EFFECT OF INHALED NEBULIZED FUROSEMIDE ON BREATHLESSNESS DURING EXERCISE IN THE SETTING OF ABNORMAL RESTRICTIVE CONSTRAINTS ON TIDAL VOLUME EXPANSION: A RANDOMIZED, DOUBLE BLIND, PLACEBO CONTROLLED, CROSSOVER, DOSE-RESPONSE STUDY

by

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B.Sc., McGill University, 2014

A THESIS SUBMITTED TO McGILL UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS OF THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF EDUCATION

(Department of Kinesiology & Physical Education)

McGILL UNIVERSITY

MONTREAL, QUEBEC, CANADA

August 2017

ACKNOWLEDGEMENTS

Without a doubt, the first person that must be acknowledged is my supervisor Dr. Dennis Jensen. Without the opportunity to pursue a Master of Science under his supervision, I would not be the aspiring researcher that I am today. I have learned such an incredible amount of important information about the research process, as well as the minor but extremely relevant details that are involved in being a modern scientist. I would have not learned these to the extent that I did without Dr. Jensen's honest, straightforward, and frequent communication. In addition, Dr. Jensen's passion for physiology and attention to detail could not be more inspiring. The constant challenge to learn more, read more, think more, criticize more, and be more up to date will leave a lasting impression with how I approach my own research career. His ability to engage me to work independently while somehow always being accessible at a moment's notice has made these past two years challenging yet nurturing. Dr. Jensen has both taught me more than he realizes and has led me to enjoy the often-stressful research environment. In this regard, I am truly grateful for his mentorship.

I must thank Dr. Benjamin Smith for being easily reachable to approve electrocardiographs and sign prescription sheets despite an ever-changing schedule. I must thank Tara Greiss for keeping a sometimes-mundane hospital research experience fun and friendly, while also always being there to provide medication to my participants (*via* nebulization) and all other random favours. I must also thank Abdullatif Alhuzaim for his help with nebulization, even at odd hours of the day. These three made research at the Glen significantly more manageable.

I want to thank Noah Marchand, Corey-Martel Perry and Amar Mainra for the tremendous amount of time they contributed toward collecting and analyzing data, as well as all the small steps in between. I do not see how I would have had time to sleep without their help. I

want to give an important shout-out to my current and former lab mates Steven Murray, Sara Abdallah, Abdullatif Alhuzaim, Josh Broadman, Jared Ferguson, and Courtney Wilkinson-Maitland. The lab environment created by this group was comfortable and entertaining, yet intellectually stimulating. I always felt that I had adequate personal space, but a great amount of comradery and brains with which to discuss ideas and concerns despite the multiple settings in which we conducted research.

I need to mention my family that is always there for me. The never-ending amount of assistance provided by my mom, brother, sister-in-law and two aunts is more than for which one can ask. This backbone of support ensured that my life outside of university was always without worry. I love them all.

Finally, there is Anne Wu. From helping coordinate the early and confusing parts of data collection, all the troubleshooting, dozens of practice blood draws (i.e., pricking each other from our burning ears), catching my mistakes, and taking the lead on data entry, to listening for a truly endless number of hours to me ramble about the progress of every single aspect of my thesis, there is not enough space here to properly acknowledge you. You are the best!

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ABSTRACT

Introduction: The worldwide prevalence of physical activity-related breathlessness among adults aged 40 years and older is ~27%, with this value expected to rise into the future as our population continues to age. In adults with advanced disease across a range of both malignant and non-malignant diagnoses (e.g., chronic obstructive pulmonary disease, interstitial lung disease, cancer), physical activity-related breathlessness is the hallmark symptom as well as an independent predictor of adverse health outcomes, including exercise intolerance, hospitalization and death. Many of these patients suffer from chronic breathlessness syndrome: breathlessness that persists despite optimal treatment of the underlying pathophysiology and that results in disability. It follows that alleviating breathlessness is amongst the primary goals in the medical management of these individuals. However, there is currently no formal pharmacotherapeutic strategy to manage chronic breathlessness syndrome.

Based on our current understanding of the neurophysiological mechanism(s) of breathlessness, any therapeutic intervention capable of altering sensory feedback information from afferent nerves in the lungs and airways has the potential to alleviate the perception of breathlessness. Experimental and clinical research suggests that inhalation of the diuretic *furosemide* can alleviate breathlessness provoked experimentally in health and disease, presumably by modifying the activity of the vagus-innervated pulmonary stretch receptors and mimicking a larger tidal volume (V_T). However, these findings are inconsistent, as the body of evidence indicating that inhaled nebulized furosemide relieves breathlessness insignificantly better than nebulized 0.9% saline placebo continues to grow. We reasoned that if inhaled nebulized furosemide relieves breathlessness by mimicking greater V_T expansion *via* altered vagal afferent feedback activity from pulmonary stretch receptors, perhaps it would best

accomplish this under conditions of abnormal restrictive constraints on V_T expansion in healthy adults where the potentially confounding influences of psycho-physiological co-morbidities, deconditioning, hypoxemia, hypercapnia, concomitant medication use, etc. are minimized.

Objective and hypothesis: The primary objective of this study was to examine the acute effect of inhaled nebulized furosemide at doses of 40 mg and 120 mg on ratings of perceived breathlessness during cycle endurance exercise testing in the setting of abnormal restrictive constraints on V_T expansion. We hypothesized that single-dose inhalation of nebulized furosemide would be associated with dose-dependent relief of exertional breathlessness compared with nebulized 0.9% saline.

Methods: Twenty-four healthy men inhaled nebulized furosemide (40 mg and 120 mg) and nebulized 0.9% saline (placebo) in a randomized, double-blind, placebo-controlled, crossover study. Following inhalation, participants completed a symptom-limited constant-load cycle endurance exercise test at 80% of their peak incremental power output in the setting of external thoracic restriction *via* chest wall strapping to reduce slow vital capacity by ~20%. Detailed assessments of breathlessness, ventilation, breathing pattern, the behaviour of dynamic operating lung volumes, and cardiometabolic function were performed at rest and during exercise. Diuresis was assessed *via* post-dose quantification of urine production rate.

Results: Compared with nebulized 0.9% saline, neither 40 mg nor 120 mg of inhaled nebulized furosemide had a statistically significant effect on Borg 0-10 scale intensity and unpleasantness ratings of breathlessness during exercise. Similarly, neither dose of inhaled nebulized furosemide

had an effect on cardiometabolic, ventilatory, breathing pattern and dynamic operating lung volume responses to exercise compared with placebo. Compared with placebo, a significant effect of 120 mg, but not 40 mg, of nebulized furosemide on urine production rate was observed. No other side effects were reported.

Conclusion: Under the experimental conditions of this study, inhalation of nebulized furosemide at doses of 40 mg and 120 mg did not alleviate the perception of breathlessness during exercise in healthy men.

RÉSUMÉ

Introduction : La prévalence mondiale de l'essoufflement lié à l'activité physique chez les adultes âgés de 40 ans et plus est de ~ 27 %, avec cette valeur attendue pour s'élever dans l'avenir que notre population continue à vieillir. Chez les adultes atteints d'une maladie avancée dans une gamme de diagnostics malins et non malins (p. ex., la maladie pulmonaire obstructive chronique, la maladie pulmonaire interstitielle, le cancer), l'essoufflement lié à l'activité physique est le symptôme distinctif ainsi qu'un prédicteur indépendant des effets néfastes sur la santé, y compris l'intolérance à l'exercice, l'hospitalisation et la mort. Beaucoup de ces patients souffrent de syndrome de dyspnée chronique : essoufflement qui persiste malgré le traitement optimal de la physiopathologie sous-jacente et qui se traduit par un handicap. Il s'ensuit que la réduction de l'essoufflement est parmi les principaux objectifs de la gestion médicale de ces individus. Cependant, il n'existe actuellement aucune stratégie formelle de pharmacothérapeutique pour gérer le syndrome de l'essoufflement chronique.

Basé sur notre compréhension actuelle du mécanisme neurophysiologique de l'essoufflement, toute intervention thérapeutique capable de modifier les informations sensorielles de rétroaction des nerfs afférents dans les poumons et les voies respiratoires a le potentiel pour atténuer la perception de l'essoufflement. Des recherches expérimentales et cliniques suggèrent que l'inhalation du furosémide diurétique peut atténuer l'essoufflement provoqué expérimentalement dans la santé et la maladie, vraisemblablement en modifiant l'activité des récepteurs de l'étirement pulmonaire innervés par le nerf vague et en imitant un plus grand volume courant (V_T). Toutefois, ces résultats sont incohérents, comme l’ensemble de preuves indiquant que le furosémide inhalé soulage l'essoufflement de manière insignifiante mieux que 0,9 % salin nébulisé (placebo) continue de croître. Nous avons raisonné que si le

furosémide nébulisé inhalé soulage l'essoufflement en imitant une plus grande expansion de V_T par l'activité altérée de rétroaction afférente des récepteurs de l'étirement pulmonaire, peut-être qu'il serait mieux d'accomplir ce dans des conditions de contraintes restrictives anormales sur l'expansion du V_T chez les adultes en bonne santé où les influences potentiellement confondants de la comorbidité psycho-physiologique, le déconditionnement, hypoxémie, hypercapnie, l'utilisation concomitante de médicaments, etc. sont minimisés.

Objectif et hypothèse : L'objectif principal de cette étude était d'examiner l'effet aigu du furosémide nébulisé inhalé à des doses de 40 mg et de 120 mg sur les classements de l'essoufflement perçu pendant l'essai d'endurance de cycle dans le cadre de contraintes restrictives anormales sur l'expansion du V_T. Nous avons émis l'hypothèse que l'inhalation d'une dose unique de furosémide nébulisé serait associée à un soulagement dose-dépendante de l'essoufflement de l'effort par rapport à la solution de 0,9 % salin nébulisée.

Méthodes : Vingt-quatre hommes sains ont inhalé furosémide (40 mg et 120 mg) et 0,9 % salin nébulisé (placebo) dans une étude randomisée, à double insu, contrôlée par placebo et croisée. À la suite de l'inhalation, les participants ont effectué un essai d'endurance à cycle constant à charge limitée par symptôme à 80 % de leur puissance incrémentale maximum dans le cadre de la restriction thoracique externe par l'intermédiaire du cerclage de la paroi thoracique pour réduire la capacité vitale lente de ~ 20 %. Des évaluations détaillées de l'essoufflement, de la ventilation, de la respiration, du comportement des volumes de poumon d'exploitation

dynamique et de la fonction cardiométabolique ont été effectuées au repos et pendant l'exercice. La diurèse a été évaluée par quantification du taux de production d'urine après la dose.

Résultats : Comparativement à salins 0,9 % nébulisés, ni 40 mg ni 120 mg de furosémide nébulisé inhalé ont eu un effet statistiquement significatif sur l'intensité de l'échelle de Borg 0-10 et les cotes de désagrément de l'essoufflement pendant l'exercice. De même, aucune dose de furosémide nébulisé inhalée n'a eu d'effet sur la cardiométabolique, la respiration, le modèle respiratoire et les réponses dynamiques du volume pulmonaire à l'exercice par rapport au placebo. Comparativement au placebo, on a observé un effet significatif de 120 mg, mais non de 40 mg, du furosémide nébulisé sur le taux de production d'urine. Aucun autre effet secondaire n'a été rapporté.

Conclusion : Dans les conditions expérimentales de cette étude, l'inhalation de furosémide nébulisé à des doses de 40 mg et de 120 mg n'a pas atténué la perception de l'essoufflement pendant l'exercice chez les hommes sains.

PREFACE AND CONTRIBUTIONS OF AUTHORS

Marcus Waskiw-Ford was the principal contributor to the collection, analysis, and interpretation of data; and was primarily responsible for thesis/manuscript preparation.

Anne Wu, Noah Marchand, and **Amar Mainra** contributed to the collection and analysis of data.

Tara Greiss and **Abdullatif Alhuzaim** contributed to the collection of data and served as respiratory therapists.

Drs. Benjamin Smith and **Jean Bourbeau** served as medical supervisors, and contributed to the review of the protocol, interpretation of data and review of the thesis/manuscript.

As principal investigator, **Dr. Dennis Jensen** secured financial support of the experiments, and contributed to all aspects of the study. He helped prepare the final draft of the thesis/manuscript. He is the guarantor of the thesis/manuscript and takes responsibility for the integrity of the data and accuracy of the data analysis.

CHAPTER ONE: REVIEW OF THE LITERATURE

1.1. Breathlessness: An Introduction

Breathlessness is "a subjective experience of breathing discomfort that consists of qualitatively distinct sensations that vary in intensity" [1]. The worldwide prevalence of physical activity-related breathlessness among adults aged 40 years and older is ~27%, and this value is expected to rise in the future as our population continues to age [2, 3]. In a community (population)-based study, 8.9% of Australian adults aged 18 years and older similarly reported that breathlessness chronically limited their ability to perform activities of daily life such as walking [4]. In the general population, breathlessness is an independent predictor of all-cause mortality [5, 6] and is second to chest pain (angina) as the most common reason for adults to seek emergency medical care [7]. Physical activity-related breathlessness is the hallmark symptom as well as an independent predictor of adverse health outcomes (e.g., exercise intolerance, hospitalization, death) among adults with advanced disease across a range of both malignant and non-malignant diagnoses [8]. It follows that alleviating breathlessness is amongst the primary goals in the medical management of adults with advanced disease [9]. Despite the high prevalence, impact and burden of physical activity-related breathlessness in health and disease, very few therapies targeted to its relief currently exist.

The overarching goal of my M.Sc. thesis was to assess the efficacy of inhaled nebulized furosemide (a powerful loop diuretic commonly used in the clinical care setting to treat hypertension and edema) as a pharmacotherapy for relief of physical activity-related breathlessness. In moving forward, I will review the neurophysiological mechanisms of breathlessness in health and disease. I will then discuss the need for researchers to identify adjunct therapies to more effectively manage the symptom of breathlessness. Finally, I will

provide experimental and clinical evidence supporting a potential role of inhaled nebulized furosemide in the management of breathlessness.

1.2. Mechanisms of Breathlessness

One of the more prominent theories explaining the symptom of breathlessness is *neuromechanical uncoupling* or *neuromechanical dissociation*. The original idea of "length-tension inappropriateness" put forward by Campbell and Howell states that breathlessness arises from an abnormal disturbance between sensory afferent information about the length of the inspiratory muscles and the resulting sensory afferent information about their tension development [10]. This concept of "length-tension inappropriateness" was later refined and expanded to specify that breathlessness likely reflects the awareness of an abnormal disparity between the magnitude of increased central neural respiratory drive and the resultant thoracic volume displacement, the appropriateness of which is conveyed to cortical and subcortical areas of the brain implicated in the perception of breathlessness by multiple sensory afferent nerves in the lungs, airways and respiratory muscles [11-13]. In other words, the *uncoupling* of the ascending sensory afferent signals from proprioceptors in the lungs, airways and respiratory muscles that comment on the mechanical/muscular response of the respiratory system, relative to the prevailing level of increased descending efferent motor command from the reflex respiratory control centers to the respiratory pump muscles, may be a key factor in the etiology of breathlessness.

Neuromechanical uncoupling is consistently seen in patients with chronic obstructive pulmonary disease (COPD) and restrictive lung disorders (RLD). For example, an extensive

review by O'Donnell and colleagues examined multiple mechanical studies of patients with COPD and interstitial lung disease (ILD, a common RLD) and hypothesized that breathlessness was most closely associated with the awareness of increased central respiratory motor output command in the setting of abnormal or insufficient dynamic mechanical or muscular responses of the respiratory system [14]. At any given power output during exercise on a cycle ergometer, patients with COPD and ILD experience higher levels of perceived breathlessness compared with healthy age-matched control subjects. This heightened perception of breathlessness can be explained by the observation that the ratio of contractile respiratory muscle effort [tidal esophageal pressure swing expressed as a percentage of maximum inspiratory pressure (Pes,tidal%PI,max)] to thoracic volume displacement [tidal volume (V_T) expressed as a percentage of predicted forced vital capacity (V_T%predFVC)], often used an index of neuromechanical uncoupling, is greater in COPD (**Fig. 1.1**) and ILD (**Fig. 1.2**) than in health [14]. This observation of neuromechanical uncoupling is further evident in the recent study by Faisal et al., as the electromyogram of the crural diaphragm (EMGdi), a surrogate for inspiratory neural drive, is exaggerated for a given level of ventilation ($\dot{V}_E$) during incremental cycle exercise testing in both COPD and ILD compared with health (**Fig. 1.3**) [15].

The notion of central corollary discharge, applied to the respiratory system, states that respiratory motor cortices send an ascending (or efferent) copy of descending motor activity to cortical and subcortical areas of the brain implicated in the conscious perception of breathing [1]. It is thus likely that dramatic increases in neural respiratory drive, perceived *via* increased central corollary discharge in the context of an inadequate ventilatory response of the respiratory system, is the proximate source of breathlessness in health and disease.

