order to account for the effect of helium on the MEFV curve, the same series of FVC maneuvers was repeated with helium instead of room air immediately after the last constant load exercise trial during visit 2. Inspiratory capacity maneuvers were performed at rest and at the end of each exercise stage during visit 1. During visit 2, inspiratory capacity maneuvers were performed at rest as well as at the 6 min mark during each constant load exercise tests. Operating lung volumes (EILV and EELV) were derived from each inspiratory capacity maneuver as previously described (143). Theoretical maximum ventilation (\dot{V}_{ECAP}) was then calculated at rest and for each exercise stage and for each constant load exercise test based on the maximum expiratory airflow throughout an composite averaged tidal breath and the corresponding operating lung volumes as previously described (144). Fractional utilization of available ventilatory capacity (\dot{V}_E/\dot{V}_{ECAP}) was determined as the quotient of \dot{V}_E and \dot{V}_{ECAP} . The

presence of EFL was determined by positioning each flow-volume within the corresponding MEFV curve according to the measured EELV lung volume, as previously described (144). The magnitude of EFL was then calculated as the % overlap between the expiratory portion of the tidal breaths and the MEFV curve.

Perceptual responses. At rest and during exercise, subjects rated the intensity of "breathing discomfort" (dyspnea) and "leg discomfort" using the modified category-ratio 0–10 Borg scale (111). Dyspnea was defined as "the sensation of labored or difficult breathing" and leg discomfort was defined as the "sensation of leg muscle fatigue". The endpoints of the scale were anchored such that 0 represented "no breathing/leg discomfort" and 10 represented "the most severe breathing/leg discomfort ever experienced or imagined". During visit 1, perceptual responses were recorded at rest and during the last 30 s of each exercise stage. During visit 2, perceptual responses were recorded at rest and at the end of each minute during the constant load exercise tests. Given the subjective nature of the perception of breathing and leg discomfort during exercise, during visit 2, perceptual responses were assessed by a blinded experimenter.

Data processing and analysis. During both visits, flow, volume, respiratory pressures, $P_{ET}CO_2$, and EMG_{di} data were collected during rest and throughout exercise using a 16-channel analog-todigital data acquisition system (PowerLab, ADInstruments, Colorado Springs, CO, USA), sampled at 2000 Hz, and recorded using LabChart 7.3.7 software. Raw EMG_{di} signals were amplified and band-pass filtered between 20 and 1000 Hz (Biomedical Amplifier, Guangzhou Yinghui Medical Equipment Co Ltd, Guangzhou, China) and converted to a root mean square (RMS) using a time constant of 100 ms and a moving average window. EMG_{di} data were analyzed on a breath-by-breath basis, where for each breath, peak RMS data was obtained by manually selecting RMS signals falling between zones of cardiac artefact (167). The electrode pair with the largest EMG_{di} amplitude for each breath was used for the analysis, and the associated EMG_{di} data were then expressed as a percent of maximum EMG_{di} activity (EMG_{di,max}), defined as the highest level of EMG_{di} observed during an inspiratory capacity maneuver at rest or during exercise (168). The ratio between EMG_{di} expressed as a function of EMG_{di,max} and V_T normalized to vital capacity was used as an index of NMU, as previously described (95,154). Flow, volume, and pressures were composite averaged using customized software, and total W_b was then calculated by integrating the area within the esophageal pressure–volume curve, as previously described (145). All cardiorespiratory and perceptual variables were analyzed during the last 2 minutes of each constant load exercise test.

Statistical analysis. Subjects characteristics, pulmonary function, and peak exercise variables were compared between the sexes using Student's unpaired *t*-test. A 2×4 (sex [male and female] by condition [CON, RES, HEL, OXY]) repeated measures analysis of variance was used to test for differences in perceptual and cardiorespiratory variables during the last 2 min of the constant load exercise tests. When significant *F* ratios were detected, Tukey's post hoc test was used to determine the location of group mean differences where appropriate. The prevalence of EFL were analysed during the constant load exercise tests as frequency statistics; comparisons were made between conditions using Fischer's Exact Test. Significance was set at *p*<0.05 and data are presented as mean \pm SEM unless otherwise stated.

4.3 Results

Subject characteristics and pulmonary function. Table 4-1 summarizes descriptive characteristics and pulmonary function data for all subjects. Resting pulmonary function was within the normal predicted range for both groups. When expressed in absolute terms, the majority of pulmonary function measures were greater in men than women (all p<0.05), with the exception of FEV₁/FVC (p=0.56), and FEF_{25-75%} (p=0.74).

