KU Leuven

Biomedical Sciences Group

Faculty of Movement and Rehabilitation Sciences

Department of Rehabilitation Sciences

EFFECTS OF RESPIRATORY MUSCLE TRAINING ON PHYSIOLOGICAL AND PSYCHOLOGICAL ASPECTS OF DYSPNEA PERCEPTION AND EXERCISE TOLERANCE IN PATIENTS WITH COPD

Sauwaluk DACHA

Jury:

Promoter:	Prof. dr. D. Langer
Co-promoters:	Prof. dr. R Gosselink
	Prof. dr. A. von Leupoldt
Chair:	Prof. dr. S. Verschueren
Jury members:	
	Prof. dr. T. Troosters
	Prof. dr. W. Janssens
	Prof. dr. I. Van Diest
	Prof. dr. W.D. Reid

Dissertation presented
in partial fulfilment of the
requirements for the degree of
Doctor in Biomedical Sciences

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

6MWD	6 minute walking distance
ABD	Abdominal muscle
ANOVA	Analysis of variance
A-vO_2	Arterio-venous oxygen content
BBQ	Breathlessness Beliefs Questionnaire
BDI	Baseline dyspnea index
BF	Breathing frequency
BFI	Blood flow index
CaO_2	Arterial oxygen content
CAT	COPD assessment test
CHF	Chronic heart failure
CI	Confidence intervals
CO	Cardiac output
COPD	Chronic obstructive pulmonary disease
CPET	Cardiopulmonary exercise testing
CV	Coefficient of Variation
CWR	Constant work rate
DH	Dynamic hyperinflation
DLCO	Diffusing capacity of the lung for carbon monoxide
ECG	Electrocardiogram
EEG	Electroencephalogram
EELV	End-expiratory lung volume
EMG	Electromyography
FEV_1	Forced expiratory volume in one second
fMRI	Functional magnetic resonance imaging
FRC	Functional residual capacity
FVC	Forced vital capacity
FWO	Research foundation – Flanders
HADS	Hospital anxiety and depression scale
Hb	Deoxygenated hemoglobin
HbO_2	Oxygenated hemoglobin
HR	Heart rate
IC	Inspiratory capacity

ICC	Intra-class correlation coefficient
ICG	Indocyanine green
IMT	Inspiratory muscle training
IRV	Inspiratory reserve volume
LMS	Least mean square
MEP	Expiratory mouth pressure
MID	Minimal important difference
MIP	Maximal inspiratory mouth pressure
mMRC	Modified medical research council dyspnea scale
MTL	Mechanical threshold loading
MVC	Maximum voluntary contraction
MVV	Maximal voluntary ventilation
NIRS	Near-infrared spectroscopy
NIV	Noninvasive ventilation
NRD	Neural respiratory drive
Pdi	Transdiaphragmatic pressure
Pes	Esophageal pressure
PET	Positron emission tomography
Pga	Gastric pressures
PI_{max}	Maximal inspiratory mouth pressure
Ptw	Potentiated quadriceps twitch force
RMS	Root mean square
RND	Respiratory neural drive
RREP	Respiratory-related evoked potentials
RV	Residual volume
Sca	Scalene muscle
SCM	Sternocleidomastoid muscle
SD	Standard deviation
SpO_2	Oxygen saturation
$StiO_2$	Tissue oxygenation
TDI	Transitional dyspnea index
Te	Expiratory time
TFRL	Tapered flow resistive load
Ti/Ttot	Inspiratory duty cycle

Ti	Inspiratory time
TLC	Total lung capacity
VE	Minute ventilation
VO_2	Oxygen consumption
Vt	Tidal volume

Chapter 1

General Introduction

1.1 Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a highly prevalent chronic health condition, which leads to disability, morbidity, and mortality (1-3). The Global Initiative for Chronic Obstructive Lung Disease (GOLD) report defines COPD as "a common, preventable, and treatable disease characterized by persistent respiratory symptoms and airflow limitation that is due to airway and/or alveolar abnormalities, usually caused by significant exposure to noxious particles or gases" (4)[P558]. The international, population-based BOLD study aims to use standardized survey methods and a spirometric criterion for COPD, enabling direct comparisons between study populations. The prevalence of spirometry-defined COPD (FEV1 /FVC < 0.7, FEV1 <80% of predicted value) is approximately 10% worldwide and it varies between European countries (5). There were 251 million cases of COPD reported globally in 2016, and there were 3.17 million deaths from COPD, which is 5% of the global mortality in 2015 (3). The treatment of COPD results in high health care costs and has become a global financial burden (1, 2). Many patients with COPD are suffering from dyspnea, which is the main symptom of COPD causing limitation of basic daily life activities (4-6).