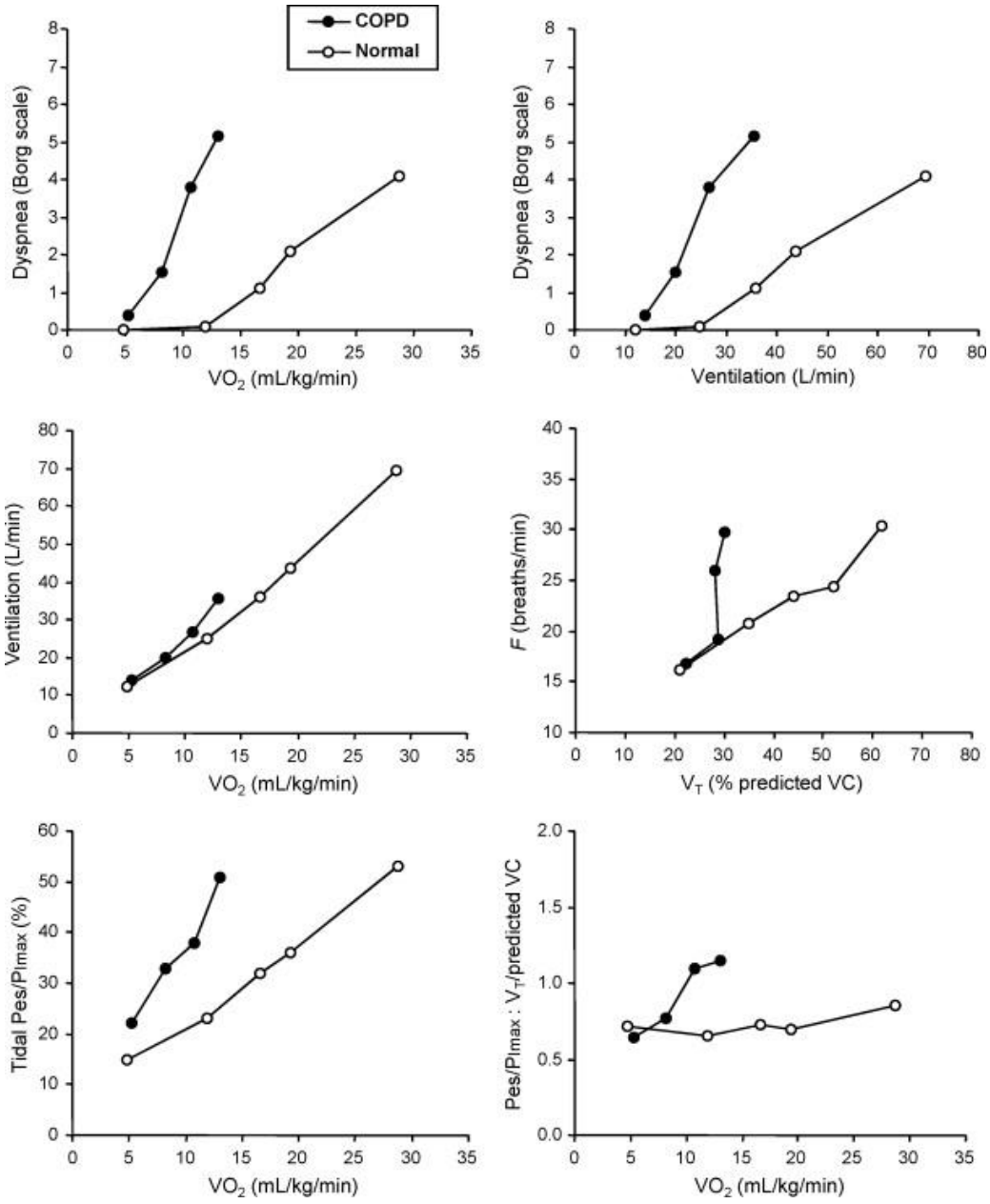

Figure 1.1. Perceptual and ventilatory responses to symptom-limited incremental cycle exercise in patients with chronic obstructive pulmonary disease (COPD, black circles) and healthy controls (white circles). In COPD, as compared to health, at a given rate of oxygen consumption (VO_2), breathlessness ratings, ventilation, contractile respiratory muscle effort (tidal esophageal pressure swing as a percentage of maximum; Tidal Pes/PImax) and the ratio of contractile respiratory muscle effort (Tidal Pes/PImax) to thoracic displacement (tidal volume as a percentage of vital capacity; V_T/predicted VC) – used as a crude index of neuromechanical uncoupling, are all significantly elevated. *F*: breathing frequency. Adapted from O'Donnell et al. [14]

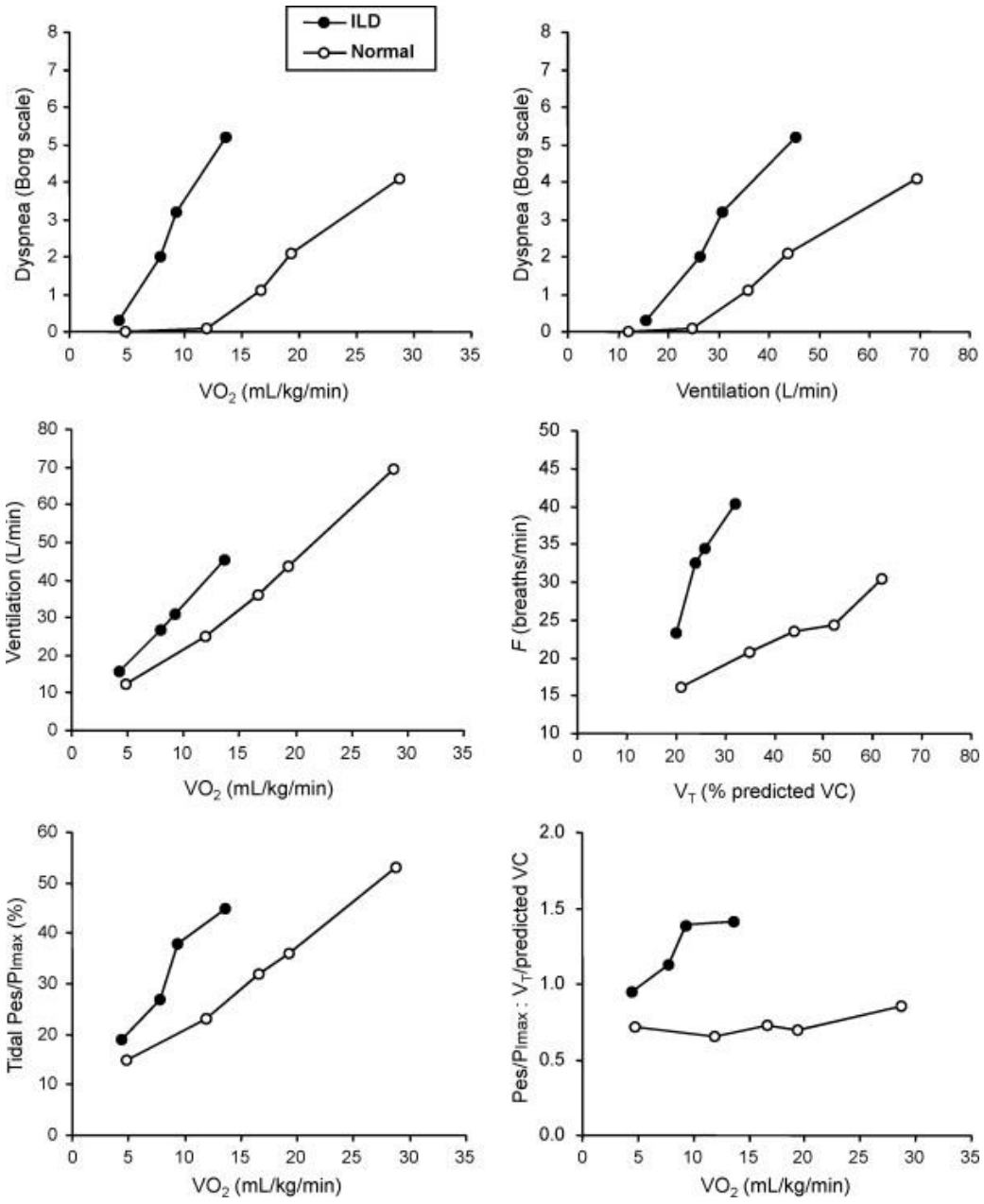

Figure 1.2. Perceptual and ventilatory responses to symptom-limited incremental cycle exercise in patients with interstitial lung disease (ILD, black circles) and healthy controls (white circles). In ILD, as compared to health, at a given rate of oxygen consumption (VO_2), breathlessness ratings, ventilation, contractile respiratory muscle effort (tidal esophageal pressure swing as a percentage of maximum; Tidal Pes/PImax) and the ratio of contractile respiratory muscle effort (Tidal Pes/PImax) to thoracic displacement (tidal volume as a percentage of vital capacity; V_T/predicted VC) – used as a crude index of neuromechanical uncoupling, are all significantly elevated. *F*: breathing frequency. Adapted from O'Donnell et al. [14].

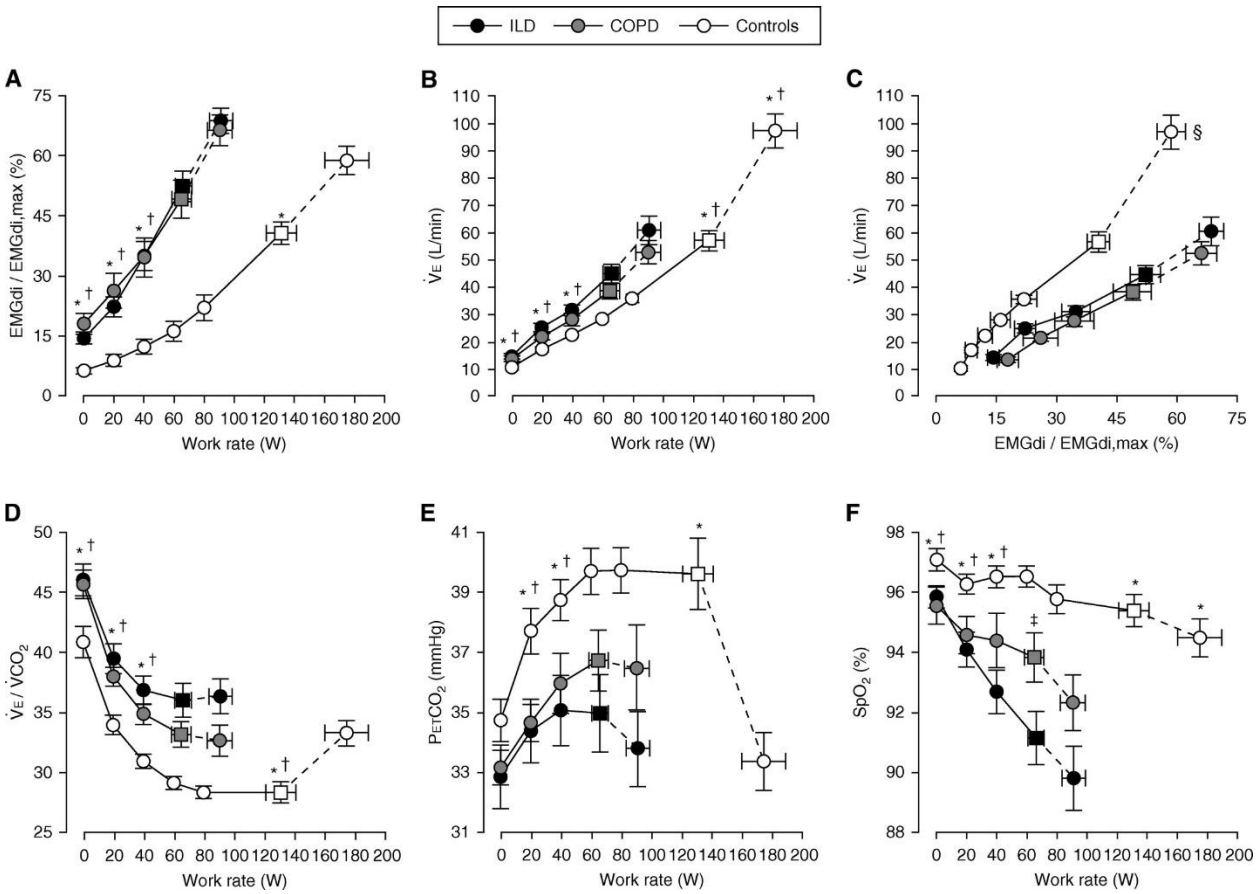

Figure 1.3. Relationship between work rate and multiple pulmonary parameters during symptom-limited incremental cycle exercise in interstitial lung disease (ILD; black circles), chronic obstructive pulmonary disease (COPD; grey circles) and healthy controls (white circles). Panel C exemplifies an exaggerated EMGdi response for a given ventilation. EMGdi/EMGdi,max (electromyography of the diaphragm as a percentage of maximum) – a crude index of neural inspiratory drive; V_E: ventilation; VCO_2: carbon dioxide production; $P_{ET}CO_2$: end-tidal carbon dioxide partial pressure; SpO_2: arterial oxygen saturation. Adapted from Faisal et al. [15].

In addition, the various qualities of breathlessness, such as *work/effort of breathing* and *air hunger/unsatisfied inspiration*, are distinguishable and likely have distinct afferent pathways [16-18]. For example, the perception of *respiratory work/effort* may stem from cervical and dorsal root afferents that innervate the chest wall and respiratory muscles, whereas the perception of *air hunger/unsatisfied inspiration* may originate from sources such as the pulmonary stretch

receptors (PSRs) and chemoreceptors, as it can be induced in the absence of respiratory muscle activity [19].

1.3. Management of Breathlessness

Effective management of breathlessness among individuals with advanced disease remains an elusive goal for most healthcare providers since modern pharmacotherapies targeted to the underlying disease produce only modest relief of breathlessness in most patients. It follows that many patients with advanced disease suffer from chronic breathlessness syndrome, which has recently been defined as breathlessness that persists despite optimal treatment of the underlying pathophysiology and that results in disability [20, 21]. Indeed, 46-91% of adults with advanced COPD suffer from chronic and disabling breathlessness at rest and on minimal exertional despite (i) optimal treatment of their expiratory flow limitation, lung hyperinflation and airway inflammation with bronchodilators, inhaled corticosteroids and/or phosphodiesterase inhibitors and (ii) remediation of arterial hypoxemia and limb muscle dysfunction with supplemental oxygen and pulmonary rehabilitation, respectively [22-25].

Considering its prevalence, burden and associated adverse psycho-physiological health effects, physicians have an ethical duty to manage chronic breathlessness syndrome [9, 26]. To this end, Currow et al. recently proposed that effective management of chronic breathlessness syndrome, like chronic refractory pain, should be viewed as a human right [26]. With the possible exception of systemic opioids (e.g., morphine) [27-30], there is currently no formal interventional strategy to manage chronic breathlessness syndrome. It follows that researchers have a responsibility to identify adjunct therapies to complement existing disease-modifying therapies to better manage chronic breathlessness syndrome.

Based on our current understanding of the neurophysiological mechanism(s) of breathlessness (see ***Section 1.1*** above), any therapeutic intervention capable of decreasing neural respiratory drive, improving dynamic respiratory mechanical/muscular function and/or altering sensory feedback information from afferent nerves in the tracheobronchial tree has the potential to alleviate the perception of breathlessness by enhancing neuromechanical coupling of the respiratory system. To date, a considerable amount of experimental and clinical research has focused on decreasing neural respiratory drive (e.g., opioids [28], hyperoxia [31, 32], exercise training [33]) and/or improving dynamic respiratory mechanical/muscular function (e.g., bronchodilators [32], helium [34], inspiratory muscle training [35]) as a therapeutic means of alleviating the perception of breathlessness in health and disease. By contrast, very little attention has been paid to sensory afferent nerves in the lungs, airways and respiratory muscles as therapeutic targets in the management of breathlessness, which is somewhat surprising in view of their role in the neuromodulation of breathlessness [12, 36-39].

Afferent receptors most widely implicated in the perception of breathlessness include: bronchopulmonary C-fibers; chest wall and respiratory muscle proprioceptors (i.e., muscle spindles and Golgi tendon organs); and slowly (SARs) and rapidly (RARs) adapting PSRs [12, 36, 40, 41]. However, as described in more detail below, a growing body of experimental and clinical research suggests that the vagus-innervated SARs and RARs play the most important role in the neuromodulation of breathlessness [42]. It follows that SARs and RARs represent promising therapeutic targets in the management of breathlessness.

1.4. Pulmonary Stretch Receptors

1.4.i. Pulmonary Stretch Receptors: A Brief Overview of their Anatomy and Physiology

The SARs and RARs, collectively known as the pulmonary stretch receptors (PSRs), can be sufficiently distinguished by their morphological and functional characteristics. The SARs are located throughout the tracheobronchial tree and can vary across mammalian species, but it is thought that they are most concentrated in the intrathoracic trachea [43]. Their nerve endings are found in the airway smooth muscle, are corpuscular in nature, arrange in a branching formation, and are connected to myelinated afferent nerves [44]. The RARs can also be found throughout the tracheobronchial tree, but tend to be found predominantly in the lobar bronchus and larger bronchi [43]. Although the morphological characteristics of the RARs have not been fully elucidated, the available evidence indicates that certain myelinated fibers that enter the airway epithelium and end in non-myelinated nerve fibers belong to the RARs [44].

SARs are stimulated by normal inflation of the lungs, with a slow adaption rate and regular discharge to maintained inflation pressure [43]. They are not known to be particularly chemosensitive. As the name implies, RARs differentiate themselves from SARs by their rapid adaptation to a maintained lung inflation or deflation, as well as irregular or random discharge patterns during normal breathing [44]. RARs are also activated by a wide variety of mechanical and chemical irritant stimuli, such as abnormal airway or lung pathological changes (i.e., pneumothorax), cigarette smoke, and inflammatory and immunological mediators, leading some to classify the RARs as “pulmonary irritant receptors” [43, 44].