Incremental exercise test data. Peak exercise data from the incremental exercise performed during visit 1 are shown in Table 4-2. At peak exercise, there was a significant effect of sex on absolute $\dot{V}O_2$, work rate, \dot{V}_E , V_T , and \dot{V}_{ECAP} (all p<0.05). When $\dot{V}O_2$ at peak exercise was expressed as a percent of predicted values, there was no significant effect of sex, indicating that subjects had statistically similar levels of relative fitness. On average, subjects in both groups achieved respiratory exchange ratios >1.10 and near maximum heart rates based on predicted normal values, indicating that maximal effort was exerted across groups. The perception of dyspnea and leg discomfort during incremental exercise in men and women are shown in Figure 4-1. Women had higher perceptions of dyspnea than men for any given work rate at or above 60 W during incremental exercise (all p<0.05). However, when work rate was expressed in relative terms, the effect of sex was no longer present (p>0.05, data not shown). There were no sex-differences in the perception of leg discomfort for any given absolute or relative work rate during incremental exercise (both p>0.05).

Exercise data at V_{Th} in both groups are presented in Table 4-3. On average, men and women reached V_{Th} at similar fractions of peak exercise $\dot{V}O_2$ (*p*=0.85), but since men were working at a higher absolute work rates, they had a higher absolute $\dot{V}O_2$, $\dot{V}CO_2$, \dot{V}_E , and V_T than

women (all p < 0.05). All other cardiorespiratory and perceptual variables at V_{Th} were similar between the sexes (all p > 0.05).

Mechanical ventilatory response to constant load exercise. The ventilatory response to constant load exercise under all four experimental conditions is depicted in panel A of Figure 4-2. There was a significant effect of condition, sex, and their interaction on \dot{V}_E (all *p*<0.05). Specifically, \dot{V}_E was significantly lower during the RES relative to CON (*p*<0.05), while \dot{V}_E during the HEL and HOX trial was not significantly different from CON (both *p*>0.05). We also observed that women had a significantly lower \dot{V}_E than men during the CON, and HEL trials (both *p*<0.05), but statistically similar \dot{V}_E during the RES and HOX trials (both *p*>0.05). The fractional utilization of $\dot{V}_E/\dot{V}_{E CAP}$ is shown in panel B of Figure 4-2. We found that there was a significantly lower during the HEL trial relative to CON (*p*=0.03), but similar to CON during the RES and HOX (both *p*>0.05). There were no significant differences in operating lung volumes on the basis of sex or condition (all *p*>0.05; Figure 4-2, panel C).

The W_b during each constant load exercise test is depicted in panel A of Figure 4-3. As expected, the RES trial significantly increased W_b relative to CON, while the HEL significantly reduced W_b relative to CON (both p<0.05). By contrast, there was no significant effect of HOX on W_b when compared to CON (p=0.14). Figure 4-4 shows the average W_b for each group during each constant load exercise test as a function of \dot{V}_E superimposed over previously determined sex- and age-specific regression equations (see Chapter 2). The pattern of EMG_{di} activity followed a similar pattern between trials as the W_b (Figure 4-3, panel B), where relative to CON the RES trial significantly increased EMG_{di} (p=0.007), the HEL significantly reduced EMG_{di} (p=0.03), but did not have a significant effect on HOX (p=0.61). Panel C of Figure 4-3 shows the average degree of NMU during each constant load exercise test. There was a significant effect of sex and condition on NMU (both p<0.05), but not their interaction (p=0.76), where men had a higher degree of NMU than women across conditions, and NMU was higher in all subjects during the RES trial relative to CON.

The frequency of EFL during each constant load exercise test is shown in Figure 4-5. During the CON trial, 7 (n=3 men, n=4 women) of 12 subjects were flow limited, and the frequency of EFL was not significantly different relative to CON during the RES (5 of 12 subjects, p=0.68) and HOX (4 of 12 subjects, p=0.41) trials. However, 0 of 12 subjects were flow limited during the HEL trial, a significant reduction in the frequency of EFL relative to CON (p=0.005).

Perceptual responses to constant load exercise. The perception of dyspnea and leg discomfort during the constant load exercise tests are shown in Figure 4-6. There was a significant effect of sex on dyspnea, where women had higher perceptions of dyspnea than men across trials (p=0.03; Figure 4-6, panel A). However, there was no significant effect of condition on the perception of dyspnea during constant load exercise (p=0.10). Moreover, there was no significant effect of sex or condition on the perception of leg discomfort (both p>0.05; Figure 4-6, panel B).

4.4 Discussion

Major findings. We determined the effect of experimentally manipulating mechanical ventilatory constraint during short bouts of exercise at V_{Th} on the perception of dyspnea in healthy older men and women. The major findings from our study are twofold. First, we found that women

had a greater perception of dyspnea during short-duration constant load exercise at V_{Th} across a series of four experimental conditions. Second, we determined that acutely increasing or decreasing the degree of mechanical ventilatory constraint during submaximal exercise at the same relative intensity in healthy older men and women does not affect the perception of dyspnea, at least during short bouts of exercise at V_{Th} . Our results do not support the hypothesis that sex-differences in respiratory mechanics during exercise are caused by sex-differences in exercise in healthy older adults, at least during submaximal exercise at V_{Th} .