Dyspnea is a multidimensional sensation (7-9). There are three major dimensions. First the sensory-perceptual domain, which includes dyspnea intensity and quality. Second, the affective distress which is focused on the perception of immediate unpleasantness or cognitive evaluation response for potential consequences of what is perceived. Moreover, the third dimension is the symptom impact burden, which reflects how dyspnea affects functional ability, disability, quality of life, or health status (6, 8). The sensory-perceptual domains of dyspnea in COPD (intensity and quality) have been studied extensively (intensity is also a major focus on this thesis), but the perception of the affective distress component of dyspnea (unpleasantness perceived for a given intensity) has not received much attention (9). The experience of dyspnea derives from interactions among multiple physiological, psychological, social, and environmental factors (10). Anxiety and fear are important psychological factors that could impact the perception of dyspnea (11-15). In patients with COPD, increased levels of (non-specific) anxiety and dyspnea-specific fear are associated with increased dyspnea (13, 14).

Dyspnea perception shares many characteristics with pain (16, 17). Both are subjective perceptions of physiological sensations, and both pain and dyspnea contain a sensory (intensity) and an affective (unpleasantness) dimension (18, 19). Studies show that perception of both dyspnea and pain are processed partly in similar areas of the brain (the insula, dorsal anterior cingulate cortex, amygdala, and medial thalamus) which have been documented for their role in emotional (fear and anxiety) processing (20, 21). Applying neurophysiologic and psychophysical concepts of pain could be beneficial

in the understanding of the mechanism of dyspnea, leading to better management in both pain and dyspnea in patients.

Respiratory and peripheral muscle weakness are important factors that contribute to dyspnea and exercise limitation in COPD (22-24). Patients who have decreased inspiratory muscle strength experience more symptoms of dyspnea during exercise, independently from the severity of airflow obstruction (25). From resting to exercise, the mechanical loading on the respiratory muscle quickly becomes overwhelming, leading to a growing disparity between the ventilatory load and the capacity of the respiratory muscles; as a result, dyspnea occurs. Improving respiratory muscle capacity (inspiratory muscle function, including strength and endurance) by inspiratory muscle training (IMT) has been shown to significantly improve dyspnea and exercise capacity (26, 27).

Inspiratory muscle training (IMT) is an intervention aiming to ameliorate the imbalance between demand for inspiratory muscle work and capacity by improving inspiratory muscle function. The muscles adapt to the training by changing their structure (28) and induce changes in muscle functions (i.e., improvements in strength, speed of contraction, power output and endurance) (29). The concept of training is to apply functional overload by requesting the muscles to work longer at a higher intensity and/or more frequently than what they are accustomed to. The adaptation elicited by training depends upon the type of stimulus. Generally, inspiratory muscles respond to high-load-low-frequency loading for strength-training and prolonged low-load-high-frequency contraction for endurance training (29).

Dyspnea and locomotor muscle fatigue are the primary causes that contribute to impaired exercise capacity in patients with COPD (34, 35). Muscle fatigue is defined as a reduction in the ability of a muscle to generate force or power output induced by exercise (30). As mention above, the improvement in the inspiratory muscle function could also improve exercise capacity. However, it is unclear how the improvement in the inspiratory muscle function could improve exercise capacity. The mechanism suggested a link between inspiratory and locomotor muscle work in which high-intensity contractions (during exercise) of both respiratory muscles and locomotor muscles cause reduction of muscle blood flow to both groups of muscles resulting in fatigue and reduction of exercise endurance (31, 32). Studies found that unloading of the respiratory muscles improved limb muscle fatigue during exercise (33, 34). Chiappa et al. found an improvement in exercising forearms and resting calf blood flow after IMT in patients with chronic heart failure (35).

From the abovementioned introduction, the evidence suggest that IMT can improve dyspnea sensation and exercise capacity in patients. However, the mechanism “how” IMT could improve these two elements has not been comprehensively investigated. In addition, the effect of IMT on locomotor

muscle fatigue and psychological factors has not been investigated. Therefore, the main aim of this thesis project is to investigate potential physio/psychological mechanisms explaining the effect of IMT on dyspnea and exercise tolerance. The main hypothesis is that improvement in dyspnea and exercise capacity in response to IMT could be explained by these physiological and psychological mechanisms. Detailed background information is provided in chapter 2 and the rationales leading to the research questions with an overview of each study are presented in chapter 3.