1.4.ii. The Role of Pulmonary Stretch Receptors in the Neuromodulation of Breathlessness

It is well-established that increasing the activity of PSRs (most likely SARs) *via* lung inflation inhibits firing of mesencephalic neurons and decreases phrenic motor nerve activity in animals [45-48], and that this effect can be abolished by vagotomy or blocking PSR activity [19, 48-50]. Eldridge and Chen have suggested that firing of mesencephalic neurons is associated with sensations of breathlessness [48, 51]. To investigate the relationship between lung inflation and breathlessness further, researchers have used breath-holding, a powerful and effective breathlessness-inducing technique [52] used for over a century [53]. In animals, breath-holding achieved by airway occlusion can be prolonged and the attendant respiratory distress delayed *via* lung expansion [54]. In humans, the breathlessness-relieving properties of PSR activity were demonstrated by using voluntary breath-holding to induce breathlessness, and then using the recommencement of normal tidal breathing to relieve it [55, 56]. Indeed, it is a consistent observation that the intensity of perceived breathlessness is inversely related to V_T expansion (i.e., PSR activation) in the setting of increased central neural respiratory drive in high-level quadriplegics (**Fig. 1.4**) [57], in healthy humans with and without constrained V_T [58-60], and in adults with chronic obstructive and restrictive pulmonary disorders [15, 61].

Data from our laboratory suggests that, in the setting of exercise with external thoracic restriction (i.e., abnormal restrictive constraints on V_T expansion), an exaggerated neural respiratory drive response (NRD) to exercise near the limits of tolerance in healthy subjects may be explained by the loss of SAR feedback inhibition [due to restricted V_T expansion at a critically low inspiratory reserve volume (IRV) of ~0.5 L] on central motor output command [60]. For instance, breathlessness intensity ratings increased very rapidly to intolerable levels once IRV reached a minimal level, and these changes occurred in parallel with changes in EMGdi (i.e.

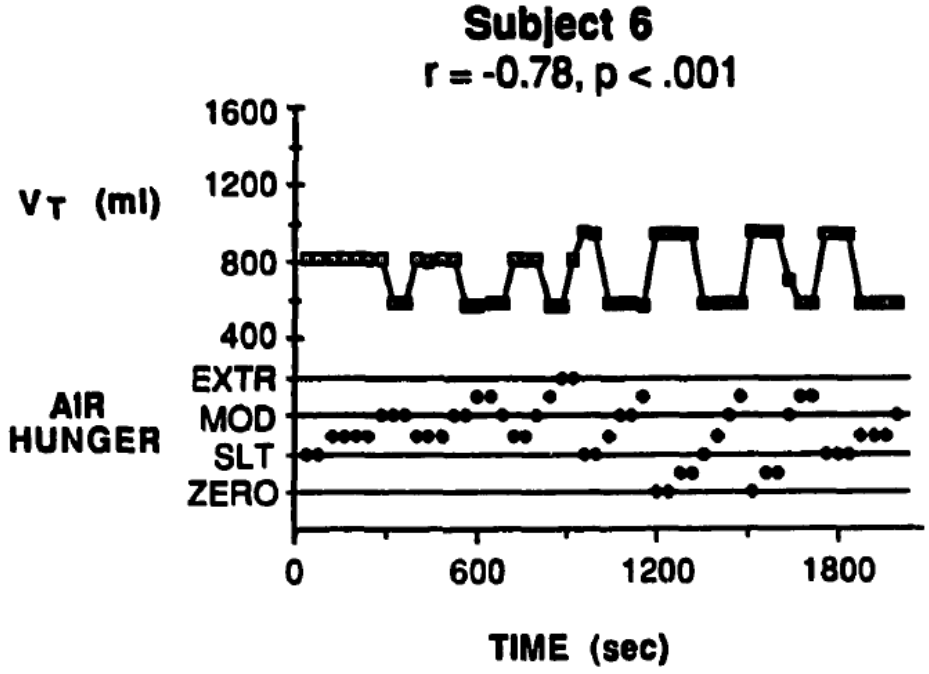

Figure 1.4. Relationship between tidal volume expansion and air hunger ratings in a group of quadriplegics mechanically ventilated at an elevated but fixed end-tidal PCO_2. A significant inverse relationship between tidal volume expansion and air hunger ratings is shown. V_T: tidal volume; EXTR: extreme; MOD: moderate; SLT: slight. Adapted from Manning et al. [57].

NRD) [60]. This supports the notion that increases in SAR activity (and decreases in RAR activity) are fundamental to decreasing phrenic nerve discharge (a measure of NRD), a common observation in animal studies [49-51]. It follows that targeting and altering PSR activity can potentially relieve breathlessness by reporting relatively greater vagal afferent feedback activity than the respiratory mechanics can elicit [39]. In other words, in the setting of abnormal restrictive constraints on V_T expansion (and PSR activation) as seen in COPD and ILD [15], a substance capable of enhancing vagal afferent feedback information by manipulating PSR activity and artificially mimicking a greater V_T has the potential to enhance neuromechanical coupling of the respiratory system with attendant reductions in breathlessness. As outlined in

detail below, research indicates that inhalation of the diuretic *furosemide* can alleviate breathlessness provoked experimentally in health and disease, presumably by modifying the activity of PSRs and mimicking greater V_T expansion [62, 63].

1.5. Inhaled Furosemide: Proposed Mechanisms of Action

Furosemide, a sulfonamide derivative, has primarily been used as a diuretic. It is capable of impeding reabsorption of salt in the thick ascending limb of Henle's loop *via* inhibition of the $Na^+/K^+/2Cl^-$ cotransporter (NKCC) that moves Na^+, K^+ and Cl^- ions across the tubular lumen [64]. If taken orally or intravenously, its diuretic effects can combat excessive fluid retention, as seen in acute pulmonary edema [65] and congestive heart failure [66].

With reports that inhalation of nebulized furosemide can inhibit cough reflexes in healthy humans [67], Sant'Ambrogio and colleagues sought to explore the specific mechanisms underlying this inhibition. They isolated the upper airways of 9 anaesthetized, spontaneously breathing dogs and exposed the larynx to isotonic dextrose, a known RAR stimulant [63]. RAR activity, directly measured *via* an electrostatic recorder, was significantly dampened if a furosemide solution was introduced into the laryngeal lumen prior to isotonic dextrose (**Fig. 1.5**) [63], suggesting that furosemide has an inhibitory effect on RAR activity.

In a study by Sudo and coworkers, a group of tracheobronchial receptors in spontaneously breathing rats were differentiated into SARs and RARs according to their adaptation rate to airway pressure [62]. Receptor activity, measured by a recording electrode and expressed as spike frequency, was plotted against airway pressure. They observed that administration of nebulized furosemide, as opposed to intravenous furosemide or vehicle,

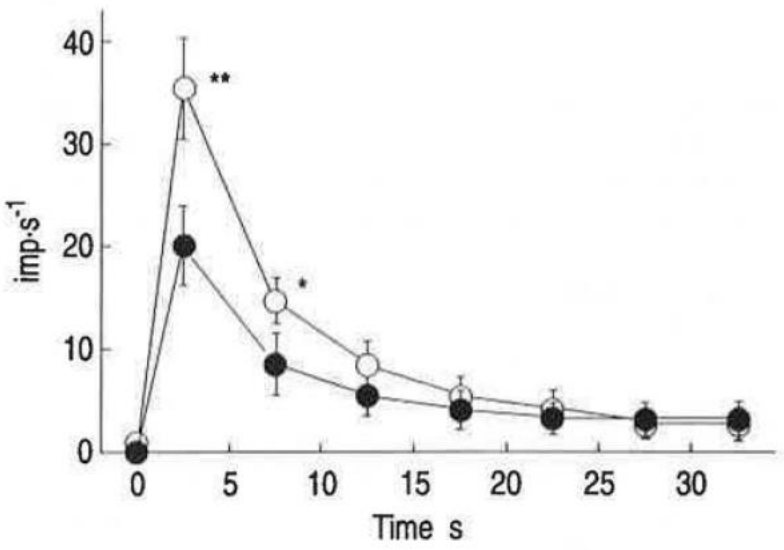

Figure 1.5. Effect of iso-osmolal solution of dextrose on laryngeal receptor activity [i.e. rapidly adapting stretch receptor (RAR) activity] before (open circles) and after (blackened circles) administration of nebulized of furosemide in rats. **: $p<0.001$; *: $p<0.05$; imp: impulses. Adapted from Sant'Ambrogio et al. [63].

resulted in a greater slope and upward shift of the SAR spike frequency-airway pressure curve, with nearly a three-fold increase in spike frequency at zero airway pressure (**Fig. 1.6**) [62]. These findings indicated that inhalation of nebulized furosemide increased SAR activity at any given stimulus. Moreover, in congruence with the data from Sant'Ambrogio et al., RAR activity was significantly attenuated from inhalation of nebulized furosemide (**Fig. 1.6**) [62].

In 1988, Bianco and colleagues first demonstrated the beneficial effects furosemide can afford to the respiratory system (e.g., protection against exercise-induced bronchoconstriction in asthmatics) if nebulized and inhaled [68]. Inhalation of a drug is perhaps the most natural and preferred method of delivery to the lungs [69], and there is strong evidence to believe that the respiratory effects of inhaling nebulized furosemide are not due to systemic diuretic activity [70].

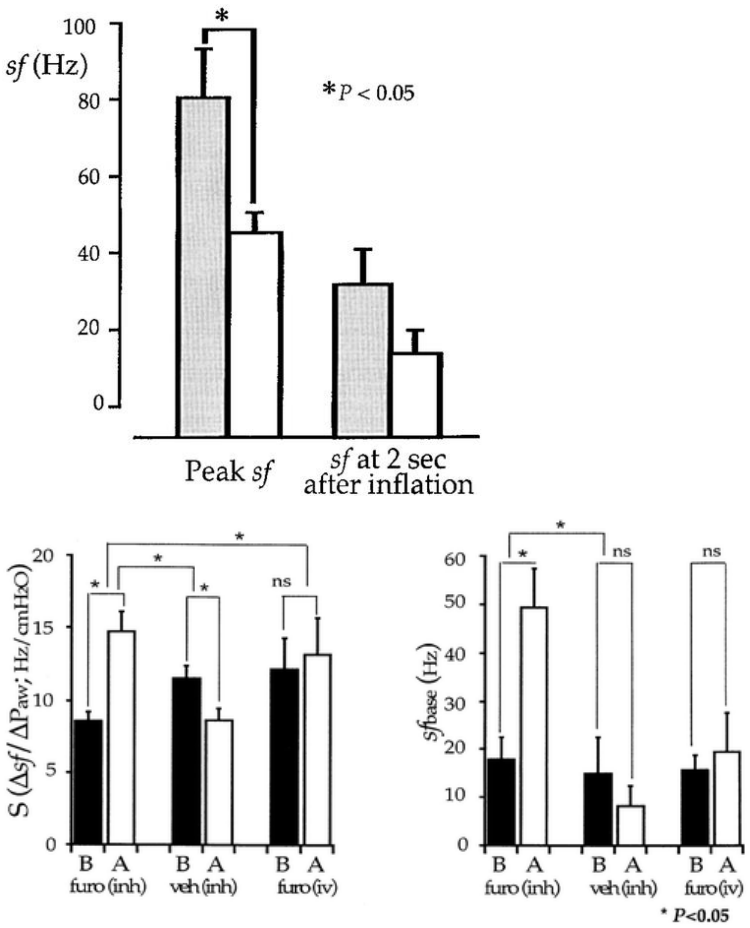

Figure 1.6. *Top panel*: Spike frequency (*sf*) response of rapidly adapting stretch receptors (RARs) to lung inflation before (shaded column) and after (open column) inhalation of furosemide. *Bottom panel*: Changes in sensitivity to lung inflation (left) and respective *sf* (right) in slowly adapting stretch receptors (SARs) before (B) and after (A) application of inhaled furosemide [furo (inh)], inhaled vehicle [veh (inh)], and intravenous furosemide [furo (iv)]. Adapted from Sudo et al. [62].

Although it is thought that a portion of nebulized furosemide is ingested during inhalation [71, 72] and may be absorbed into the circulation through the gastrointestinal tract (explaining reports of diuresis following inhalation of nebulized furosemide [73]), there is an overwhelming pool of evidence against any diuretic effects should nebulized furosemide be inhaled [74-76], with the vast majority of interventions not reporting any excessive urination, at least not at nebulized doses of 40-80 mg. In addition, furosemide administered orally or intravenously does not elicit

the same respiratory effects as when nebulized and inhaled [62, 63, 68], supporting a direct (topical) effect of furosemide on sensory afferent nerves in the airways.

The mechanism(s) of action of inhaled furosemide on the airways remain unclear. Analogous to its effects on the renal system, inhaled nebulized furosemide likely acts *via* NKCC in the tracheobronchial tree by travelling into the large airways (and to a lesser extent, the small airways), passing between the tight junctions of the airway wall and accessing the submucosal extracellular fluid (**Fig. 1.7**) [52]. By impeding the function of NKCC at the epithelial cells, inhaled furosemide can prevent movement of Na^+ and Cl^- from the submucosal extracellular fluid into the airway lumen, effectively increasing their concentrations in the submucosal extracellular space [77, 78].

1.5.i. Slowly Adapting Stretch Receptors

Matsumo and coworkers reported that increases in Na^+ concentrations in the submucosal extracellular fluid can trigger increases in SAR activity, presumably by independently exciting or amplifying already excited SAR nerve endings [79]. Through this mechanism, inhaled nebulized furosemide may augment SAR activity by raising the concentration of Na^+ in their local environment, which would ultimately signal to the central nervous system a greater-than-present V_T through the vagus nerve.

Indeed, nebulized furosemide delayed the onset of escape behaviour to respiratory distress induced by tracheal occlusion (a form of involuntary breath-holding) in animals [80], presumably by enhancing afferent feedback activity through its local effects on SARs. In the same study, it was also observed that vagotomy abolished the rescue effect on respiratory

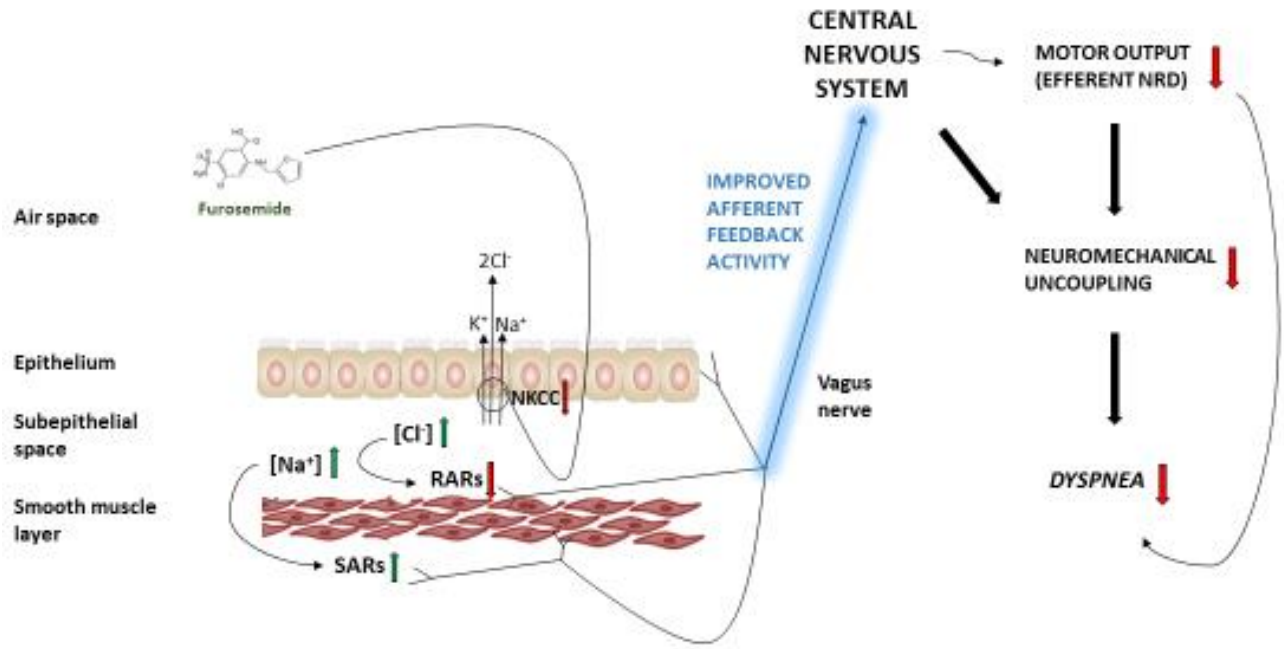

Figure 1.7. Proposed mechanism of action of inhaled nebulized furosemide on breathlessness. SARs: slowly adapting stretch receptors; RARs: rapidly adapting stretch receptors; NKCC: sodium-potassium-chloride channel; NRD: neural respiratory drive; dyspnea: breathlessness.

distress provided by nebulized furosemide [80]. The fact that the rescue effect of lung inflation, in an identical experimental setting, also disappears with vagotomy [54], suggests that inhalation of nebulized furosemide may impact SARs similarly to direct lung inflation. This has been substantiated by research in healthy humans, as inhaled furosemide similarly relieved respiratory distress (measured *via* breathlessness ratings) during voluntary breath-holding (**Fig. 1.8**) [81].