Sex-differences in the perception of dyspnea. During incremental exercise, we found that women had a significantly higher dyspnea for a given absolute work rate at or above 60 W (Figure 4-1), an observation that is consistent with our previous data (see Chapter 2) and that of others (74,109). During the incremental exercise test, women reported levels of dyspnea at V_{Th} that were on average 1.2 Borg units greater than in men; however, this difference did not reach statistical significance (*p*=0.18, Table 4-3). However, during the constant load exercise tests at V_{Th} , women reported significantly higher levels of dyspnea than men by an average of 1.3 Borg units across experimental conditions (Figure 4-6). The discrepancy between the presence of a significant sex-difference in dyspnea during constant load exercise tat V_{Th} and the absence thereof at the same exercise intensity during incremental exercise likely reflects differences in exercise modality, the timing of dyspnea measurements, and the fact that multiple observations were made for each individual. Nevertheless, the salient question is what mechanism causes the observed sex-differences in dyspnea during exercise in healthy older individuals?

The neurophysiological mechanisms of dyspnea are complex and multifactorial (177). During exercise, dyspnea in older individuals is thought to reflect in the perception of increased respiratory effort required to meet the body's increased ventilatory demands. Sex-differences in the mechanical ventilatory response to exercise in healthy younger and older adults have previously been observed (74,85). Regardless of age, women have a higher W_b for a given $\dot{V}_E \ge 50-65 \ 1 \cdot \text{min}^{-1}$ than men (see Chapter 2), which has been ascribed to women having a higher resistive component of W_b (20,93). Additionally, older women appear to have a higher propensity towards EFL during exercise than older men (see Chapter 2). Therefore, we reasoned that if older women have a greater degree of mechanical ventilatory constraint during exercise than do older men, which would impose a greater load on their respiratory muscles, then the perception of increased respiratory effort may serve to explain the sex-differences in exertional dyspnea in healthy older men and women.

Manipulations of mechanical ventilatory constraint during exercise. We experimentally altered the magnitude of mechanical ventilatory constraint during a series of short-duration constant load exercise tests in a group of healthy older men and women. During the RES trial, we increased the resistive component of W_b such that the total W_b remained within the normal physiological range. Based on data from our previous work in older men and women (see Chapter 2), we increased W_b in older men to a similar extent than would be observed in older women at the same absolute \dot{V}_E . We also increased the resistive and total W_b in women to a similar extent than in men, albeit at a significantly lower absolute \dot{V}_E (Figure 4-5). Thus, if the sex-difference in W_b contributed to sex-differences in dyspnea, we would expect to observe an increase in dyspnea in men to a similar level than women during the CON trial, and an increase in dyspnea in women over and above their CON trial. However, regardless of sex, dyspnea did not increase relative to CON during the RES trial (Figure 4-6). Experimental studies in healthy

young individuals involving the addition of an external resistive loads during exercise have shown that dyspnea increases in a resistance-dependent fashion (124,178). However, the level of added resistance ranged from 33-73 cmH₂O·1·s⁻¹, which greatly exceeds the resistance of the intrathoracic airways of individuals with even the most profound degrees of pathological airway obstruction (179) thereby limiting the generalizability of the findings. Conversely, another study used a similar experimental protocol but with a much lower level of added resistance (2.7 cmH₂O·1·s⁻¹) and found that healthy individuals did not report higher levels of dyspnea during exercise with an added external resistance relative to control (180). In our study, the level of external inspiratory resistance was 5.7 ± 0.3 cmH₂O·1·s⁻¹, which resulted in an increase in W_b that never exceeded the absolute values observed during maximal exercise in a similar group of healthy older individuals (see Chapter 2). Thus, our data suggest that increasing the W_b during short-duration constant exercise at V_{Th} does increase the perception of dyspnea in healthy older adults, regardless of sex.

By using a normoxic helium-oxygen inspirate, we were able to reduce the magnitude of mechanical ventilatory constraint during the HEL trial. Breathing helium during exercise has been shown to eliminate EFL and reduce the resistive W_b in healthy younger individuals (104,133,181). Since older women have a higher propensity towards EFL and a higher resistive component of W_b than older men (see Chapter 2), we surmised the HEL trial should result in a reduction in dyspnea in women to a level equivalent to that of men during the CON trial, while only having a small positive effect on dyspnea relative to the CON trail in men. However, HEL had no significant effect on dyspnea (Figure 4-6).

Healthy aging is known to reduce pulmonary gas exchange efficiency at rest (58,182,183). While body temperature corrected blood gas data in healthy older individuals

during exercise are relatively sparse (14), it is reasonable to postulate that older individuals, particularly those of above-average fitness, may experience EIAH (184). Moreover, women may be more susceptible to EIAH than men due to sex-differences in mechanical ventilatory constraints (104). The HOX trial, which involved breathing a hyperoxic gas mixture (FiO_2 = 26%) was intended to relieve any attendant EIAH (if present) without substantially reducing the drive to breathe. This experimental approach has previously been used in healthy younger men and women during constant load exercise to exhaustion (185). However, dyspnea was unaffected by the HOX condition (Figure 4-6).