1.2 Introduction to the individual chapters

Chapter 2: Respiratory muscle function and exercise limitation in patients with chronic obstructive pulmonary disease: a review

The aims of this review are 1) to provide a summary of physiological mechanisms linking respiratory muscle dysfunction to dyspnea and exercise limitation, 2) to provide an overview of available therapeutic approaches to maintain load-capacity balance of respiratory muscles during exercise, and 3) to review potential mechanisms that could explain the effects of interventions aimed to optimize dynamic respiratory muscle function including inspiratory muscle training. Additionally expert commentary was provided to indicate underexplored areas and potential future research questions.

Chapter 3: General methodology

In this chapter, a detailed overview of the main study as well as its components that offer answers to the specific research questions formulated in the different chapters. The general methodology is sketched out as well as the different specific research questions and hypothesis.

Chapter 4: Comparison between manual and (semi-) automated analyses of esophageal diaphragm electromyography during endurance cycling in patients with COPD

Chapter 4 offers a methodology study in which an algorithm uses commercially available software to make analyses more time-efficient, transparent, and freely accessible to other researchers was developed. This chapter aims to investigate the inter-rater reliability of the previous developed manual method, to explore the agreement between the manual and the proposed new method both cross-sectionally (to evaluate validity) and changes in response to intervention (to evaluate responsiveness). This new method was used in the analysis of respiratory muscle activation in chapter 5.

Chapter 5: Effects of inspiratory muscle training on dyspnea and respiratory muscle function at rest and during exercise in patients with COPD

In chapter 5, we investigate the effects of IMT on the activation of respiratory muscles in relation to dyspnea sensation during exercise and during an IMT session in patients with COPD. Furthermore, in an explorative fashion, we tested whether the activation of the extra-diaphragmatic inspiratory muscles would be higher than the activation of the diaphragm during an IMT session compared to during exercise.

Chapter 6: Impact of inspiratory muscle training on respiratory and locomotor muscle perfusion, oxygenation and locomotor muscle fatigue during exercise in patients with COPD

This chapter aims to evaluate the effects of IMT on changes in respiratory and locomotor muscle blood flow and locomotor muscle fatigue during cycling exercise.

Chapter 7: Impact of inspiratory muscle training on the perception and neural processing of respiratory sensations in COPD

Chapter 7 focuses on the perception of the respiratory distress component of dyspnea. This chapter investigated the effects of IMT on changes in the unpleasantness of dyspnea, general anxiety and dyspnea specific fear.

Chapter 8: **General discussion**

Chapter 8, elaborates on the findings of the three mechanisms proposed in chapter 5, 6 and 7, the role of IMT in clinical practice, future perspective of IMT, and introduce the potential future research questions to be explored.