1.5.ii. Rapidly Adapting Stretch Receptors

Increases in Cl^- concentration in the submucosal extracellular space can depolarize the nerve endings of RARs [82], and may thus explain how inhalation of nebulized furosemide decreases RAR activity.

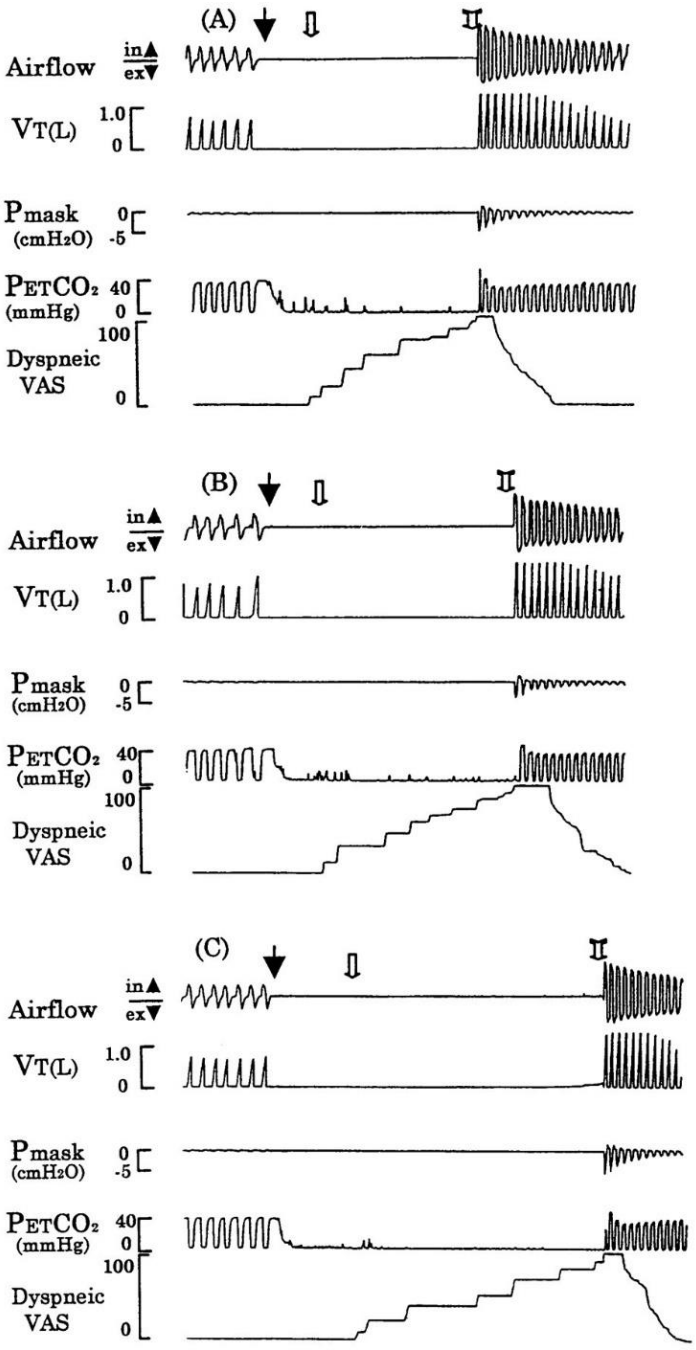

Figure 1.8. Recordings illustrating changes in visual analog scale (VAS) ratings and other parameters during voluntary breathholding. *A*: before inhalation of placebo. *B*: after inhalation of placebo. *C*: after inhalation of furosemide. The three arrows of different shape indicate the start of breathholding, the onset of unpleasant sensation, and the cessation of breathholding, respectively. V_T: tidal volume; P_{mask}: mask pressure; $P_{ET}CO_2$: partial pressure of end-tidal carbon dioxide. Adapted from Nishino et al. [81].

Evidence from animal studies indicates that declines in dynamic lung compliance (typically achieved through reductions in end-expiratory pressure) may increase RAR activity [83-86]. If inhalation of nebulized furosemide can weaken the signal of high lung stiffness by dampening RAR activity, it might decrease the resultant NRD needed to overcome a less compliant lung, thereby reducing the intensity of perceived breathlessness. This is of particular importance to the RLD population as low lung and/or chest wall compliance is the hallmark of the disorder. In other words, by signaling a smaller restriction to V_T expansion to the central nervous system in a patient with RLD, inhaled nebulized furosemide may decrease NRD and consequently ratings of perceived breathlessness. However, this hypothesis needs to be further investigated.

Considering that RARs are involved in the genesis of the cough reflex [87], and that cough can exacerbate already-existing sensations of breathlessness, it is possible that inhaled nebulized furosemide partially relieves breathlessness through its inhibitory effect on cough [63, 67]. In fact, a study of emphysematous rabbits revealed heightened baseline RAR activity relative to controls, and this was associated with greater phrenic nerve activity (i.e., NRD) [88], evoking the concept that controlling inappropriately high RAR activity may be fundamental to the alleviation of breathlessness in humans [89].

1.5.iii. Impaired Pulmonary Stretch Receptor Activity

There is an emerging theme that inhaled nebulized furosemide requires intact airway tracts (i.e., intact PSRs) to function optimally. For instance, inhalation of 40 mg of furosemide was unable to relieve breathlessness better than 0.9% saline (placebo) in patients with a history

of sulfur mustard gas exposure [90], a toxin with destructive effects on the respiratory tracts and PSRs [91, 92]. This is supported by data from Jensen et al., where a subgroup analysis revealed that COPD patients with a milder disease stage and presumably healthier airways responded significantly better to inhaled nebulized furosemide treatment than their counterparts with more severe symptoms [93].

1.5.iv. Airway Function

There is strong evidence to suggest that inhalation of nebulized furosemide can relieve breathlessness through non-vagal mediated mechanisms. For example, a study of 20 symptomatic patients with moderate-to-severe COPD by Jensen and colleagues demonstrated that inhalation of 40 mg of nebulized furosemide not only alleviated breathlessness during constant-load cycle exercise testing, but lead to statistically significant improvements in V_T expansion, inspiratory capacity (IC), and mean tidal expiratory flow rates [93], suggesting reductions in airway obstruction *via* possible release of cholinergic tone of the airway smooth muscle.

It has been well-established that an osmolaric perturbation of the airways is a powerful bronchoconstrictive agent *via* the release of inflammatory mediators, particularly in asthmatics [94-96]. Considering that inhaled furosemide likely has no direct effect on airway smooth muscle [97, 98], it is reasonable to postulate that inhaled nebulized furosemide indirectly modulates airway caliber by controlling the NKCC-mediated secretion of ions into the airway lumen. Indeed, furosemide has remarkable protective properties against bronchoconstrictive challenges *in vitro* [99], in mice [100], and in human asthmatics [68, 101-103]. The multiple complex

mechanisms underlying these protective effects include: enhancing production of bronchodilating prostaglandins by the airway epithelium [104]; moderating production of bronchoconstricting prostaglandins by the vascular endothelium [104, 105]; preventing the release of anaphylactic agents from mast cells [106]; reducing airway responsiveness to neurokinin A [103] (i.e., interfering with airway neurotransmission to decrease cholinergic tone [107]); and modulating airway temperature fluctuations [108]. Further, pulmonary epithelial permeability, a potent signal for airway inflammation, is often compromised in acute and chronic lung conditions (e.g., asthma) [109, 110] and reverts back to normal with administration of inhaled nebulized furosemide in asthmatics but not in healthy volunteers [110, 111].

These ameliorations in airway function can lead to alleviation of sensations of breathlessness in COPD [93, 112] and asthmatics [113]. However, it should be noted that in the great majority of the studies presented here, inhaled nebulized furosemide corrected airway function in the setting of chronic inflammatory airway disease. Thus, it is unclear whether inhalation of nebulized furosemide, specifically through its ability to combat bronchoconstriction, can enhance airway function (e.g., improve FEV_1) in airways already functioning normally.

In summary, although the mechanisms by which inhaled nebulized furosemide act to alleviate breathlessness are becoming increasingly evident, further investigation remains necessary. From the observations presented, we believe there are at least two key modes of action by which inhaled nebulized furosemide may alleviate breathlessness:

1) Attenuation of neuromechanical uncoupling *via* reflex inhibition of NRD secondary to enhanced PSR activity (i.e., increased SAR activity to mimic a larger-than-present V_T, and decreased RAR activity to signal a smaller-than-present restriction to V_T expansion) (**Fig. 1.7**);

2) Modulation of airway caliber and obstruction through control of inflammatory mediators, airway osmolaric flux, and airway neurotransmission (may be limited to populations with abnormal airway inflammation).

1.6. Effect of Inhaled Furosemide on Breathlessness: Evidence from Experimental and Clinical Trials

In the experimental and clinical settings, reviews state that inhalation of nebulized furosemide generally affords positive outcomes on breathlessness ratings with few adverse side effects [74, 76, 114]. Key interventions that have investigated the effects of inhaled nebulized furosemide on breathlessness are discussed below.

1.6.i. Effects of Inhaled Furosemide on Experimentally-Induced Breathlessness

An early study by Nishino and colleagues looked at the effects of inhaled nebulized furosemide on voluntary breath-holding time and the associated breathlessness (measured by visual analogue scale) in 12 healthy subjects [81]. Inhaled nebulized furosemide, as compared to before or after inhalation of nebulized 0.9% saline (placebo), significantly prolonged breath-holding time, as well as reduced ratings of breathlessness (**Fig. 1.8**) [81]. Immediately following the breath-holding test, Nishino et al. induced respiratory discomfort *via* the combination of hypercapnia and inspiratory flow resistive loading and observed significant reductions in breathlessness following inhalation of nebulized furosemide relative to nebulized 0.9% saline [81].

In a different intervention, the same group of researchers sought to explore the potential impact of inhaled nebulized furosemide on CO_2 sensitivity [115]. Although increases in either central and peripheral chemoreceptor activity are closely related to breathlessness *via* its effect on NRD [36], they determined that it is unlikely that inhaled nebulized furosemide alleviates breathlessness by decreasing chemoreceptor afferent activity, as inhalation of 40 mg had no impact on the ventilatory response to hypercapnic hyperpnea in 10 healthy volunteers [115]. They also observed that inhalation of nebulized furosemide relieved breathlessness during hypercapnic hyperpnea relative to pre-furosemide values (**Fig. 1.9)** [115]. However, it is important to note that from the reported data, it is unclear whether inhalation of nebulized furosemide relieved breathlessness significantly better than nebulized 0.9% saline (**Fig. 1.9**), since post-dose breathlessness intensity ratings appeared quantitatively similar [115].

In a trial by Moosavi and coworkers, a combination of constrained ventilation and hypercapnia were used to induce sensations of air hunger in 10 healthy subjects [73]. In comparison to nebulized 0.9% saline, inhalation of 40 mg of nebulized furosemide trended toward the relief of air hunger, but was just short of reaching statistical significance (**Fig. 1.10**) [73].

Laveneziana and colleagues investigated the effects of 40 mg and 80 mg of inhaled nebulized furosemide on the work/effort of breathing during the combination of expiratory flow resistive loading and incremental cycle exercise testing in 9 healthy adults [116]. In contrast to the results of Nishino et al. [81], Minowa et al. [115], and Moosavi et al. [73], this group of investigators reported that neither dose of inhaled nebulized furosemide was effective at altering the perception of the work/effort of breathing (measured by a 10-point modified Borg scale) when compared to nebulized 0.9% saline or pre-furosemide values [116].

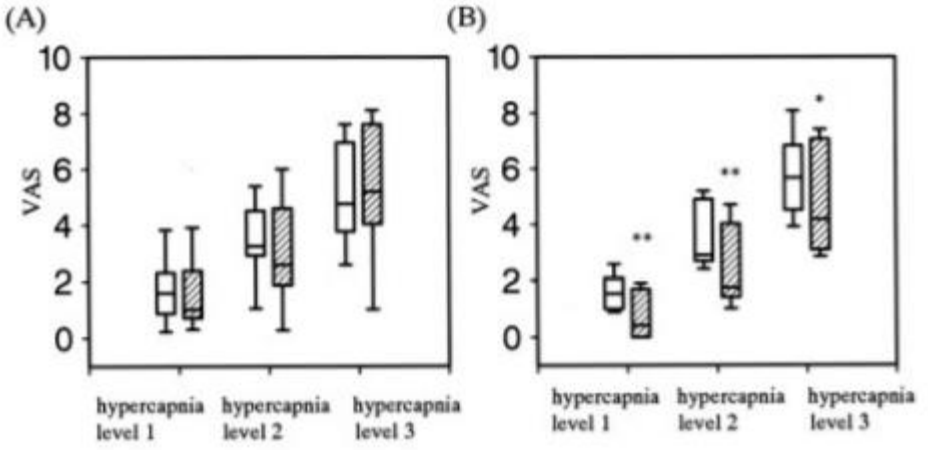

Figure 1.9. Changes in ratings of breathlessness by visual analogue scale (VAS) following inhalation of placebo (A) and furosemide (B). Open boxes represent VAS score before inhalation and shaded boxes represent VAS scores after inhalation. **P* < 0.05, ***P* < 0.01. Adapted from Minowa et al. [115].

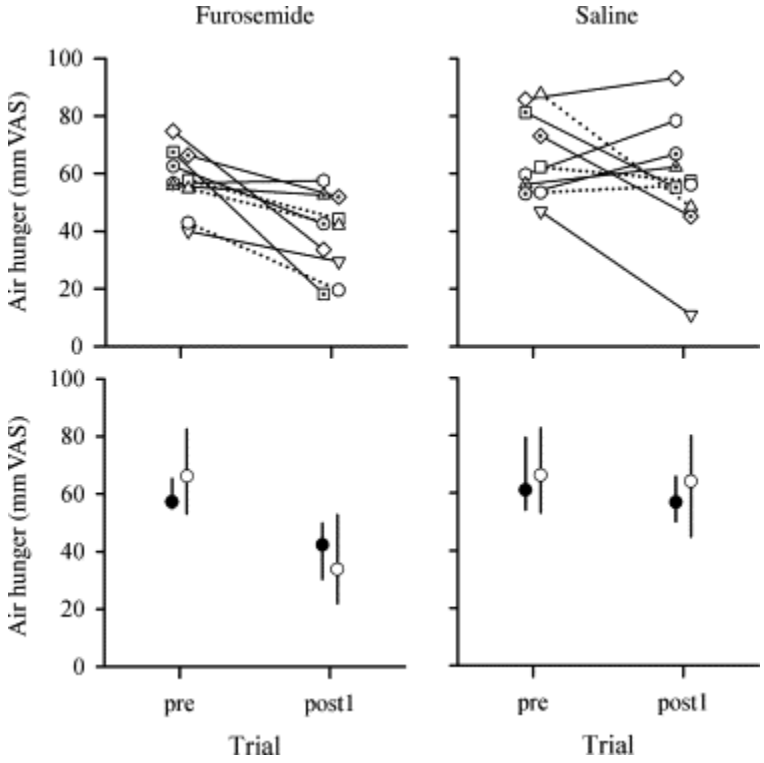

Figure 1.10. Top panels: Individual visual analog score (VAS) ratings of air hunger at a selected period of interest before (pre) and after (post1) treatment with inhaled furosemide (furosemide) and placebo (saline). Bottom panels: Closed circles indicate median air hunger ratings and open circles show corresponding data from an earlier study [81]. Adapted from Moosavi et al. [73].

Finally, recent studies by O'Donnell et al. [117] and Morélot-Panzini et al. [118] have postulated that optimal delivery of nebulized furosemide at doses of 40 mg and 80 mg using a mechanical ventilator at controlled inspiratory flow rates (300-500 ml/sec) and levels of V_T expansion (15% of predicted vital capacity) would reliably relieve breathlessness induced by the combination of hypercapnia and constrained ventilation. However, they observed that 40 mg and 80 mg doses of inhaled nebulized furosemide did not produce more consistent or greater relief of breathlessness compared with nebulized 0.9% saline in 12 healthy adults [117, 118].

In summary, the effects of inhaled nebulized furosemide vs. nebulized 0.9% saline on experimentally-induced breathlessness in healthy adults have been positive in some interventions, although this particularly body of literature suffers from high variability, inconclusive reports and relatively small sample sizes of less than or equal to 12.