Mechanisms of dyspnea in healthy aging. During exercise, dyspnea is thought to arise as a result of a mismatch between respiratory motor output and the mechanical response to this output (132), also known as NMU (114). Previous studies have used EMG_{di} as a surrogate for neural respiratory drive to the diaphragm (172). While this approach has well documented limitations (186), it is commonly employed in combination with normalized measures of V_T to provide a quantitative estimate of NMU in both health and disease (95,154,187,188). In the present study, we manipulated the degree of mechanical ventilatory constraint during exercise in healthy older men and women, which correspondingly altered EMG_{di} (Figure 4-3, panel B). However, changes in EMG_{di} were not accompanied by proportional changes in normalized measures of V_T , which implies that we also experimentally manipulated NMU (Figure 4-3, panel C). Based on our results, we conclude that NMU is not the primary determinant of dyspnea during exercise in healthy older adults, at least during short bouts of exercise at relatively low intensities. Moreover, our results do not support the hypothesis that sex-differences in exertional dyspnea are caused by sex-differences in mechanical ventilatory constraint. The question then

becomes, if mechanical ventilatory constraint does not explain sex-differences in dyspnea during exercise in healthy older individuals, then what does?

Dyspnea is a complex sensation that arises through the interaction of mechanical, chemical, neural, affective, and sociocultural factors (177). Our study aimed to experimentally manipulate just one of these many contributing factors, and therefore several others are likely to explain the observed sex-differences in exertional dyspnea. If we narrow our perspective to physiological factors that are known differ during exercise on the basis of biological sex, one possible explanation relates to the pulmonary vascular response to constant load exercise. Recent data in healthy older men and women during moderate intensity exercise at approximately 72-74% of age-predicted maximal heart rate shows that older women have a greater pulmonary artery wedge pressure response compared to older men at the same relative exercise intensity (189). It is possible, albeit speculative, that the increased dyspnea in older women observed in the present study were the result of sex-differences in the hemodynamic response to exercise in healthy older adults. Nevertheless, the multifaceted nature of the mechanisms of dyspnea cannot be overstated. The notion that a single causal factor would explain the sex-differences in the perception of dyspnea in older individuals is appealing due to its conceptual tidiness, but is likely an oversimplification. Therefore, future studies that consider a large host of potentially dyspneogenic factors in a large population of healthy men and women are required in order to improve our understanding of the mechanistic basis of sex-differences in breathlessness during exercise.

Limitations. There are several limitations of our study that merit discussion. First, the constant load exercise bouts in the present study were performed at a moderate exercise intensity and the

results of our study cannot be generalized to exercise intensities above V_{Th}. Nevertheless, we chose to perform experimental trials at V_{Th} since it is commensurate with the intensity at which most individuals perform continuous endurance exercise training. Second, given the moderate intensity and short-duration of each exercise bout, the absolute dyspnea ratings in our study were relatively low ('slight' to 'moderate'). Therefore, it is possible that there is a perceptual (or perhaps even mechanical) threshold above which acute alterations in mechanical ventilatory constraints may influence dyspnea. Third, as is the case with any study involving a subjective primary outcome variable, the placebo effect may have confounded our results. However, we took great care in ensuring that the subjects and the experimenter tasked with asking the subjects to rate their perceptions of dyspnea were blinded to the experimental conditions. This was accomplished by standardizing the gas delivery method throughout trials, performing the same calibration procedures in the same order between trials, and instructing subjects not to speak for at least 1 min after the end of each trial due to the effect of helium on voice pitch. Fourth, in contrast to our previous study (see Chapter 2), we used the flow-volume overlap method of assessing the presence of EFL, a technique which has well documented limitations (144). However, we took several precautions to ensure that our assessments of EFL were as accurate as possible, including: i) familiarizing subjects to the performance of inspiratory capacity maneuvers during exercise, ii) assessing the quality of inspiratory capacity maneuvers based on Peso, and iii) constructing a composite MEFV curve for each individual by accounting for the effects of thoracic gas compression and bronchodilation (136). Lastly, our manipulations of mechanical ventilatory constraint were relatively modest. Although we could have utilized a proportional assist ventilator to further reduce the W_b (185,190), the reduction in W_b would have been the result of a reduction in the both the resistive and viscoelastic components of W_b, and

would have made blinding subjects to the experimental condition impossible. Similarly, we could have used a higher resistive load, however this would not have been representative of the resistive work associated with exercise hyperphoea in healthy individuals.

Perspectives. In our study, acutely manipulating the degree of mechanical ventilatory constraint during short bouts of exercise at V_{Th} did not have a significant effect on the perception of dyspnea in healthy older men and women. We recognize that the contextual nature of our study and the relatively small sample size employed limit the generalizability of our findings. Therefore, we provide the following perspectives. First, the perception of dyspnea during the constant load exercise tests was considered 'slight' to 'moderate' according to the Borg scale (111). Although the degree of mechanical ventilatory constraint did not appear to contribute to dyspnea at V_{Th}, our findings cannot be extrapolated to higher exercise intensities where the perception of dyspnea is much greater. Moreover, we cannot rule out the possibility that the degree of mechanical ventilatory constraint influences dyspnea at exercise intensities above V_{Th}. Second, our comparisons between older men and older women were made at a similar relative exercise intensities, but only for short periods of time (i.e. 6 min). It is unknown how the time course of the perception of dyspnea would evolve during longer bouts of exercise, nor how mechanical ventilatory constraint might influence this time course. Lastly, the finding of sexdifferences in the perception of dyspnea during exercise is not unique to healthy older individuals; sex-differences in the perception of activity-related breathlessness have been reported in studies involving healthy young individuals (94,95), asthmatics (191), and individuals with COPD (192-194). While it is tempting to generalize our findings to other populations, we emphasize that this is not possible. It is currently unclear how aging, biological

sex, and respiratory disease interact to influence the perception of breathing discomfort during physical exertion.