References

1. Halpin DM, Miravitlles M. Chronic obstructive pulmonary disease: the disease and its burden to society. Proc Am Thorac Soc. 2006;3(7):619-23.
2. Vestbo J, Hurd SS, Agusti AG, Jones PW, Vogelmeier C, Anzueto A, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med. 2013;187(4):347-65.
3. Mathers CD, Loncar D. Projections of Global Mortality and Burden of Disease from 2002 to 2030. PLoS Med. 2006;3(11):e442.
4. Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017 Report. GOLD Executive Summary. Am J Respir Crit Care Med. 2017;195(5):557-82.
5. The burden of lung disease. 2013. In: The European Lung White Book Respiratory Health and Disease in Europe. [Internet]. The European Respiratory Society
[2-14]. Available from: http://www.erswhitebook.org/chapters/the-burden-of-lung-disease/.
6. Parshall MB, Schwartzstein RM, Adams L, Banzett RB, Manning HL, Bourbeau J, et al. An official American Thoracic Society statement: update on the mechanisms, assessment, and management of dyspnea. Am J Respir Crit Care Med. 2012;185(4):435-52.
7. Laveneziana P, Similowski T, Morelot-Panzini C. Multidimensional approach to dyspnea. Curr Opin Pulm Med. 2015;21(2):127-32.
8. Laviolette L, Laveneziana P. Dyspnoea: a multidimensional and multidisciplinary approach. Eur Respir J. 2014;43(6):1750-62.
9. Lansing RW, Gracely RH, Banzett RB. The multiple dimensions of dyspnea: review and hypotheses. Respir Physiol Neurobiol. 2009;167(1):53-60.
10. Dyspnea. Mechanisms, assessment, and management: a consensus statement. American Thoracic Society. Am J Respir Crit Care Med. 1999;159(1):321-40.
11. Lou P, Zhu Y, Chen P, Zhang P, Yu J, Zhang N, et al. Prevalence and correlations with depression, anxiety, and other features in outpatients with chronic obstructive pulmonary disease in China: a cross-sectional case control study. BMC Pulm Med. 2012;12:53.
12. Oga T, Tsukino M, Hajiro T, Ikeda A, Nishimura K. Analysis of longitudinal changes in dyspnea of patients with chronic obstructive pulmonary disease: an observational study. Respir Res. 2012;13:85.
13. von Leupoldt A, Taube K, Lehmann K, Fritzsche A, Magnussen H. The impact of anxiety and depression on outcomes of pulmonary rehabilitation in patients with COPD. Chest. 2011;140(3):730-6.
14. Janssens T, De Peuter S, Stans L, Verleden G, Troosters T, Decramer M, et al. Dyspnea perception in COPD: association between anxiety, dyspnea-related fear, and dyspnea in a pulmonary rehabilitation program. Chest. 2011;140(3):618-25.
15. Keil DC, Stenzel NM, Kühl K, Vaske I, Mewes R, Rief W, et al. The impact of chronic obstructive pulmonary disease-related fears on disease-specific disability. Chron Respir Dis. 2014;11(1):31-40.
16. von Leupoldt A, Dahme B. Cortical substrates for the perception of dyspnea. Chest. 2005;128(1):345-54.
17. Gracely RH, Undem BJ, Banzett RB. Cough, pain and dyspnoea: similarities and differences. Pulm Pharmacol Ther. 2007;20(4):433-7.
18. von Leupoldt A, Dahme B. Experimental comparison of dyspnea and pain. Behav Res Methods. 2007;39(1):137-43.
19. Wilson RC, Jones PW. Differentiation between the intensity of breathlessness and the distress it evokes in normal subjects during exercise. Clin Sci (Lond). 1991;80(1):65-70.
20. Schon D, Rosenkranz M, Regelsberger J, Dahme B, Buchel C, von Leupoldt A. Reduced perception of dyspnea and pain after right insular cortex lesions. Am J Respir Crit Care Med. 2008;178(11):1173-9.

21. von Leupoldt A, Sommer T, Kegat S, Baumann HJ, Klose H, Dahme B, et al. Dyspnea and pain share emotion-related brain network. Neuroimage. 2009;48(1):200-6.
22. Gosselink R, Troosters T, Decramer M. Peripheral muscle weakness contributes to exercise limitation in COPD. Am J Respir Crit Care Med. 1996;153(3):976-80.
23. Hamilton AL, Killian KJ, Summers E, Jones NL. Muscle strength, symptom intensity, and exercise capacity in patients with cardiorespiratory disorders. Am J Respir Crit Care Med. 1995;152(6):2021-31.
24. Gea J, Pascual S, Casadevall C, Orozco-Levi M, Barreiro E. Muscle dysfunction in chronic obstructive pulmonary disease: update on causes and biological findings. J Thorac Dis. 2015;7(10):E418-E38.
25. Killian KJ, Jones NL. Respiratory muscles and dyspnea. Clin Chest Med. 1988;9(2):237-48.
26. Gosselink R, De Vos J, van den Heuvel SP, Segers J, Decramer M, Kwakkel G. Impact of inspiratory muscle training in patients with COPD: what is the evidence? Eur Respir J. 2011;37(2):416-25.
27. Beaumont M, Forget P, Couturaud F, Reychler G. Effects of inspiratory muscle training in COPD patients: A systematic review and meta-analysis. Clin Respir J. 2018;12(7):2178-88.
28. Ramirez-Sarmiento A, Orozco-Levi M, Guell R, Barreiro E, Hernandez N, Mota S, et al. Inspiratory muscle training in patients with chronic obstructive pulmonary disease: structural adaptation and physiologic outcomes. Am J Respir Crit Care Med. 2002;166(11):1491-7.
29. McConnell A. Respiratory muscle training: Theory and practice. Edinburgh: Churchill Livingstone; 2013.
30. Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. Physiol Rev. 2001;81(4):1725-89.
31. Sheel AW, Boushel R, Dempsey JA. Competition for blood flow distribution between respiratory and locomotor muscles: implications for muscle fatigue. J Appl Physiol. 2018;125(3):820-31.
32. Dempsey JA, Romer L, Rodman J, Miller J, Smith C. Consequences of exercise-induced respiratory muscle work. Respir Physiol Neurobiol. 2006;151(2-3):242-50.
33. Chiappa GR, Queiroga F, Jr., Meda E, Ferreira LF, Diefenthaeler F, Nunes M, et al. Heliox improves oxygen delivery and utilization during dynamic exercise in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2009;179(11):1004-10.
34. Amann M, Regan MS, Kobitary M, Eldridge MW, Boutellier U, Pegelow DF, et al. Impact of pulmonary system limitations on locomotor muscle fatigue in patients with COPD. Am J Physiol Regul Integr Comp Physiol. 2010;299(1):R314-24.
35. Chiappa GR, Roseguini BT, Vieira PJ, Alves CN, Tavares A, Winkelmann ER, et al. Inspiratory muscle training improves blood flow to resting and exercising limbs in patients with chronic heart failure. J Am Coll Cardiol. 2008;51(17):1663-71.