1.6.ii. Effects of Inhaled Furosemide on Breathlessness in Clinical Trials

In asthmatics, inhaled furosemide attenuates the early and late bronchoconstrictive responses to exercise [68] and other bronchoconstricting agents such as ultrasonically nebulized water (**Fig. 1.11**) or specific antigens [71, 101, 119]. Research on inhaled nebulized furosemide for the relief of breathlessness in this population is limited. A study by Hinckley and colleagues indicated that inhaled nebulized furosemide has an insignificant effect on breathlessness at rest when used in adjunct to optimal asthma medication such as prednisone (a corticosteroid) and albuterol (a short-acting β_2-adrenergic receptor agonist bronchodilator) as compared to these medications alone [120]. In contrast, in patients with reactive airway disease (i.e., an undiagnosed airway disorder), 40 mg of inhaled nebulized furosemide taken with salbutamol

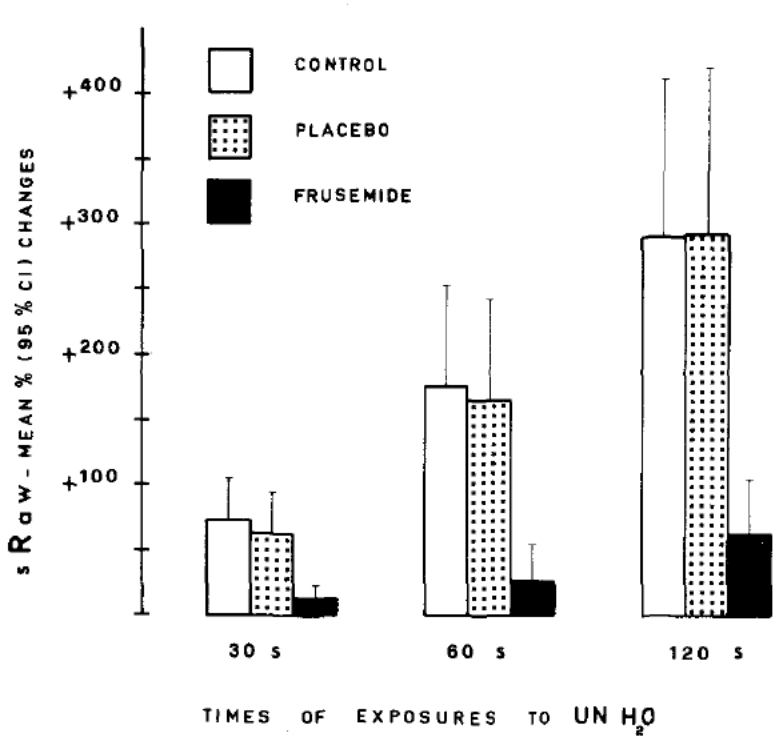

Figure 1.11. Effect of inhaled furosemide on changes in sRaw (airway resistance multiplied by thoracic gas volume) induced by ultrasonically nebulized distilled water (UNH_20) in asthmatics. Adapted from Robuschi et al. [71]

(albuterol) was more effective at relieving breathlessness than salbutamol alone [121], demonstrating its potential role as an adjunct therapy for breathlessness.

The efficacy of inhaled nebulized furosemide for the alleviation of breathlessness in patients with cancer is also unclear. For example, despite standard treatment having little effect, 20 mg of inhaled furosemide was able to improve breathlessness at rest one hour post-treatment in 12 of 15 terminally ill cancer patients [122]; similar results have been observed in this population in both the short-term (single-dose) [123] and long-term (multiple doses per day) [124]. However, these studies were uncontrolled and prone to placebo effects. An incomplete placebo-controlled clinical trial by Stone and coworkers reported a trend toward 20 mg of

inhaled nebulized furosemide *worsening* breathlessness at rest relative to nebulized 0.9% saline in 7 patients with lung cancer [125]. Furthermore, a placebo-controlled clinical trial by Wilcock et al. reported no effect of 40 mg of inhaled nebulized furosemide vs. nebulized 0.9% saline on breathlessness during number reading and arm chair exercise tests in 15 patients with lung cancer and mesothelioma [126].

For patients with COPD, there is a bit more consistency surrounding the efficacy of inhaled nebulized furosemide on breathlessness. During a COPD-related exacerbation in 100 patients with COPD, inhalation of 40 mg of nebulized furosemide, in adjunct to conventional treatments (i.e. supplemental oxygen, bronchodilators, corticosteroids, etc.), afforded an approximately three unit decrease in breathlessness ratings on a 0-10 visual analog scale one hour post-administration at rest in comparison to nebulized 0.9% saline and conventional treatments; pulmonary function [measured by forced expiratory volume in 1 second (FEV_1)], heart rate and mean blood pressure improved as well [127]. In a trial by Jensen et al., 20 patients with moderate-to-severe COPD completed a constant-load cycle endurance exercise test after receiving either 40 mg of inhaled nebulized furosemide or nebulized 0.9% saline [93]. Intensity ratings of breathlessness and measures of dynamic airway function (i.e., IC, V_T, mean tidal expiratory flow rates) were improved at a standardized submaximal time during exercise following furosemide inhalation relative to saline (**Fig. 1.12**); these improvements in breathlessness ratings and airway function were also associated with increased exercise endurance time [93]. In keeping with these results, a study of 19 patients with moderate to severe COPD by Ong et al., demonstrated that 40 mg of inhaled nebulized furosemide, compared to nebulized 0.9% saline, both enhanced resting respiratory function [FEV_1 and forced vital capacity (FVC)] and relieved respiratory discomfort at a standardized time during constant-load

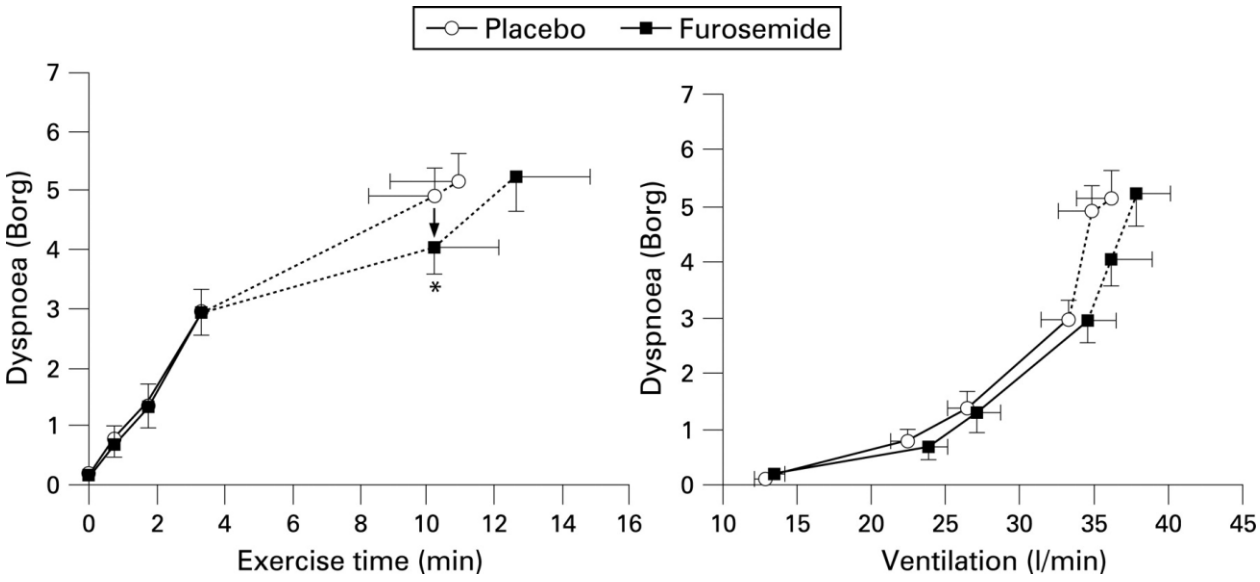

Figure 1.12. Effect of inhaled furosemide on breathlessness ratings vs. exercise time and breathlessness ratings vs. ventilation during constant-load cycle exercise in COPD patients. Adapted from Jensen et al. [93].

(but not incremental) cycle exercise testing (**Fig. 1.13**) [128]. Lastly, Panahi and colleagues looked at the effects of 40 mg of inhaled nebulized furosemide on breathlessness in 41 patients with chronic bronchitis and bronchiolitis due to sulfur mustard gas exposure [90]. Changes in pulmonary function [FEV_1, FVC and peak expiratory flow rate (PEF)] and breathlessness were not significantly different following nebulized furosemide vs. nebulized 0.9% saline 4 hours post-inhalation [90]. However, it is possible that the effects of inhaled furosemide dissipated within an hour post-treatment [73].

Evidently, although the alleviation of breathlessness *via* inhaled furosemide has a strong theoretical basis and is backed by promising data [74, 76, 114], several inconsistencies still exist. Indeed, the body of evidence indicating that inhaled nebulized furosemide relieves breathlessness insignificantly better than nebulized 0.9% saline placebo continues to grow [73, 90, 116-118, 120, 125, 126]. It is possible that suboptimal experimental conditions to observe an effect from

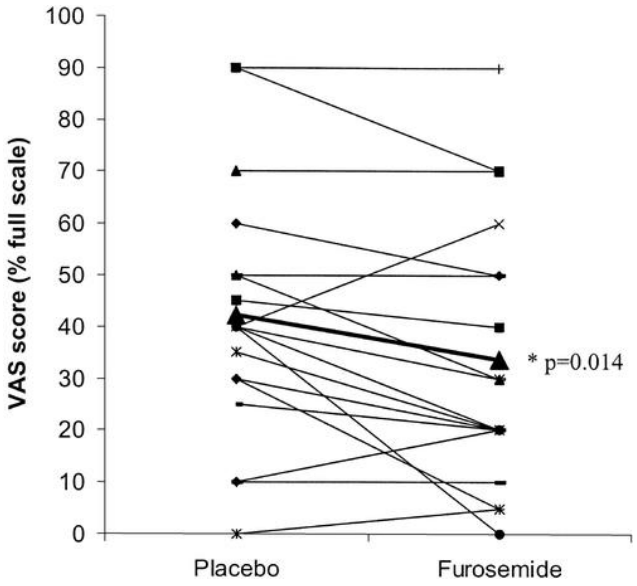

Figure 1.13. Visual analog scale (VAS) ratings at a standardized exercise time during constant work rate cycle exercise testing in COPD patients after inhalation of furosemide or placebo. The thin lines indicate individual ratings, and the thick line indicates mean values. Adapted from Ong et al. [128].

inhaled nebulized furosemide were implemented in many of these trials. For example, if inhaled nebulized furosemide predominantly relieves breathlessness *via* stimulation of SARs and mimicking a larger V_T, perhaps it would best accomplish this under conditions where the SARs are insufficiently stimulated by V_T expansion. Further, the majority of studies have used 20-40 mg doses of inhaled nebulized furosemide, which may have been insufficient to reach and alter the activity of PSRs, and thereby elicit a consistent effect of nebulized furosemide on breathlessness across different subjects/studies. It is also unclear if there is a dose-dependent relationship between inhaled nebulized furosemide and breathlessness. Finally, in many of the placebo-controlled trials, sample sizes were generally small ($n \simeq 10$) [73, 81, 115, 116, 118, 125, 126, 129].

1.7. Objectives & Hypotheses

Thus, in light of the variability and lack of consensus in the inhaled nebulized furosemide literature, the objectives and hypotheses of this randomized, double-blind, placebo-controlled, cross-over study were as follows:

Objective 1: To examine the acute effect of inhaled nebulized furosemide on ratings of perceived breathlessness during cycle endurance exercise testing in the setting of abnormal restrictive constraints on V_T expansion.

- **Hypothesis 1:** Compared with nebulized 0.9% saline (placebo), single-dose inhalation of nebulized furosemide will decrease ratings of exertional breathlessness.

Objective 2: To determine whether the acute effect of inhaled nebulized furosemide on ratings of perceived breathlessness during cycle endurance exercise testing in the setting of abnormal restrictive constraints on V_T expansion is dose-dependent.

- **Hypothesis 2:** Compared with nebulized 0.9% saline (placebo), the magnitude of relief of exertional breathlessness following single-dose inhalation of nebulized furosemide will be dose-dependent; that is, greater relief following inhalation of 120 mg furosemide vs. 40 mg furosemide vs. 0.9% saline.

Objective 3: To identify the physiological mechanism(s) underlying relief of exertional breathlessness following single-dose inhalation of nebulized furosemide.

- **Hypothesis 3:** Relief of exertional breathlessness following inhaled nebulized furosemide compared with 0.9% saline will occur independent of concurrent improvements in

cardiometabolic, gas exchange, ventilatory, breathing pattern and dynamic operating lung volume responses to exercise.

To address these objectives and test these hypotheses, we examined the effect of 40 mg and 120 mg of inhaled nebulized furosemide on detailed assessments of breathlessness, ventilation, breathing pattern, and dynamic operating lung volumes during high-intensity constant work-rate cycle exercise testing in healthy men aged 18-40 years in the setting of external thoracic restriction sufficient to reduce vital capacity (VC) by ~20% and constrain V_T expansion. External thoracic restriction was used because it acutely but adequately and reliably decreases VC, IC, and IRV and elicits breathlessness that is (i) severely intense and unpleasant, (ii) described as a heightened sense of "unsatisfied inspiration" and (iii) identified as a main exercise-limiting symptom [60, 130-133]. Lastly, external thoracic restriction in healthy adults can evoke these effects without the confounding influences to breathlessness such as hypoxemia, depressive symptoms and physical deconditioning often found in patient populations that may hide the potential benefits of inhaled nebulized furosemide.

CHAPTER TWO: EFFECT OF INHALED NEBULIZED FUROSEMIDE ON BREATHLESSNESS DURING EXERCISE IN THE SETTING OF ABNORMAL RESTRICTIVE CONSTRAINTS ON TIDAL VOLUME EXPANSION: A RANDOMIZED, DOUBLE BLIND, PLACEBO CONTROLLED, CROSSOVER, DOSE-RESPONSE STUDY

ABSTRACT

Introduction: Breathlessness that persists despite optimal treatment of the underlying pathophysiology lacks a formal interventional strategy with which it can be managed. Experimental and clinical research suggests that inhalation of *furosemide* can alleviate breathlessness provoked experimentally in health and disease, but these effects are inconsistent. We postulated that inhaled nebulized furosemide would best relieve breathlessness under conditions where the pulmonary stretch receptors are insufficiently stimulated by tidal volume (V_T) expansion. Thus, we tested the hypotheses that inhaled nebulized furosemide decreases ratings of perceived breathlessness during cycle exercise testing in the setting of abnormal restrictive constraints on V_T expansion, and that breathlessness relief is dose-dependent.

Methods: Twenty-four healthy men inhaled nebulized furosemide (40 mg and 120 mg) and nebulized 0.9% saline (placebo) in a randomized, double-blind, placebo-controlled, crossover study. Following inhalation, participants completed a symptom-limited constant-load cycle endurance exercise test at 80% of peak power output in the setting of external thoracic restriction *via* chest wall strapping to reduce slow vital capacity by ~20%.

Results: Compared with placebo, neither 40 mg nor 120 mg of inhaled nebulized furosemide had a statistically significant effect on Borg 0-10 scale intensity ratings of breathlessness, cardiometabolic, ventilatory, breathing pattern or dynamic operating lung volume responses during exercise. Compared with placebo, an effect of 120 mg, but not 40 mg, of nebulized furosemide on urine production rate was observed.

Conclusion: Under the experimental conditions of this study, inhalation of nebulized furosemide at doses of 40 mg and 120 mg did not alleviate the perception of breathlessness during exercise in healthy men.

2.1. INTRODUCTION

The population-based prevalence of adults reporting breathlessness that limits activities of daily life (e.g., walking) is ~10% [3, 4, 8]. Breathlessness is ubiquitous in advanced disease across a range of both malignant and non-malignant diagnoses [8]; for example, Müllerová et al. [22] reported that >75% of adults with very severe chronic obstructive pulmonary disease (COPD) experienced physical activity-limiting breathlessness. Notwithstanding the high prevalence and burden of breathlessness in the general population and amongst adults with advanced disease, effective management of this symptom remains a challenge for healthcare providers. For instance, Sundh and Ekström [24] found that 57% of adults with COPD experienced persistent disabling physical activity-related breathlessness despite treatment of their underlying pathophysiology with inhaled triple therapy (i.e., chronic breathlessness syndrome) [21]. With the possible exception of low-dose systemic opioids [28, 30], which are rarely prescribed for breathlessness [134] and are often associated with concerns of adverse side effects (e.g., respiratory depression, constipation) [135-137], there is currently no evidence-based pharmacotherapy indicated for use in the management of chronic breathlessness syndrome.

Randomized, double-blind, placebo-controlled crossover studies have demonstrated that inhalation of nebulized furosemide (40 mg) compared with nebulized 0.9% saline (placebo) decreased intensity ratings of perceived breathlessness provoked by a variety of respiratory stimuli at rest in healthy adults [73, 81, 115] or by constant-load cycle endurance exercise testing in COPD [93, 128]. A randomized, double-blind, parallel group study by Sheikh Motahar Vahedi et al. [127] similarly reported that inhaled nebulized furosemide (40 mg) was superior to nebulized 0.9% saline as an adjunct to conventional therapies for alleviating breathlessness at rest in adults admitted to the emergency department with an acute exacerbation of COPD.

Although the mechanisms underlying relief of breathlessness with inhaled nebulized furosemide remain unclear, changes in the activity of pulmonary stretch receptors (PSRs) that provide sensory feedback information on tidal volume (V_T) or lung expansion *via* the vagus nerve to cortical and subcortical regions of the brain implicated in the perception of breathlessness are likely contributory [52, 138]. To this end, Sudo et al. [62] showed that inhalation of nebulized furosemide enhanced the activity of slowly adapting PSRs (SARs) and suppressed the activity of rapidly adapting PSRs (RARs) during lung inflation in anaesthetized rats. In keeping with these observations, Nehashi et al. [80] reported that inhalation of nebulized furosemide inhibited respiratory distress induced by airway occlusion in anaesthetized cats. Sakurai et al. [54] similarly reported that lung expansion inhibited respiratory distress induced by airway occlusion in a dose-related manner and that bilateral vagotomy totally abolished this effect, presumably *via* loss of sensory feedback from PSRs. On the basis of these observations and the purported role of PSRs in the neuromodulation of breathlessness in humans [57, 58, 139], it has been proposed that inhaled nebulized furosemide alleviates breathlessness by altering pulmonary vagal afferent activity from PSRs, presumably mimicking greater V_T expansion [52, 62, 73, 80, 81].