Conclusion. Acutely manipulating the magnitude of mechanical ventilatory constraint during short bouts of moderate-intensity exercise in healthy older men and women did not have an effect on the perception of dyspnea. We conclude that although sex-differences in respiratory mechanics are evident in healthy older adults, they do not appear to contribute to the magnitude of exertional dyspnea, at least during short bouts of submaximal exercise. Thus, the higher levels of dyspnea observed in older women relative to older men may be caused by physiological measures that were not assessed in the present study, or by non-physiological factors. Future work is required to determine the mechanisms that lead to sex-differences in dyspnea perception in older adults.

	Men	Women	
Age, y	69±3	65±2	
Height, cm	174±3	165±3	*
Body Mass, kg	77±5	61±4	*
BMI, kg m ⁻²	26±2	23±2	
FVC, 1	4.37±0.19	3.45±0.27	*
FVC, % predicted	102±3	105±6	
FEV ₁ , 1	3.03 ± 0.08	2.59±0.22	*
FEV ₁ , % predicted	100±3	107±7	
FEV ₁ /FVC	70±2	75±2	*
FEV ₁ /FVC, %predicted	98±4	102±3	
PEF, $1 \cdot \sec^{-1}$	8.10±0.46	7.02±0.23	*
FEF_{25-75} , $1 \cdot \text{sec}^{-1}$	2.04 ± 0.18	2.17±0.36	
FEF ₂₅₋₇₅ , %predicted	81±10	86±13	
TLC, 1	6.92±0.28	5.26±0.37	*
TLC, % predicted	103±3	101±5	
VC, 1	4.53±0.20	3.54±0.25	*
VC, % predicted	102±3	107±6	
IC, 1	3.16±0.35	2.28±0.27	*
IC, % predicted	104±12	92±10	
FRC, 1	3.76±0.38	2.98±0.17	*
FRC, % predicted	84±7	100±10	
RV, 1	2.39±0.11	1.72±0.18	*
RV, % predicted	96±3	88±9	
DL _{co} , ml·min ⁻¹ ·mmHg ⁻¹	26±2	19±2	*
DL _{co} , % predicted	103±5	94±5	
MIP, cmH_2O	-111±8	-83±8	*
MIP, % predicted	106±7	115±11	
MEP, cmH_2O	135±19	95±8	*
MEP, % predicted	70±10	70±6	

Table 4-1. Baseline subject characteristics and pulmonary function data

Abbreviations: BMI, body mass index; FVC, forced vital capacity; FEV₁, forced expired volume in 1 s; PEF, peak expiratory flow; FEF₂₅₋₇₅, forced expired flow between 25 and 75% of FVC; TLC, total lung capacity; VC, vital capacity; IC, inspiratory capacity; FRC, functional residual capacity; RV, residual volume; DLco, diffusion capacity of the lung for carbon monoxide; MIP, maximal inspiratory pressure; MEP, maximal expiratory pressure. All data are presented as mean \pm SE.* *p*<0.05, men vs. women.

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	Men	Women	
$\dot{V}O_2$, $1 \cdot min^{-1}$	2.43±0.25	1.71±0.11	*
$\dot{V}O_2 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$	31.6±4.4	28.0±1.3	
^{VO} ₂, % predicted	107±9	112±6	
$\dot{V}CO_2$, $1 \cdot min^{-1}$	2.74±0.30	1.87 ± 0.08	*
RER	1.13±0.02	1.11±0.03	
HR, beats \cdot min ⁻¹	158±5	159±2	
HR % predicted	105±4	101±2	
$S_pO_2, \sqrt[9]{6}$	97±1	99±1	
V _T , 1	2.36±0.10	1.73±0.14	*
F_b , breaths min ⁻¹	44±5	39±2	
\dot{V}_{E} , $1 \cdot min^{-1}$	103±9	67±5	*
$\dot{V}_E/\dot{V}O_2$	43.2±3.6	39.4±3.0	
$\dot{V}_E/\dot{V}CO_2$	39.1±2.6	35.6±2.1	
P _{ET} CO ₂ , mmHg	31.8±1.6	33.9±1.9	
Work rate, W	187±24	140±7	*
EELV (% TLC)	57±3	57±3	
EILV (% TLC)	91±2	91±2	
\dot{V}_{ECAP} (1·min ⁻¹)	128.6±10.6	105.3±4.2	
$\dot{V}_{E}/\dot{V}_{ECAP}$ (%)	82.7±11.0	64.2±6.51	
Dyspnea, Borg scale	5.5±1.3	6.0±0.5	
Leg Discomfort, Borg scale	7.8±0.9	6.8±0.9	