Chapter 2

Respiratory Muscle Function and Exercise Limitation in Patients with Chronic Obstructive Pulmonary Disease: a Review

Noppawan Charususin**, **Sauwaluk Dacha****, Rik Gosselink, Marc Decramer,

Andreas Von Leupoldt, Thomas Reijnders, Zafeiris Louvaris & Daniel Langer

Expert Rev Respir Med. 2018;12(1):67-79

**This chapter also included in the thesis of Noppawan Charususin*

***Contributed equally*

2.1. Abstract

Introduction: Respiratory muscle dysfunction is common and contributes to dyspnea and exercise limitation in patients with chronic obstructive pulmonary disease (COPD). Improving dynamic function of respiratory muscles during exercise might help to reduce symptoms and improve exercise capacity.

Areas covered: The aims of this review are to 1) summarize physiological mechanisms linking respiratory muscle dysfunction to dyspnea and exercise limitation; 2) provide an overview of available therapeutic approaches to better maintain load-capacity balance of respiratory muscles during exercise; and 3) to summarize current knowledge on potential mechanisms explaining effects of interventions aimed at optimizing dynamic respiratory muscle function with a special focus on inspiratory muscle training.

Expert commentary: Several mechanisms which are potentially linking improvements in dynamic respiratory muscle function to symptomatic and functional benefits have not been studied so far in COPD patients. Examples of underexplored areas include the study of neural processes related to the relief of acute dyspnea and the competition between respiratory and peripheral muscles for limited energy supplies during exercise. Novel methodologies are available to non-invasively study these mechanisms. Better insights into the consequences of dynamic respiratory muscle dysfunction will hopefully contribute to further refine and individualize therapeutic approaches in patients with COPD.

Keywords: Chronic obstructive pulmonary disease, dyspnea, exercise capacity, respiratory muscle dysfunction, therapeutic approaches

2.2. Introduction

Chronic obstructive pulmonary disease (COPD) is a common, preventable, and treatable condition, characterized by persistent airflow limitation (1). Notably, the most prominent exercise-limiting symptom of COPD is dyspnea (2), which is associated with avoidance of activities and subsequent deconditioning (3). Dyspnea is defined as an uncomfortable sensation of breathing (2). Dyspnea and exercise limitation in patients are multifactorially determined. Both have been closely linked to increased respiratory neural drive (RND), dynamic mechanical constraints during exercise breathing, and functional respiratory muscle weakness leading to neuroventilatory dissociation (2-4), in variable combinations. It has long been postulated that functional inspiratory muscle weakness, per se, contributes to dyspnea causation in advanced COPD (5). Respiratory muscle dysfunction is moreover frequently observed in patients with COPD (6).

Several factors contribute to respiratory muscle dysfunction in these patients including lung hyperinflation, hypoxemia, hypercapnia, inflammation, malnutrition, long-term use of corticosteroids, physical inactivity, and changes in fiber-type distribution in the respiratory muscles (7-10). Lung hyperinflation is a major cause of respiratory muscle dysfunction in patients with obstructive lung disease since it places the inspiratory muscles at a mechanical disadvantage. An increased load on the respiratory muscles on the other hand is already present during resting breathing in these patients and will be further exacerbated during periods of tachypnea. There are compensatory mechanisms by which the respiratory muscles of these patients adapt to these mechanical disadvantages and increased loads. The chest wall reconfigures to accommodate the overdistended lungs, and the diaphragm partially preserves its ability to generate pressure by adapting to its shortened operating length. The diaphragm also becomes more fatigue resistant (3, 11-13). These compensatory mechanisms can however quickly become overwhelmed during tachypnea-induced dynamic hyperinflation (DH).