However, relief of breathlessness following inhalation of nebulized furosemide is not a universal finding, with randomized, double (and triple)-blind, crossover studies reporting no statistically significant effect of inhaled nebulized furosemide (40-80 mg) compared with nebulized 0.9% saline on intensity ratings of perceived breathlessness during arm exercise tests in symptomatic adults with lung cancer or mesothelioma [126], at rest in sulfur mustard gas-exposed adults with irreversible obstructive airway disease [90], during expiratory flow-limited incremental cycle exercise testing in healthy adults [116] and during the combination of

hypercapnia and constrained ventilation in healthy adults [117, 118]. Thus, the efficacy of inhaled nebulized furosemide on breathlessness remains uncertain and requires further investigation. In particular, it remains unclear whether the efficacy of inhaled nebulized furosemide on breathlessness is dose-dependent.

The objective of this randomized, double-blind, placebo controlled, crossover study was to examine the acute effect of inhaled nebulized furosemide at doses of 40 mg and 120 mg on exertional breathlessness in healthy men. Considering the possibility that inhaled nebulized furosemide alleviates breathlessness by mimicking greater V_T expansion *via* altered pulmonary vagal afferent activity from PSRs, we studied the potential dose-response effect of inhaled nebulized furosemide on ratings of perceived breathlessness during constant-load cycle endurance exercise testing in the presence of external thoracic restriction when V_T expansion is constrained and breathlessness is (i) severely intense and unpleasant, (ii) described as a heightened sense of "unsatisfied inspiration" and (iii) identified as a main exercise-limiting symptom [60, 130, 132, 133]. We hypothesized that, under these experimental conditions, inhalation of nebulized furosemide would be associated with dose-dependent relief of exertional breathlessness compared with nebulized 0.9% saline.

2.2. METHODS

2.2.i. Participants. Participants included healthy, non-smoking, non-obese (body mass index <30 kg/m^2) men aged 18-40 years with a forced expiratory volume in 1-sec (FEV_1) ≥80% predicted [140] and a FEV_1-to-FVC ratio (FEV_1/FVC) ≥70%. Participants were excluded if they: had a known or suspected cardiovascular, metabolic, pulmonary, musculoskeletal, endocrine and/or neuromuscular disorder; were taking doctor prescribed medications; were allergic or hypersensitive to furosemide and/or any other sulfonamide-derived medication(s); had an anion gap of <10 or >16 mEq/L at rest [141]; were hypokalemic, defined as an arterialized capillary blood [K^+] of <3.5 mEq/L at rest; and/or were severely hypotensive, defined as a systolic blood pressure of ≤90 mmHg and/or diastolic blood pressure of ≤60 mmHg at rest.

2.2.ii. Study design. This was a single-center, randomized, double-blind, placebo-controlled, crossover study (ClinicalTrials.gov identifier: NCT01851980) wherein participants visited the laboratory on 4 separate occasions over a period of 2-4 weeks. Visits were conducted at approximately the same time of day (±1-hr) for each participant and separated by ≥48-hr to minimize the possibility of a potentially confounding 'carry-over' effect between *Visits 2, 3 and 4* (i.e., treatment periods A, B and C). Participants were instructed to avoid strenuous exercise and alcohol on each test day. Health Canada (File No. HC6-24-c193768) and the Research Ethics Board of the Research Institute of the McGill University Health Centre (15-370-MUHC) approved the study protocol and consent form. Written and informed consent was obtained from all participants prior to study initiation.

Visit 1 included: screening for eligibility criteria; routine clinical assessment of heart rate and rhythm by 12-lead electrocardiography (GE Marquette's CardioSoft® 12-lead ECG system;

CareFusion, Yorba Linda, CA), blood pressure by auscultation of the brachial artery with an automated blood pressure monitor (Carescape™ V100 Dynamap® monitor; GE Healthcare, Freidburg, Germany) and oxyhemoglobin saturation by finger pulse oximeter (Carescape™ V100 Dynamap® monitor); collection of arterialized capillary blood samples from a warmed earlobe (Finalgon® Cream, Boehringer Ingelheim GmbH) into a pre-heparinized capillary tube (safeCLINITUBES, D957P-70-125; Radiometer Copenhangen, Denmark) for measurement of $[K^+]$, $[Na^+]$, $[Cl^-]$ and $[HCO_3^-]$ - and subsequent calculation of the anion gap [i.e., $([Na^+] + [K^+]) - ([Cl^-] + [HCO_3^-])$] - using an OPTI™ CCA-TS2 analyzer (OPTI Medical Systems Inc., Roswell, GA, USA); pulmonary function testing, including spirometry and slow vital capacity (SVC) maneuvers; external thoracic restriction by chest wall strapping (CWS) to reduce SVC by ~20% of its baseline (unrestricted) value at rest [60, 133]; spirometry and SVC maneuvers after ~5-min of acclimatization to the CWS; and a symptom-limited incremental cardiopulmonary cycle exercise test (CPET) in the presence of CWS to determine peak power output (PPO) as well as to familiarize participants to CPET with CWS.

After randomization of treatments (*Visits 2-4*) according to a computer-generated block randomization schedule prepared by an independent third-party, participants inhaled a 12 ml solution containing either 0.9% saline placebo (12 ml), 40 mg of furosemide [4 ml of 10 mg/ml furosemide (Sandoz, Boucherville, QC, Canada) + 8 ml of 0.9% saline] or 120 mg of furosemide (12 ml of 10 mg/ml furosemide) administered by means of an Omron® NE-C30 CompAir® Elite Compact Compressor Nebulizer (Omron Healthcare, Inc., Blannockburn, IL, USA) that produced particles with a mass median diameter of ~5 μm at a nebulization rate of ~0.35 ml/min. During nebulization, participants were instructed to take deep and slow breaths through a pediatric (open) facemask that surrounded the mouth with nasal passages occluded by a nose

clip. Within ≤10-min after nebulization, participants were fitted with the CWS, which was adjusted to decrease SVC to within ±10% of the value recorded prior to CPET at *Visit 1*. After ~5-min of acclimatization to the CWS, participants performed spirometry and SVC maneuvers, followed immediately thereafter by a symptom-limited constant-load CPET at 80% of the PPO determined at *Visit 1*. Following ~10-min of recovery from CPET, participants performed two SVC maneuvers to determine whether the CWS had loosened during CPET.

To help mask the taste of the nebulized solutions and promote blinding of treatments, participants rinsed their mouth with an alcohol-free mint flavored mouthwash for 20-sec immediately before the start of nebulization, after ~20-min (i.e., midpoint) of nebulization, and at the end of nebulization. Participants were instructed to empty their bladder immediately before inhaling the nebulized solutions. Following inhalation of the nebulized solutions, participants were asked to empty their bladder into a urine collection container for determination of urine production rate [an index of diuresis and calculated as cumulative urine volume (ml) ÷ total duration of the observation period (min) beginning at the start of nebulization] immediately before the start of CPET, following 30-min of recovery from CPET and/or whenever necessary. Immediately after inhaling the nebulized solutions, participants were asked, "Do you have an urge to urinate?" If "yes", participants rated the intensity of their perceived "urge to urinate" using a 100-mm visual analog scale (VAS), where "0" represented "absolutely no urge to urinate" and "100" represented "the most intense urge to urinate imaginable or ever experienced". If "no", the intensity of perceived "urge to urinate" was assumed to be "0". Upon completing all study-related procedures at the end of *Visit 4*, participants were asked to identify during which visit they believed that they inhaled the placebo, 40 mg furosemide and 120 mg furosemide solutions.

2.2.iii. Pulmonary function tests. Spirometry and SVC maneuvers were performed with participants seated using automated equipment (Vmax Encore^{TM} 29c; CareFusion, Yorba Linda, CA) according to recommended techniques [142, 143].

2.2.iv. External thoracic restriction. An inelastic strap (Nike Structured Strength Training Belt; Beaverton, OR, USA) was fitted just beneath the axillae and around the chest to envelope the rib cage [60, 133]. The desired degree of lung volume restriction was achieved by tightening a Velcro strap at the back of the CWS while participants expired to residual volume, followed shortly thereafter by a series of SVC maneuvers.

2.2.v. Cardiopulmonary exercise testing. Exercise tests were performed on an electronically braked cycle ergometer (Lode Corival; Lode B.V. Medical Tech., Groningen, The Netherlands) using a computerized CPET system (Vmax Encore^{TM} 29c). Incremental CPETs consisted of a steady-state resting period of ≥6-min, followed by 25 W increases in power output (starting at 25 W) every 2-min: PPO was defined as the highest power output that the participant was able to sustain for ≥30-sec. Constant-load CPETs consisted of a steady-state resting period of ≥6-min, followed by a 1-min warm-up at 25% of PPO and then a step increase in power output to 80% of PPO. During both incremental and constant-load CPETs, pedal cadence was maintained between 60-90 rev/min and participants were verbally encouraged to exercise to the point of symptom-limitation (i.e., volitional fatigue).

Standard respiratory and gas exchange parameters were collected breath-by-breath while participants breathed through a rubber mouthpiece and low-resistance flow transducer with nasal

passages occluded by a nose clip. Heart rate and rhythm were monitored by 12-lead electrocardiography (GE Marquette's CardioSoft® 12-lead ECG system), while oxyhemoglobin saturation was monitored by finger pulse oximeter (Carescape™ V100 Dynamap® monitor). Inspiratory capacity (IC) maneuvers were performed at rest, within the last 30-sec of every 2-min interval during CPET and at end-exercise. Assuming that total lung capacity does not change during CPET with CWS in healthy men, changes in IC and inspiratory reserve volume [IRV = IC $-V_T$] reflect changes in dynamic end-expiratory and end-inspiratory lung volume, respectively.

Using Borg's modified 0-10 category ratio scale (CR10) [144], participants were asked to rate the intensity and unpleasantness of their perceived breathlessness as well as the intensity of their perceived leg discomfort and chest tightness at rest, within the last 30-sec of every 2-min interval during CPET and at end-exercise. Prior to each CPET, participants were familiarized with Borg's CR10 scale and its endpoints were anchored such that "0" represented "no intensity (unpleasantness) at all" and "10" represented "the most severe intensity (unpleasantness) you have ever experienced or could ever imagine experiencing." In addition, a script derived from Price et al. [145] was read to each participant prior to each CPET to help distinguish between the intensity and unpleasantness of breathlessness. Participants verbalized their main reason(s) for stopping exercise; quantified the percentage contribution of breathlessness, leg discomfort and chest tightness to exercise cessation; and identified qualitative phrases that best described their breathlessness at end-exercise [132].

2.2.vi. Analysis of exercise end-points. All physiological parameters were averaged in 30-sec intervals at rest and during CPET. These parameters, averaged over the first 30-sec of the 2nd minute of every 2-min interval during CPET, were linked with IC and symptom measurements

collected during the last 30-sec of the same minute. Three main time points were used for the evaluation of measured parameters: (1) *pre-exercise rest*, defined as the average of the last 60-sec of the steady-state period after ≥3-min of breathing on the mouthpiece while seated on the cycle ergometer before the start of CPET; (2) *isotime*, defined as the average of the first 30-sec of the 2nd minute of the highest equivalent 2-min interval of constant-load CPET completed by a given participant; and (3) *peak exercise*, defined as the average of the last 30-sec of loaded pedaling during constant-load CPET at 80% of PPO. Exercise endurance time (EET) was defined as the duration of loaded pedaling during constant-load CPET at 80% of PPO.

2.2.vii. Sample size estimation and statistical analyses. Using a formula for a balanced analysis of variance (ANOVA) with crossover design in combination with Tukey's HSD adjustment method [146], we estimated that 24 participants were needed to detect a ±1 Borg CR10 scale unit difference in breathlessness intensity ratings during constant-load CPET at isotime (primary outcome variable) across the 3 treatments, assuming a two-tailed test of significance, a within-subject standard deviation of ±1 Borg CR10 scale units, α=0.05 and β=0.80.

A one-way repeated measures ANOVA with Tukey's HSD post-hoc test was used to examine the effect of treatment with 0.9% saline placebo, 40 mg furosemide and 120 mg furosemide on: the duration of nebulization; the amount of time between the end of nebulization and the end of CPET; post-dose SVC and spirometric pulmonary function test parameters recorded prior to CPET; urine production rate; intensity ratings of the perceived "urge to urinate"; the percentage contribution of breathlessness, leg discomfort and chest tightness to exercise cessation; and EET. A two-way repeated measures ANOVA with Tukey's HSD post-hoc test was used to examine treatment, time (e.g., rest, isotime, peak exercise) and

treatment*time effects on physiological and perceptual parameters measured at rest and during constant-load CPETs. Fisher's exact test was used to examine the effect of treatment with (i) placebo vs. 40 mg furosemide, (ii) placebo vs. 120 mg furosemide and (iii) 40 mg furosemide vs. 120 mg furosemide on the percentage of participants reporting an "urge to urinate" after inhaling the nebulized solution. A chi-squared test was used to compare the selection frequency of the individual reasons for stopping exercise as well as the selection frequency of the individual descriptors of breathlessness at end-exercise across treatments. All analyses were performed using SigmaStat® (Version 3.5; Systat® Software, San Jose, CA, USA) and statistical significance was set at $p<0.05$. Data are presented as means ± SEM.

2.3. RESULTS

2.3.i. Participant characteristics. Participants included 24 healthy, non-obese (body mass index, 23.9 ± 0.6 kg/m^2) men aged 25.3 ± 1.2 yrs with normal spirometry (FEV_1, 95 ± 3% predicted; FEV_1/FVC, 79.7 ± 1.3%) and a symptom-limited PPO and peak rate of oxygen uptake ($\dot{V}O_{2peak}$) on incremental CPET with CWS of 205 ± 10 W and 41.9 ± 1.9 ml/kg/min, respectively.

2.3.ii. Time course of nebulization and post-dose CPET. The duration of nebulization was not significantly different across treatments: placebo, 35.7 ± 1.0 min; 40 mg furosemide, 36.4 ± 1.2 min; and 120 mg furosemide, 39.2 ± 1.1 min (p=0.075). The amount of time between the end of nebulization and the end of CPET was not significantly different across treatments: placebo, 50.8 ± 1.3 min; 40 mg furosemide, 53.2 ± 1.4 min; and 120 mg furosemide, 55.3 ± 1.7 min (p=0.086).

2.3.iii. Diuresis. As illustrated in **Fig. 2.1**, inhalation of the 40 mg furosemide solution had no statistically significant effect on urine production rate, the percentage of participants reporting an "urge to urinate" and/or the intensity of perceived "urge to urinate" compared with placebo. By contrast, urine production rate, the percentage of participants reporting an "urge to urinate" and the intensity of perceived "urge to urinate" were all significantly greater after inhaling the 120 mg furosemide solution compared with both placebo and 40 mg solutions. No other systemic or adverse effects were reported following inhalation of the 40 mg and 120 mg furosemide solutions.

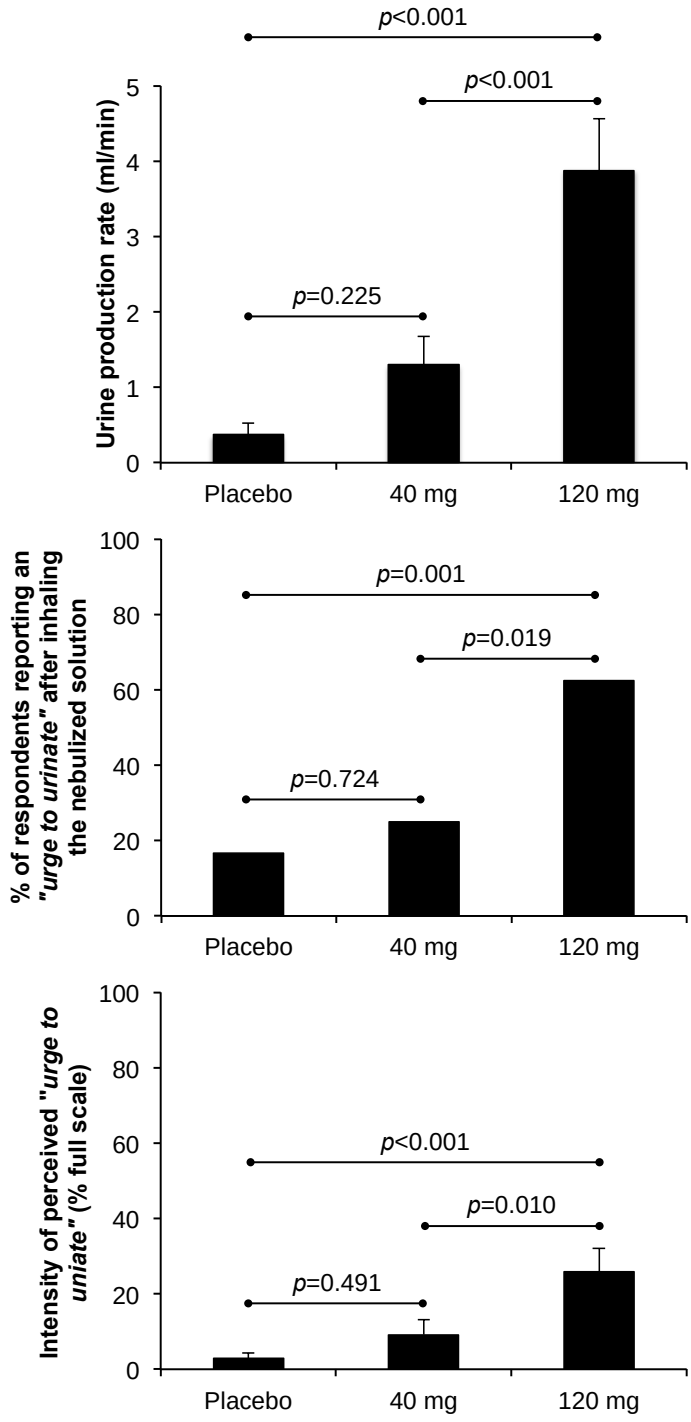

Figure 2.1. Effect of single-dose inhalation of nebulized furosemide (40 mg and 120 mg) on urine production rate (mean ± SE), the percentage of participants reporting an "urge to urinate" and the intensity of perceived "urge to urinate" (mean ± SE). Cumulative urine output was 37.8 ± 15.2 ml, 135.8 ± 39.9 ml and 423.9 ± 77.3 ml over an observation period of 98.5 ± 1.4 min, 101.0 ± 1.8 min and 107.6 ± 2.5 min during 0.9% saline placebo, 40 mg furosemide and 120 mg furosemide treatment visits, respectively.