Table 4-2. Cardiorespiratory and perceptual variables at peak exercise during the incremental exercise test performed on visit 1

Abbreviations: $\dot{V}O_2$, oxygen uptake; $\dot{V}CO_2$; carbon dioxide output; RER; respiratory exchange ratio; HR, heart rate; S_pO_2 , oxygen saturation by pulse oximetry; V_T , tidal volume; F_b , breathing frequency; \dot{V}_E , minute ventilation; $\dot{V}_E/\dot{V}O_2$, ventilatory equivalent for oxygen; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent for carbon dioxide; $P_{ET}CO_2$, end-tidal carbon dioxide; EELV, end-expiratory lung volume; EILV, end-inspiratory lung volume; W_b , work of breathing; \dot{V}_{ECAP} , ventilatory capacity. All data are presented as mean±SE. * p<0.05, men vs. women.

•	Men	Women	
$\dot{V}O_2$, $1 \cdot min^{-1}$	1.80±0.20	1.25±0.08	*
$\dot{V}O_2 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$	23.9±3.9	20.6±1.0	
VO₂, %max	74±3	74±3	
$\dot{V}CO_2$, $1 \cdot min^{-1}$	1.79±0.22	1.21±0.08	*
RER	0.99±0.03	0.97±0.03	
HR, beat \cdot min ⁻¹	123±12	126±5	
HR %max	77±6	79±3	
S _p O ₂ , %	97±1	98±1	
V _T , 1	2.06±0.14	1.43±0.12	*
F_b , breaths min ⁻¹	28±3	26±1	
\dot{V}_{E} , $1 \cdot min^{-1}$	56±6	38±3	*
$\dot{V}_E/\dot{V}O_2$	31.6±2.2	29.9±1.6	
$\dot{V}_E/\dot{V}CO_2$	32.6±1.6	30.9±1.3	
P _{ET} CO ₂ , mmHg	39.4±1.2	40.9±1.5	
Work rate, W	117±17	90±4	*
EELV (% TLC)	52±2	54±3	
EILV (% TLC)	82±3	81±4	
\dot{V}_{ECAP} (1·min ⁻¹)	95.5±11.0	93.6±10.9	
$\dot{V}_{E}/\dot{V}_{ECAP}$ (%)	61.2±6.4	43.5±7	
Dyspnea, Borg scale	1.92 ± 0.55	3.1±0.6	
Leg Discomfort, Borg scale	2.67±0.4	3.3±0.7	

Table 4-3. Cardiorespiratory and perceptual variables at V_{Th} during the incremental exercise test performed on visit 1

Abbreviations: V_{Th} ; first ventilatory threshold; $\dot{V}O_2$, oxygen uptake; $\dot{V}CO_2$; carbon dioxide output; RER; respiratory exchange ratio; HR, heart rate; S_pO_2 , oxygen saturation by pulse oximetry; V_T , tidal volume; F_b , breathing frequency; \dot{V}_E , minute ventilation; $\dot{V}_E/\dot{V}O_2$, ventilatory equivalent for oxygen; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent for carbon dioxide; $P_{ET}CO_2$, end-tidal carbon dioxide; EELV, end-expiratory lung volume; EILV, end-inspiratory lung volume; \dot{V}_{ECAP} , ventilatory capacity. All data are presented as mean±SE. * *p*<0.05, men vs. women.



Figure 4-1. Perceptions of dyspnea and leg discomfort during incremental cycle exercise in men and women.

For both groups, dyspnea data are shown in Panel A, and leg discomfort data are shown in Panel B. The highest equivalent work rate achieved by all subjects was 100 W. Dashed lines within each group connect the 100 W data point to the peak exercise data point. The work rates corresponding to V_{Th} is depicted by the grey dotted line for men and the block dotted line for women. All data are presented as mean±SE. * p<0.05, men vs. women.



Figure 4-2. Group mean \dot{V}_E , $\dot{V}_E/\dot{V}_{E CAP}$, and operating lung volumes during the last 2 min of each constant load exercise test.

For both groups, \dot{V}_E data are shown in Panel A, \dot{V}_E/\dot{V}_{ECAP} data are shown in Panel B, and operating lung volume data are shown in Panel C. In panel C, the grey shaded area represents average resting operating lung volumes for all subjects. \dot{V}_E , minute ventilation; \dot{V}_E/\dot{V}_{ECAP} , fractional utilization of ventilatory capacity; CON, control trial; RES, resistor trial; HEL, helium trial; HOX, 26% oxygen trial. **p*<0.05, main effect of condition, † *p*<0.05, main effect of sex. ‡ *p*<0.05, interaction between condition and sex.



Figure 4-3. Group mean W_b, EMG_{di}, and NMU during the last 2 min of each constant load exercise test.