Several pharmacological and non-pharmacological treatment options are available to enable the respiratory muscles to better maintain load–capacity balance under these circumstances, either by attempting to decrease the load on the respiratory muscles (e.g. bronchodilation, ventilatory support, breathing exercises), or by improving static and dynamic respiratory muscle function (e.g. inspiratory muscle training [IMT], breathing exercises) (14, 15). IMT has been frequently applied to improve inspiratory muscle function (both pressure-generating capacity and endurance) in COPD patients in order to reduce dyspnea and to improve exercise capacity (16).

The aims of this review are to (1) summarize physiological mechanisms linking respiratory muscle dysfunction to symptoms of dyspnea and exercise limitation in COPD; (2) provide an overview of

available therapeutic approaches (both pharmacological and non-pharmacological) to improve load capacity balance and (3) summarize current knowledge on potential mechanisms linking improvements in respiratory muscle function to reductions in symptoms and improvements in exercise capacity in patients with COPD. Gaps in the current literature and priorities for future research in this area will be identified and highlighted.

2.3. Load–capacity balance of the respiratory muscles, dyspnea, and exercise limitation in patients with COPD

2.3.1. Respiratory muscles during resting breathing

Respiratory muscle dysfunction is highly prevalent in patients with COPD and can be defined as the presence of at least one of the following conditions: weakness or reduced endurance which predisposes muscles to fatigue earlier (17). Respiratory muscle strength is usually determined by assessing voluntary maximal pressure-generating capacity. Maximal inspiratory mouth pressure (PImax) is commonly measured in clinical practice (18). Respiratory muscle endurance is defined as the ability of the muscles to sustain breathing at a given level of minute ventilation (ventilator endurance) or a given level of inspiratory pressure (18). Muscle fatigue is defined as a loss of the capability to generate skeletal muscle force and/or contractile velocity at a given resistance that is characterized by recovery after resting (18). Several factors are known to contribute to respiratory muscle dysfunction in COPD including cigarette smoking, malnutrition, systemic inflammation, aging, comorbidities, concomitant treatments, exacerbations, and reduced physical activity (17). Gas exchange abnormalities, hypoxemia, or reduced oxygen delivery can lead to impaired muscle strength and endurance in COPD patients (19). Chronic corticosteroid use might also lead to steroid induced myopathy which can affect both respiratory and limb muscles (20). Static lung hyperinflation due to airway narrowing and loss of elastic recoil in COPD patients leads to air trapping during expiration, resulting in a progressive increase in functional residual capacity (FRC) and a reduction in inspiratory reserve capacity (21). Hyperinflation has a pronounced effect on the function of the respiratory muscles. It chronically shortens the inspiratory muscles, thereby reducing their pressure generating capacity (22). Total diaphragm length is shorter in patients with COPD compared to healthy subjects both at residual volume and at FRC (12). On the other hand, it has been shown that the diaphragm of COPD patients with hyperinflated lungs can develop greater pressures than healthy subjects when they are forced to increase their lung volume to similar absolute levels. However, when comparing pressure-generating capacity at FRC, reductions are observed in COPD patients with hyperinflated lungs when compared with healthy subjects (Figure 2.1) (11, 13, 23). Moreover, patients have less diaphragm mobility than healthy subjects due to their limited ability to further shorten their diaphragm

fibers from this chronically shortened resting position (24). Muscles with higher percentages of type I fibers are best suitable for sustained, low-intensity efforts, whereas those with type II predominance are capable of fast, high-powered, but nonsustainable, contractile work. The respiratory muscles have a mixture of these properties, ensuring both the fatigue resistance necessary for continuous lifelong tidal breathing and sufficient power development needed for high-velocity contractions for example during exercise tachypnea (25). Major morphological abnormalities in respiratory muscles of patients with COPD include atrophy of all fiber types and a shift toward more oxidative and fatigue-resistant type I fibers. These changes, while maintaining endurance capacity of the diaphragm on the one hand are also associated with reduced maximal force development and reduced maximal shortening velocity, i.e. reduction in muscle power that can be developed during contractions (9, 10).