2.3.iv. Effect of external thoracic restriction and inhaled nebulized furosemide on pulmonary function test parameters. The effect of CWS and inhaled nebulized furosemide on SVC and spirometric pulmonary function test parameters are presented in **Table 2.1**. By design, CWS decreased SVC recorded prior to CPET by 21 ± 1% (range: -15 to -31%), 22 ± 1% (range: -13 to -31%), 21 ± 1% (range: -15 to -31%) and 21 ± 1% (range: -13 to -33%) of its baseline (unrestricted) value at *Visit 1* and at the placebo, 40 mg furosemide and 120 mg furosemide treatment visits, respectively. The SVC values recorded prior to CPET at the placebo, 40 mg furosemide and 120 mg furosemide visits were closely matched to the target SVC value recorded prior to CPET at *Visit 1*: 99 ± 1% (range: 94-105%; p=0.092 by paired t-test); 100 ± 1% (range: 95-111%; p=0.810 by paired t-test); and 100 ± 1% (range: 95-109%; p=0.769 by paired t-test), respectively. The intra-subject, between-day (or between-treatment) coefficient of variability in the SVC value recorded prior to CPET was 2.2 ± 0.2% (range: 0.7-4.5%). The SVC values recorded before vs. after CPET were not significantly different at *Visit 1* (4.41 ± 0.16 L vs. 4.54 ± 0.19 L; p=0.386 by paired t-test) and at the placebo (4.36 ± 0.15 L vs. 4.47 ± 0.16 L; p=0.106 by paired t-test), 40 mg furosemide (4.41 ± 0.16 L vs. 4.47 ± 0.16 L; p=0.115 by paired t-test) and 120 mg furosemide visits (4.40 ± 0.15 L vs. 4.42 ± 0.15 L; p=0.106 by paired t-test). Compared with placebo, neither dose of inhaled nebulized furosemide had an effect on SVC and spirometric pulmonary function test parameters recorded prior to CPET (**Table 2.1**).

2.3.v. Effect of inhaled nebulized furosemide on perceptual and physiological responses to CPET. Compared with placebo, neither dose of inhaled nebulized furosemide had an effect on EET or an effect on perceptual and physiological parameters recorded at rest and during CPET (**Table 2.2, Figs. 2.2 and 2.3**).

The relative contributions of breathlessness [placebo, 41.9 ± 3.4%; 40 mg furosemide, 40.2 ± 3.6%; 120 mg furosemide, 39.4 ± 4.0% (p=0.876)], leg discomfort [placebo, 55.4 ± 4.1%; 40 mg furosemide, 54.4 ± 4.2%; 120 mg furosemide, 51.7 ± 4.6% (p=0.746)] and chest tightness [placebo, 2.4 ± 1.5%; 40 mg furosemide, 0.8 ± 0.8%; 120 mg furosemide, 6.0 ± 3.3% (p=0.170)] to exercise cessation were not significantly different across treatments. The distribution of reasons for stopping exercise was similar across treatments: breathlessness [placebo, n=2; 40 mg furosemide, n=4; 120 mg furosemide, n=3 (p=0.683)]; leg discomfort [placebo, n=8; 40 mg furosemide, n=9; 120 mg furosemide, n=8 (p=0.941)]; combination of breathlessness and leg discomfort [placebo, n=14; 40 mg furosemide, n=10; 120 mg furosemide, n=10 (p=0.410)]; and other (placebo, n=0; 40 mg furosemide, n=1; 120 mg furosemide, n=3). The selection frequencies of the qualitative descriptors of breathlessness at end-exercise were also similar across treatments: *"My breath does not go in all the way"* [placebo, 65.2%; 40 mg furosemide, 69.6%; 120 mg furosemide, 65.2% (p=0.937)]; *"Breathing in requires effort"* [placebo, 91.3%; 40 mg furosemide, 87.0%; 120 mg furosemide, 73.9% (p=0.245)]; *"I feel a need for more air"* [placebo, 91.3%; 40 mg furosemide, 82.6%; 120 mg furosemide, 87.0% (p=0.682)]; *"My breathing is heavy"* [placebo, 95.7%; 40 mg furosemide, 100%; 120 mg furosemide, 95.7% (p=1.00)]; *"I cannot take a deep breath in"* [placebo, 60.9%; 40 mg furosemide, 60.9%; 120 mg furosemide, 78.3% (p=0.352)]; *"My chest feels tight"* [placebo, 95.7%; 40 mg furosemide, 91.3%; 120 mg furosemide, 91.3% (p=0.806)]; *"My breathing requires more work"* [placebo, 91.3%; 40 mg furosemide, 87.0%; 120 mg furosemide, 91.3% (p=0.853)]; *"I feel a hunger for more air"* [placebo, 87.0%; 40 mg furosemide, 87.0%; 120 mg furosemide, 82.6% (p=0.890)]; *"I feel that my breathing is rapid"* [placebo, 91.3%; 40 mg furosemide, 78.3%; 120 mg furosemide, 91.3% (p=0.317)]; *"My breathing feels shallow"* [placebo, 78.3%; 40 mg

furosemide, 73.9%; 120 mg furosemide, 69.6% (p=0.798)]; and *"I cannot get enough air in"* [placebo, 82.6%; 40 mg furosemide, 69.6%; 120 mg furosemide, 73.9% (p=0.579)].

2.3.vi. Debriefing. There was no statistically significant difference in the percentage of participants that correctly identified the visit at which they inhaled the placebo, 40 mg furosemide and 120 mg furosemide solutions: 45.8%, 41.7% and 62.5%, respectively (p=0.311 by chi-squared test).

Table 2.1. Effect of external thoracic restriction and inhaled nebulized furosemide on slow vital capacity (SVC) and spirometric pulmonary function test parameters.

Parameter	Unrestricted	Visit 1	Placebo	40 mg Furosemide	120 mg Furosemide
SVC, L	5.59 ± 0.19	4.41 ± 0.16	4.36 ± 0.15	4.41 ± 0.16	4.40 ± 0.15
FVC, L	5.48 ± 0.18	4.28 ± 0.14	4.25 ± 0.15	4.28 ± 0.14	4.26 ± 0.14
FEV_1, L	4.34 ± 0.13	3.39 ± 0.09	3.46 ± 0.12	3.49 ± 0.11	3.48 ± 0.11
FEV_1/FVC, %	79.7 ± 1.3	79.9 ± 1.5	81.8 ± 1.5	82.2 ± 1.4	82.1 ± 1.5
PEF, L/sec	8.06 ± 0.28	6.54 ± 0.22	7.21 ± 0.22	7.43 ± 0.23	7.10 ± 0.24
$FEV_{25-75\%}$, L/sec	4.25 ± 0.19	3.38 ± 0.15	3.46 ± 0.19	3.57 ± 0.18	3.60 ± 0.20

Values are means ± SE. FVC, forced vital capacity; FEV_1, forced expiratory volume in 1-sec; PEF, peak expiratory flow; $FEF_{25-75\%}$, forced expiratory flow between 25% and 75% of the forced vital capacity maneuver.

Table 2.2. Effect of inhaled nebulized furosemide on physiological and perceptual parameters at rest and during constant-load cycle endurance exercise testing at 80% of the symptom-limited peak power output achieved during incremental cycle exercise testing in the presence of external thoracic restriction, equivalent to 166 ± 8 W.

	REST			ISOTIME			PEAK		
Parameter	**Placebo**	**40 mg Furosemide**	**120 mg Furosemide**	**Placebo**	**40 mg Furosemide**	**120 mg Furosemide**	**Placebo**	**40 mg Furosemide**	**120 mg Furosemide**
Exercise time, min	-	-	-	8.5 ± 0.8	8.5 ± 0.8	8.5 ± 0.8	11.1 ± 0.8	10.1 ± 0.9	10.3 ± 0.8
Breathlessness intensity, Borg CR10 units	0.5 ± 0.2	0.6 ± 0.2	0.7 ± 0.2	7.0 ± 0.4	6.8 ± 0.5	6.8 ± 0.5	8.5 ± 0.4	8.0 ± 0.4	8.3 ± 0.4
Breathlessness unpleasantness, Borg CR10 units	0.5 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	6.7 ± 0.5	6.8 ± 0.5	6.6 ± 0.5	8.2 ± 0.4	7.8 ± 0.5	7.9 ± 0.4
Leg discomfort, Borg CR10 units	0.4 ± 0.2	0.4 ± 0.1	0.3 ± 0.1	8.2 ± 0.4	8.0 ± 0.3	7.7 ± 0.4	9.5 ± 0.2	9.0 ± 0.3	8.8 ± 0.4
Chest tightness, Borg CR10 units	1.4 ± 0.2	1.8 ± 0.2	1.7 ± 0.3	6.6 ± 0.5	6.3 ± 0.5	6.6 ± 0.5	7.6 ± 0.4	7.3 ± 0.4	7.8 ± 0.4
$\dot{V}O_2$, ml/kg/min	4.4 ± 0.2	4.5 ± 0.2	4.7 ± 0.2	38.4 ± 1.8	38.6 ± 1.9	38.5 ± 2.0	40.5 ± 1.8	39.7 ± 1.9	39.0 ± 1.8
HR, beats/min	73 ± 2	76 ± 3	77 ± 2	171 ± 3	174 ± 2	173 ± 3	176 ± 3	177 ± 3	177 ± 3
O_2 pulse, ml O_2/beat	4.5 ± 0.2	4.5 ± 0.3	4.6 ± 0.3	17.7 ± 1.2	18.2 ± 1.3	18.2 ± 1.2	17.9 ± 1.2	18.0 ± 1.3	18.2 ± 1.2
$\dot{V}_E/\dot{V}CO_2$	43.4 ± 1.4	43.6 ± 1.3	42.3 ± 1.4	30.1 ± 0.6	30.2 ± 0.5	30.3 ± 0.6	32.2 ± 0.7	32.1 ± 0.5	32.1 ± 0.5
$P_{ET}CO_2$, mmHg	35.1 ± 0.6	35.2 ± 0.5	34.7 ± 0.6	36.0 ± 0.6	35.9 ± 0.5	35.8 ± 0.6	33.8 ± 0.6	33.8 ± 0.5	34.0 ± 0.5
SpO_2, %	98.3 ± 0.3	98.0 ± 0.3	98.0 ± 0.3	97.2 ± 0.4	97.3 ± 0.4	97.6 ± 0.4	97.0 ± 0.4	97.0 ± 0.3	97.0 ± 0.4
$\dot{V}_E$, L/min	11.7 ± 0.5	12.2 ± 0.4	12.6 ± 0.8	90.2 ± 4.2	91.6 ± 4.2	90.6 ± 4.0	97.3 ± 4.4	97.9 ± 4.1	95.1 ± 3.9
V_T, L	0.71 ± 0.03	0.72 ± 0.04	0.77 ± 0.06	2.12 ± 0.1	2.14 ± 0.1	2.10 ± 0.1	2.00 ± 0.10	2.00 ± 0.10	1.97 ± 0.10
V_T, %SVC	16.4 ± 0.7	16.5 ± 0.8	17.3 ± 0.9	48.6 ± 1.2	48.4 ± 1.1	47.68 ± 1.3	45.81 ± 1.4	45.48 ± 1.1	44.62 ± 1.5
f_R, breaths/min	17.5 ± 0.8	18.2 ± 0.9	17.6 ± 0.9	43.6 ± 1.9	43.9 ± 1.8	43.7 ± 1.6	50.1 ± 2.3	49.8 ± 1.6	50.0 ± 2.1
IC, L	2.82 ± 0.13	2.85 ± 0.14	2.85 ± 0.15	3.08 ± 0.12	3.01 ± 0.11	3.14 ± 0.12	2.97 ± 0.13	2.91 ± 0.10	2.91 ± 0.13
IC, %SVC	64.8 ± 2.1	64.7 ± 2.0	64.4 ± 2.2	70.7 ± 1.8	70.2 ± 2.2	71.3 ± 1.6	68.0 ± 2.0	67.9 ± 2.1	66.2 ± 2.1
Change in IC from rest, L	-	-	-	0.26 ± 0.09	0.26 ± 0.06	0.29 ± 0.08	0.16 ± 0.08	0.16 ± 0.06	0.06 ± 0.11
IRV, L	2.11 ± 0.12	2.12 ± 0.13	2.07 ± 0.12	0.96 ± 0.08	0.89 ± 0.09	1.03 ± 0.07	0.97 ± 0.09	0.93 ± 0.08	0.93 ± 0.08
IRV, %SVC	48.4 ± 2.1	48.17 ± 2.1	47.1 ± 2.1	22.1 ± 1.8	21.6 ± 2.4	23.7 ± 1.6	22.4 ± 1.9	22.4 ± 2.1	21.5 ± 1.8

Values are means ± SE. $\dot{V}O_2$, rate of oxygen uptake; HR, heart rate; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent for carbon dioxide; $P_{ET}CO_2$, end-tidal partial pressure of carbon dioxide; SpO_2, oxyhemoglobin saturation; $\dot{V}_E$, minute ventilation; V_T, tidal volume; SVC, slow vital capacity measured prior to start of exercise; f_R, respiratory frequency; IC, inspiratory capacity; IRV, inspiratory reserve volume.

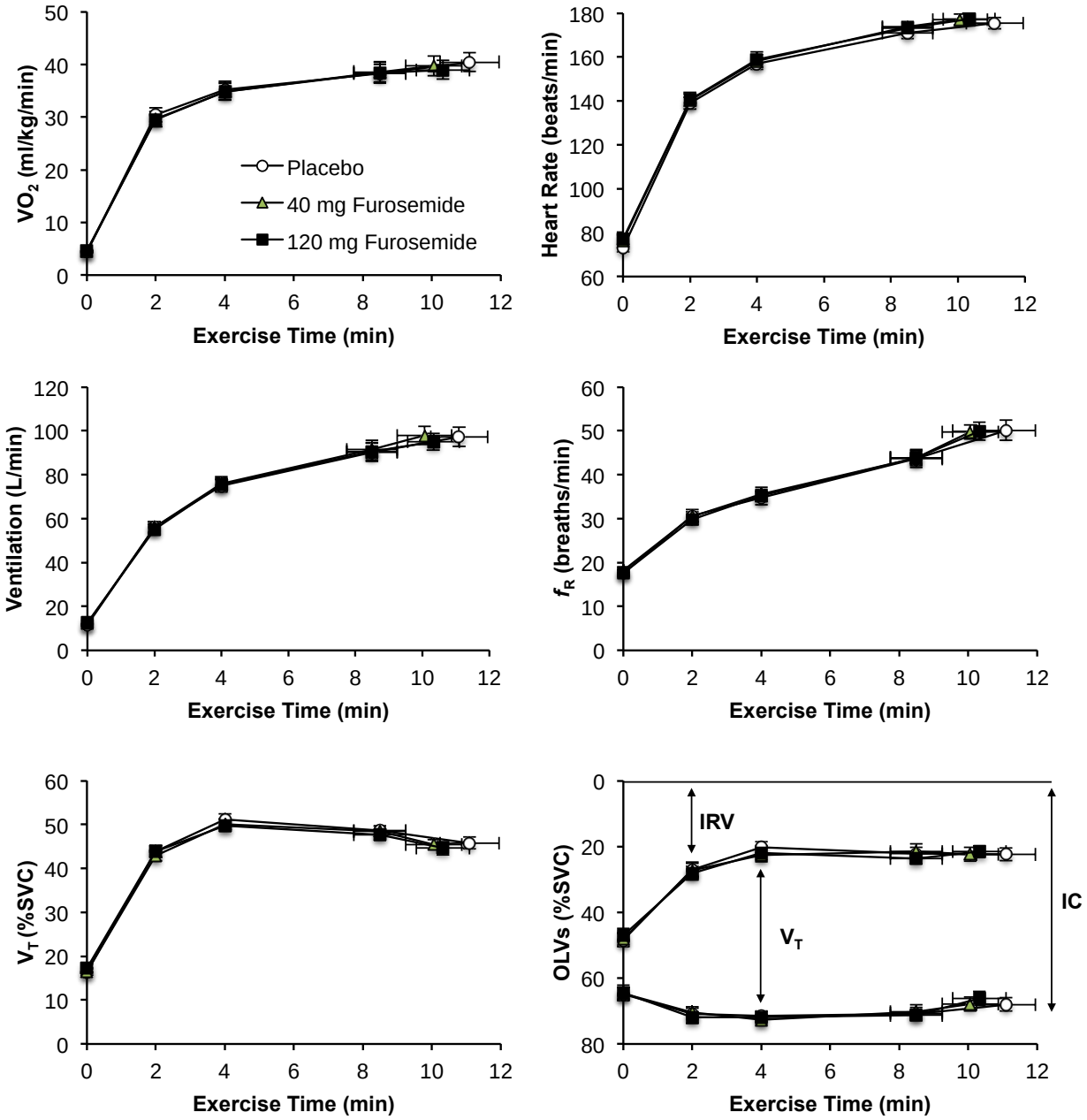

Figure 2.2. Effect of inhaled nebulized furosemide at doses of 40 mg and 120 mg on metabolic, cardiac, ventilatory, breathing pattern and dynamic operating lung volume parameters at rest and during constant-load cycle endurance exercise testing at 80% of the symptom-limited peak power output achieved during incremental cycle exercise testing in the presence of external thoracic restriction, equivalent to 166 ± 8 W. VO_2, rate of oxygen uptake; f_R, respiratory frequency; V_T, tidal volume; SVC, slow vital capacity measured prior to the start of exercise; OLVs, operating lung volumes; IRV, inspiratory reserve volume; IC, inspiratory capacity. Values are means ± SE at rest, at standardized submaximal time points of 2-min, 4-min and 8.5 ± 0.8-min (isotime), and at peak exercise.