For both groups, W_b data are shown in Panel A, EMG_{di} data are shown in Panel B, and NMU data are shown in Panel C. W_b, work of breathing; EMG_{di}, diaphragm electromyography; EMG_{di,max}, maximum EMG_{di} activity ; NMU, neuromechanical uncoupling; V_T, tidal volume; VC, vital capacity; CON, control trial; RES, resistor trial; HEL, helium trial; HOX, 26% oxygen trial. *p<0.05, main effect of condition, † p<0.05, main effect of sex. ‡ p<0.05, interaction between condition and sex.



Figure 4-4. W_b during the last 2 min of each constant load exercise test.

Group averages are shown for each condition and juxtaposed over regression lines for older men and older women (see Chapter 2). W_b, work of breathing; CON, control trial; RES, resistor trial; HEL, helium trial; HOX, 26% oxygen trial.



Figure 4-5. The number of flow limited subjects in each group during the last 2 min of each constant load exercise test.

EFL; expiratory flow limitation; CON, control trial; RES, resistor trial; HEL, helium trial; HOX, 26% oxygen trial. *p<0.05, significant difference between conditions.



Figure 4-6. Group mean perceptions of dyspnea and leg discomfort during the last 2 min of each constant load exercise test.

For both groups, dyspnea data are shown in Panel A, and leg discomfort data are shown in Panel B. CON, control trial; RES, resistor trial; HEL, helium trial; HOX, 26% oxygen trial. *p<0.05, main effect of condition, † p<0.05, main effect of sex. ‡ p<0.05, interaction between condition and sex.

Chapter 5: Conclusions

5.1 Overall Summary

According to the World Health Organization, the fastest-growing portion of the world's population is above the age of 60 (195). For example, in Canada, the proportion of population aged 65 and above has increased from 5% in 1921 to 14.8% in 2011, and is projected to reach approximately 23% by 2041 (196). Consequently, there are increasing economic, social, ethical, and political pressures to understand the mechanisms and etiology of aging, particularly when considering its impact on health. Clinicians and scientists are familiar with the pathophysiology of various diseases commonly encountered in geriatric patients, but are generally less aware of the physiologic changes that occur as part of the normative aging process (21). It is well known that healthy aging adversely affects the respiratory system (197) and that these changes cannot be mitigated by habitual exercise (198). Nevertheless, exercise is widely recognized as an integral component of healthy aging primarily due to its many prophylactic effects (199). It is therefore essential to clearly determine how healthy aging of the respiratory system affects the ventilatory response to exercise. Although a great deal of work has been conducted in this area (14,42), our understanding remains incomplete.

It has recently become evident that there exist important sex-differences in the structure of the respiratory system that affect mechanical ventilatory and perceptual responses to exercise. However, most of the data relating to this topic is based on studies performed in healthy young subjects. Indeed, there is a profound lack of data that describes how these sex-differences evolve with advancing age (18). The results of this thesis provide important information which contributes to improving our understanding of how healthy aging of the respiratory system might differentially affect women. First, I performed a comprehensive study to compare the mechanical ventilatory and perceptual responses to exercise between groups of healthy younger and older, men and women (see Chapter 2). We found that sex-differences in the degree of mechanical ventilatory constraint during exercise are present in healthy older individuals. Specifically, older women have a higher W_b for a given \dot{V}_E and a higher propensity towards EFL during exercise than older men. We also reproduced the findings from other laboratories by showing that women report higher levels of dyspnea during cycle exercise at a given absolute work rate at or above 80 W than older men. We further demonstrated that the perception of dyspnea during cycle exercise at a fixed intensity was significantly related to indices of mechanical ventilatory constraint. These findings lead to the hypothesis that the magnitude of mechanical ventilatory constraint may be the mechanism for sex-differences in dyspnea during exercise in older individuals.

Second, I analyzed the electromyographic activity of three inspiratory muscles during exercise in the same cohort of participants as described above in order to characterize the independent and combined effects of biological sex and healthy aging on inspiratory muscle recruitment patterns (see Chapter 3). I found that during incremental exercise, women had a higher activation of the scalene, and sternocleidomastoid muscles than men for a given absolute or relative \dot{V}_E . We also observed that older individuals have a higher activation of sternocleidomastoid muscles given absolute or relative \dot{V}_E during exercise. The results from this second study extend those of the first by identifying that the effects of biological sex and age on the mechanical ventilatory response to exercise also exert significant independent effects on the pattern of inspiratory muscle recruitment.

Third, I tested the hypothesis that sex-differences in mechanical ventilatory constraint during exercise in older adults contribute to sex-differences in the perception of dyspnea. By experimentally manipulating the degree of mechanical ventilatory constraint during exercise, I was able to show that sex-based differences in exertional dyspnea in healthy older individuals are not related to differences in respiratory mechanics, at least during short during exercise at V_{Th} . These results emphasize the importance of considering other factors (both physiological and non-physiological) in future studies aimed at determining the causes of sex-differences in exertional dyspnea in healthy older adults.