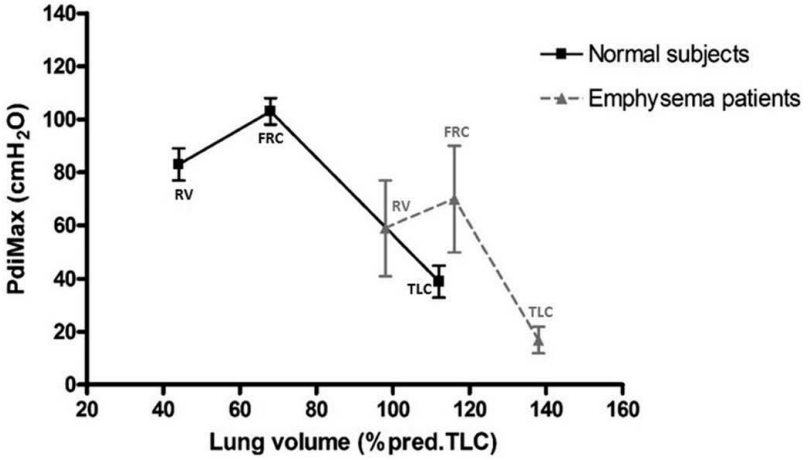

Figure 2.1 Relationships between maximal transdiaphragmatic pressure during static inspiratory efforts (PdiMax) and lung volume in normal subjects and emphysema patients. FRC = functional residual capacity; RV = residual volume; TLC = total lung capacity. (Adapted from Bellemare et al. (23).

The pressures that respiratory muscles are required to generate during tidal breathing represent the load on the respiratory system. The total load on the respiratory muscles in patients with COPD can be subdivided into the following components: first, elastic loads refer to the elastic recoil forces of the lungs and chest wall. These loads are increased when patients are forced to breathe further away from relaxation volume (FRC). The associated decreases in lung and chest wall compliance at higher lung volumes require the respiratory muscles to generate greater pleural pressures during respiration (Figure 2.2) (25, 26). Threshold loads imposed by intrinsic positive end-expiratory pressure are a direct consequence of dynamic lung hyperinflation with expiration being incomplete and inward recoil of the lungs and chest wall persisting at the end of expiration (Figure 2.2) (15). Second, resistive loads are caused by narrowing of peripheral airways. This imposes a resistance to airflow and results in a continuously increased load on both the inspiratory and expiratory muscles during breathing (15).

Increases in load on the respiratory muscles will be most pronounced during periods when breathing frequency and hyperinflation are acutely increased (e.g. exercise, disease exacerbations, periods of agitation, or anxiety).

2.3.2. Respiratory muscles during exercise

During exercise, several factors contribute to further decrease the (already reduced) capacity of the respiratory muscles. While in chronic (static) hyperinflation, the inspiratory muscles may adapt by dropout of sarcomeres, such that, at a given lung volume pressure generation is well preserved or even increased (Figure 2.1), which will not be possible during acute hyperinflation (15). Functional weakening will occur during exercise due to dynamic alterations in the length–tension (pressure–volume) and force–velocity (pressure flow) characteristics of these muscles (27). Changes in pressure–flow characteristics during exercise are caused by increases in inspiratory flow during exercise tachypnea. Alterations in the length tension characteristics of the respiratory muscles are a direct consequence of DH which forces respiratory muscles to operate at shorter lengths (28). Simultaneously, DH also results in substantial increases in the elastic and threshold loads on the inspiratory muscles (29) (Figure 2.2). Acute hyperinflation thus further reduces the pressure-generating capacity of the inspiratory muscles, while at the same time the load on the muscles is further increased. The combination of excessive mechanical loading and increased velocity of shortening of the inspiratory muscles can result in a fatiguing contraction pattern during exercise.