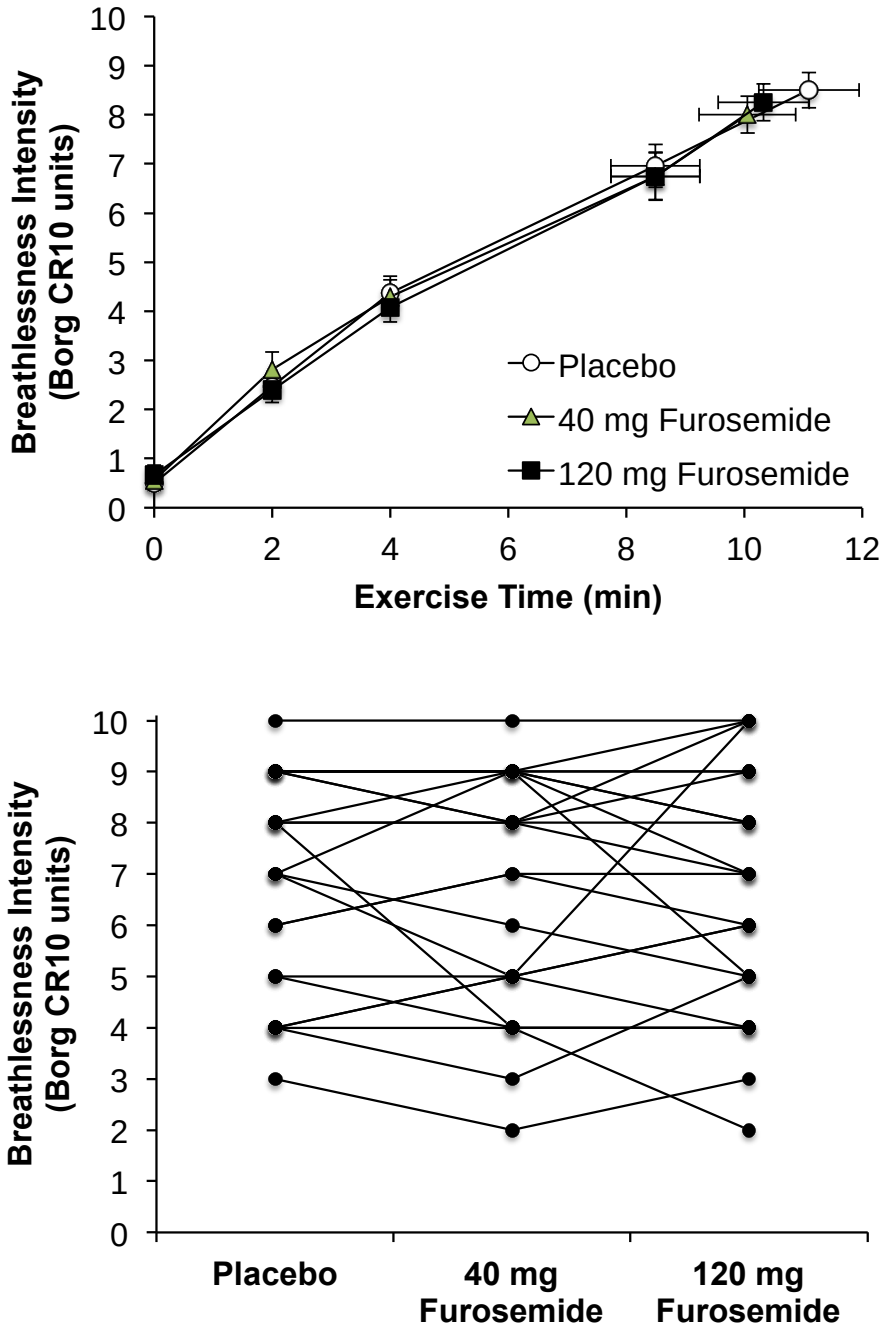

Figure 2.3. Effect of inhaled nebulized furosemide at doses of 40 mg and 120 mg on intensity ratings of perceived breathlessness at rest and during constant-load cycle endurance exercise testing at 80% of the symptom-limited peak power output achieved during incremental cycle exercise testing in the presence of external thoracic restriction, equivalent to 166 ± 8 W. *Top panel*: Values are means ± SE at rest, at standardized submaximal time points of 2-min, 4-min and 8.5 ± 0.8-min (isotime), and at peak exercise. *Bottom panel*: Individual participant post-dose breathlessness intensity ratings during exercise at isotime.

2.4. DISCUSSION

The primary finding of this randomized, double-blind, placebo-controlled, crossover study is that inhalation of nebulized furosemide at doses of 40 mg and 120 mg had no demonstrable effect on ratings of perceived breathlessness during constant-load cycle endurance exercise testing in the setting of abnormal restrictive constraints on V_T expansion (i.e., external thoracic restriction) in healthy, young men.

The results of our study are in contrast to those of earlier randomized, double-blind, crossover studies by: (1) Nishino et al. [81] who reported that inhalation of nebulized furosemide (40 mg) compared with nebulized 0.9% saline decreased the intensity of breathlessness induced by voluntary breath-holding and by a combination of hypercapnia and inspiratory resistive loading in 12 healthy subjects; (2) Minowa et al. [115] who demonstrated that the magnitude of increase in breathlessness intensity ratings during hypercapnic hyperpnea was not significantly different after vs. before inhalation of nebulized 0.9% saline, but was significantly reduced after vs. before inhalation of nebulized furosemide (40 mg) in 10 healthy subjects; (3) Moosavi et al. [73] who demonstrated that the magnitude of the pre- to post-dose decrease in breathlessness intensity ratings was marginally greater for inhaled nebulized furosemide (40 mg) compared with nebulized 0.9% saline (p=0.052) in 10 healthy subjects; and (4) Ong et al. [128] and Jensen et al. [93] who found that breathlessness intensity ratings were significantly lower (by ~20%) at a standardized submaximal time during constant-load cycle endurance exercise testing after inhalation of nebulized furosemide (40 mg) compared with nebulized 0.9% saline in 19 and 20 adults with COPD, respectively. Potential reasons for the discrepant results between these earlier studies and our own [with the obvious exception of our comparatively larger sample size (n=24)] are discussed in the *Methodological Considerations* section below.

Clear evidence of diuresis was apparent after our participants inhaled the 120 mg nebulized furosemide solution compared with both 40 mg furosemide and 0.9% saline solutions. Nevertheless, inhalation of the 120 mg nebulized furosemide solution had no effect on exertional breathlessness, suggesting that systemic absorption of furosemide from the gastrointestinal tract cannot explain earlier reports of breathlessness relief following inhalation of nebulized furosemide (40 mg) compared with 0.9% saline [73, 81, 93, 115, 128].

Our findings confirm and extend those of earlier randomized, double (and triple)-blind, crossover studies by: (1) Wilcock et al. [126] who found that intensity ratings of perceived breathlessness during arm exercise were not significantly different following inhalation of nebulized furosemide (40 mg) compared with nebulized 0.9% saline in 15 symptomatic patients with lung cancer or mesothelioma; (2) Panahi et al. [90] who demonstrated that the magnitude of the pre- to post-dose decrease in breathlessness intensity ratings recorded at rest was not significantly different for inhalation of nebulized furosemide (40 mg) compared with nebulized 0.9% saline in 41 adults with irreversible obstructive airway disease due to sulfur mustard gas exposure; and (3) Laveneziana et al. [116] who reported that inhalation of nebulized furosemide (40 mg and 80 mg) compared with nebulized 0.9% saline had no effect on intensity ratings of breathlessness during expiratory flow-limited incremental cycle CPET in 9 healthy adults.

2.4.i. Methodological considerations. As reviewed in the introduction, relief of breathlessness following inhalation of nebulized furosemide has been mechanistically linked to altered pulmonary vagal afferent activity from PSRs (most likely SARs), presumably mimicking greater V_T expansion [52, 62, 73, 80, 81]. On this basis, we reasoned that the potential dose-response effect of inhaled nebulized furosemide on breathlessness might be uniquely revealed during

constant-load CPET in the presence of an external thoracic restriction when V_T expansion (and presumably also activation of SARs) is reduced and breathlessness is (i) severely intense and unpleasant, (ii) described as a heightened sense of "unsatisfied inspiration" and (iii) identified as a main exercise-limiting symptom [60, 130, 132, 133]. By studying healthy men as opposed to symptomatic adults with advanced disease, we also reasoned that the potentially confounding influences of psycho-physiological comorbidities, deconditioning, hypoxemia, hypercapnia, concomitant medication use, etc. on exertional breathlessness would be minimized, thereby increasing our ability to demonstrate a potential dose-response effect of nebulized furosemide on exertional breathlessness. Nevertheless, neither 40 mg nor 120 mg doses of nebulized furosemide had an effect on exertional breathlessness compared with nebulized 0.9% saline.

It could be argued that external thoracic restriction may have masked a potential effect of nebulized furosemide on exertional breathlessness *via* stimulation of pulmonary irritant receptors and/or RARs by way of alveolar collapse (atelectasis) and/or breathing at abnormally low lung volumes, respectively. However, studies by Sant'Ambrogio et al. [63] and Sudo et al. [62] showed that inhalation of nebulized furosemide inhibited the activity of laryngeal irritant receptors to stimulation by inhalation of low-chloride solutions in anaesthetized, spontaneously breathing dogs and suppressed the activity of RARs during lung inflation in anaesthetized rats, respectively. On the basis of these observations, it is reasonable to contend that our use of external thoracic restriction likely served to increase the probability of demonstrating an effect of nebulized furosemide compared with nebulized 0.9% saline on exertional breathlessness. Furthermore, if CWS caused a meaningful degree of atelectasis (and attendant ventilation-perfusion mismatching), then it is reasonable to assume that the ventilatory equivalent for carbon dioxide ($\dot{V}_E/\dot{V}CO_2$) – an index of exercise ventilatory efficiency – would be elevated during

exercise with vs. without CWS; however, this does not appear to be the case since earlier studies by O'Donnell et al. [132], Mendonca et al. [60] and Kotrach et al. [133] reported no statistically significant effect of CWS sufficient to reduce vital capacity by ~20-35% of its baseline (unrestricted) value on the $\dot{V}_E/\dot{V}CO_2$ response to symptom-limited incremental and constant-load cycle CPET.

By design, CWS decreased SVC recorded prior to CPET at each treatment visit by an average of 21-22% of its baseline (unrestricted) value and to within an average of ≤1% of the target SVC value recorded prior to CPET at *Visit 1*. The intra-subject, between-day (or between-treatment) coefficient of variability in the SVC value recorded prior to CPET was very low at just 2.2 ± 0.2%, confirming our ability to reproducibly restrict lung volumes *via* CWS across treatment visits. Thus, it is unlikely that intra-subject, between-day (or between-treatment) variability in the extent of external thoracic restriction confounded our ability to demonstrate a potential dose-response effect of nebulized furosemide on exertional breathlessness.

Even though our participants were asked to rate their perception of breathlessness (intensity and unpleasantness) separately from their perception of chest tightness, we cannot rule out the possibility that conflation of these ratings concealed a potential dose-response effect of nebulized furosemide on exertional breathlessness. However, the percentage contributions of chest tightness and of breathlessness to exercise cessation were markedly different across treatments (≤6% vs. 39-42%, respectively), even though Borg CR10 scale ratings of these two symptoms were quantitatively similar during CPET. Thus, it seems unlikely that the null results of our study can be readily explained by conflation of ratings of chest tightness and of breathlessness.

We took several steps to optimize delivery of nebulized furosemide to the airways and lungs. First, we studied healthy men with normal spirometry (and presumably also normal airway geometry) and without airway inflammation, obstruction and/or secretions [72]. Second, nebulized solutions were delivered through the mouth alone (albeit with an open facemask) during deep and slow tidal inspirations [72, 147]. Third, we used a compressed air (jet) nebulizer that produced particles with a mass median diameter of ~5 μm, which are within the "respirable range" for therapeutic aerosols [72]. Fourth, we used a nebulized dose of furosemide (120 mg) that was 3-fold higher than the 40 mg dose used in previous randomized, placebo controlled studies [73, 81, 90, 93, 115, 116, 126-128]. Despite these considerations and for reasons described in detail elsewhere [72], it is reasonable to assume that only a small fraction (~10%) of the available furosemide was actually deposited into the airways and lungs of our participants during nebulization. The possibility therefore exists that the null results of our study reflect, at least in part, limited delivery of nebulized furosemide into the airways and lungs of our participants (i.e., nebulized furosemide failed to reach and subsequently act on PSRs). However, earlier studies reporting a beneficial effect of just 40 mg of nebulized furosemide compared with nebulized 0.9% saline on the perception of breathlessness in health [73, 81, 115] and COPD [93, 127, 128] all employed compressed air (jet) nebulizers with similar performance characteristics (i.e., particle size range, nebulization rate) as the one used in our study. Furthermore, the results of studies by Morélot-Panzini et al. [118] and O'Donnell et al. [117] indicated that optimal delivery of nebulized furosemide at doses of 40 mg and 80 mg using a mechanical ventilator at controlled inspiratory flow rates (300-500 ml/sec) and levels of V_T expansion (15% of predicted vital capacity) did not produce more consistent and/or greater relief of laboratory-induced breathlessness compared with nebulized 0.9% saline in 12 healthy adults. Thus, it seems unlikely

that factors related to suboptimal delivery of nebulized furosemide to the airways and lungs can explain the null results of our study.

According to the results of their randomized, single-blind, parallel group study of patients admitted to the hospital for an exacerbation of COPD, Khan and O'Driscoll [148] concluded that nebulized 0.9% saline cannot be used as an inert placebo in studies assessing relief of breathlessness with therapeutic aerosols. In support of this conclusion, randomized, double (and triple)-blind, crossover studies by Panahi et al. [90], O'Donnell et al. [117] and Morélot-Panzini et al. [118] have reported statistically significant pre- to post-dose relief of breathlessness for both nebulized 0.9% saline and nebulized furosemide (40-80 mg). In each of these studies, the magnitude of relief produced by nebulized 0.9% saline was comparable to that produced by nebulized furosemide [90, 117, 118]. O'Donnell et al. [129] have recently provided evidence that relief of breathlessness following inhalation of nebulized 0.9% saline (i.e., "placebo effect") can be largely explained by participants' expectation of a treatment effect. As part of the informed consent procedure, the men who participated in our study were told that our primary objective was to determine whether inhalation of nebulized furosemide decreases the perception of breathlessness during exercise. In light of the above, and despite the fact that there was no statistically significant difference in the percentage of participants that identified correctly the nebulized solution they received at study *Visits 2, 3 and 4*, we cannot rule out the possibility that the null results of our study may be due, at least in part, to a "placebo effect."

While the duration of action of inhaled nebulized furosemide is not known, Moosavi et al. [73] reported that relief of breathlessness following single-dose inhalation of nebulized furosemide (40 mg) compared with nebulized 0.9% saline dissipated after an average of 1-hr. In our study, constant-load CPETs across all 3 treatments were completed within an average of 85-

95 min and 50-55 min from the start and from the end of the nebulization period, respectively. It follows that the null results of our study may be due to diminution of furosemide's effect on pulmonary vagal afferent activity from PSRs. However, a randomized, placebo-controlled, crossover study by Novembre et al. [149] found that doubling the dose of nebulized furosemide from 15 mg to 30 mg prolonged (but did not enhance) its preventive effect on exercise-induced asthma in children from at least 2-hrs to at least 4-hrs. Thus, it seems unlikely that the lack of effect of 40 mg and especially 120 mg of nebulized furosemide on exertional breathlessness in our study can be explained by diminution of furosemide's effect on pulmonary vagal afferent activity from PSRs.

2.4.ii. Conclusion. Under the experimental conditions of this randomized, double-blind, placebo-controlled, crossover study, inhalation of nebulized furosemide at doses of 40 mg and 120 mg did not alleviate the perception of breathlessness during exercise in healthy men.

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