5.2 Significance

The findings of this thesis are important for several reasons. First, the basic physiology of human aging and its effect on the respiratory system is not fully understood. Moreover, the vast majority of studies have been conducted in men rather than women, particularly in the context of exercise physiology (200). Thus, by characterizing the mechanical ventilatory response to exercise in healthy older men and women, the results contained within Chapters 2 and 3 contribute to advancing our understanding of human physiology as it pertains to the female respiratory system. Second, the results of this thesis constitute a significant step towards identifying the mechanistic basis for sex-based differences in exercise in symptom perception are important if we are to provide a scientific basis for the prescription of exercise in health and disease.

5.3 Strengths and Limitations

This thesis has several strengths and limitations that merit further discussion beyond those outlined in detail within Chapters 2, 3, and 4.

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For the studies described in Chapter 2 and 3, collecting a comprehensive set of data in a large cohort (n=42) of healthy older and younger, men and women allowed us to assess the independent and combined effect of sex-differences in the mechanical and perceptual responses to exercise. The design of the studies described in Chapters 2 and 3 gave us good statistical power and allowed us to contextualize our findings relative to previous work individually performed in younger subjects (11,93) and older subjects (4,5). An additional strength was our use of the most objective and validated methods that were feasible for each study to assess our primary outcome variables. For example, we used an esophageal catheter to assess EMG_{di} (see Chapters 3 and 4), rather than skin surface electrodes placed in the 7th or 8th intercostal spaces, and we used the NEP technique to assess the presence of EFL (see Chapter 2) rather than the more conventional flow-volume loop overlap method. The use of such novel methods, while technically demanding, greatly improved the precision with which we could assess our primary outcome measures.

The primary limitation of the work contained within this thesis concerns selection bias and generalizability. When conducting research on the physiological response to exercise in human subjects, it is important to appreciate that participants may self-select and therefore might not be representative of the general population, particularly in the case of invasive experiments. This issue is exacerbated in studies involving older adults. Based on the inclusion criteria of our studies, we likely ended up with a pool of participants that were healthier and more physically active than the general population. Thus, how our findings might apply to other populations such sedentary or obese individuals and those with chronic diseases remains unknown. Another limitation related to the sample size for the study outlines in Chapter 4. Although the number of subjects included in the study in is line with previous invasive physiological studies, it is possible that we were statistically underpowered. Finally, we did not assess non-physiological factors that are known to affect dyspnea, such as psychological and/or sociocultural differences between subjects. We acknowledge that since the observed sex-differences in dyspnea in older adults do not appear to be related to differences in respiratory mechanics, they are likely related to other factors.

5.4 Future Directions

While the results of this thesis answer several important questions, several unanswered questions remain. For example, what are the functional and metabolic consequences of sexdifferences in respiratory mechanics during exercise in healthy older individuals? Precisely measuring the oxygen cost of breathing during exercise in healthy older men and women is required in order to answer this question. While some data exists to suggest that older women have a higher oxygen cost of breathing during exercise than do older men (153), methodological flaws make the interpretation of these data difficult (201). However, if we assume that older women have a higher oxygen cost of breathing during exercise than do older men, and that aging negatively impacts structure and function of human respiratory muscles (163), a logical question that arises is do sex-differences in exercise-induced diaphragm fatigue still occur in older adults? Finally, despite the fact that respiratory mechanics do not appear to cause sex-differences in dyspnea, there are many other candidate physiological mechanisms that were not assessed in the context of this thesis, such as sex-differences in the pulmonary vascular response to exercise in older adults (189). Future studies should consider these factors, as well as a host of others in order to improve our understanding of the causes of dyspnea during exercise in older individuals as a whole, as well as the specific factors responsible for increasing the perception of dyspnea in

older women relative to older men. This information is essential in order to develop innovative approaches for the palliation exertional dyspnea in healthy older individuals, as well as patient populations. Answering these questions will require a substantial degree of work using unique experimental designs and methodologies.

5.5 Conclusion

The overall purpose of this thesis was to provide a comprehensive assessment of the mechanical ventilatory and perceptual responses to exercise in healthy older men and women and to determine if sex-differences in mechanical ventilatory constraint cause sex-differences in exertional dyspnea in healthy older adults. We found that healthy aging and biological sex independently increase the magnitude of ventilatory constraint during exercise in healthy adults. We also observed that older women have a higher perception of dyspnea during exercise than older men, which could be explained by the combined effects of age and sex on mechanical ventilatory constraint during exercise. However, acutely manipulating the magnitude of mechanical ventilatory constraint during moderate-intensity exercise did not have an effect of the perception of dyspnea. We conclude that although evident, sex-differences in respiratory mechanics during exercise do not contribute to sex-differences in the perception of dyspnea in older individuals. Future work is required to expand on these observations by determining the metabolic consequences of sex-differences in mechanical ventilatory constraint during exercise in healthy exercise in healthy exercise in healthy exercise in healthy exercise did not have an effect of the perception of dyspnea. We conclude that although evident, sex-differences in respiratory mechanics during exercise do not contribute to sex-differences in the perception of dyspnea in older individuals. Future work is required to expand on these observations by determining the metabolic consequences of sex-differences in mechanical ventilatory constraint during exercise in healthy older adults.

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