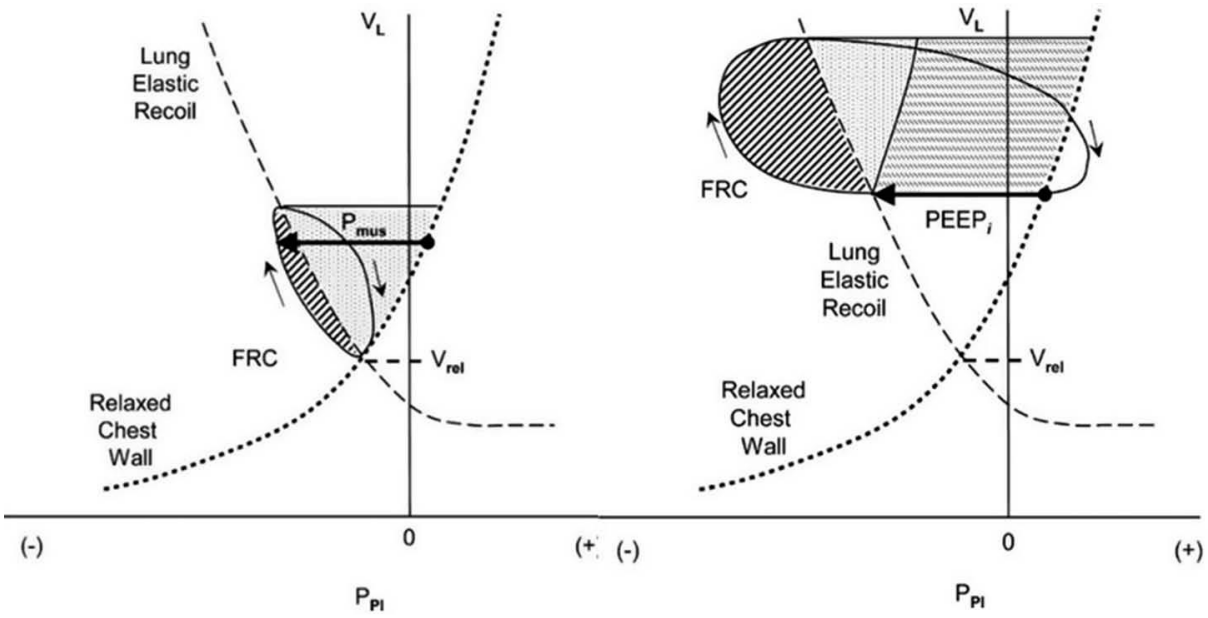

Figure 2.2 (a) Campbell diagram in a normal subject showing the volume of lung or chest wall (VL) plotted against pleural pressure (PPl). FRC is functional residual capacity, here equal to Vrel, the relaxation volume of the respiratory system. The continuous line (loop) traces a complete breath from FRC; arrows show direction. The dashed line shows the elastic characteristic of the lung (the negative of elastic recoil pressure), and the dotted line shows the elastic characteristic of the relaxed chest wall. Pmus is pressure change generated by inspiratory muscles (shown by the length of the horizontal arrow). The diagonally hatched area is work done against resistance, and the stippled area is work done against elastance of lung and chest wall. (b) Campbell diagram to illustrate the effects of dynamic hyperinflation on inspiratory muscle work. FRC is increased above relaxation volume. PEEPi is the intrinsic positive end-expiratory pressure that must be overcome before inspiratory flow can begin (shown as the length of the horizontal arrow). Diagonally hatched area is work done against resistance, stippled area is work done against elastance of lung and chest wall, and horizontally hatched area is work to overcome PEEPi. (In this illustration, work done against inspiratory resistance is increased from that shown in Figure 2.2(a), but elastic characteristics of lung and chest wall are unchanged.) (Loring SH et al. Pulmonary characteristics in COPD and mechanisms of increased work of breathing. J Appl Physiol. 2009; 107: 309–314.).

2.3.3. The role of respiratory muscles in dyspnea perception and exercise limitation

Dyspnea perception, the most common complaint in COPD patients, is defined as a subjective experience of breathing discomfort that consists of qualitatively distinct sensations notably perceived work/effort, tightness, and air hunger (30, 31) that vary in intensity, and limit physical activity (2, 32). Dyspnea typically occurs not at rest but during activities when the load–capacity balance of the respiratory muscles is disturbed (33).

In the presence of (functional) respiratory muscle weakness, pressure generated during exercise tidal breathing can represent a large fraction of the reduced maximal pressure generation capacity of the inspiratory muscles. Under these circumstances, a sense of excessive work of breathing or increased perceived inspiratory effort can develop (34). DH further amplifies this perceived effort for inspiration (increased load/reduced capacity) and is associated with dyspnea during walking (35) and cycle ergometry exercise (26). In an attempt to maintain adequate pressure generation in face of the dynamic functional weakness of the inspiratory muscles, RND from the central motor cortex will be increased (Figure 2.3). This uncoupling of the drive to the respiratory muscles from mechanical and

ventilatory output has been termed neuroventilatory dissociation (36). In other words, when the afferent feedback originating from the muscular and mechanical response of the respiratory system about changes in intrathoracic pressure, respiratory muscle length, and chest wall or lung movement are interpreted as insufficient for the corresponding efferent motor command, dyspnea sets in (Figure 2.3) (37).

DH brings end inspiratory lung volume during exercise close to total lung capacity and reduces inspiratory reserve volume. Consequently, patients develop a rapid and shallow breathing pattern. Impairments in dynamic respiratory muscle function might further exacerbate the rapid and shallow breathing of these patients during exercise by promoting further restriction of tidal volume (VT) expansion (38). In addition, the relative contribution to chest wall motion of the rib cage and neck muscles in comparison to the diaphragm in COPD patients is increased (39). With increasing lung hyperinflation in COPD patients, the inspiratory muscles of rib cage and accessory respiratory muscles are increasingly recruited even at relatively low work rates (40). These alterations in breathing pattern and respiratory muscle recruitment should be taken into consideration when implementing therapeutic strategies aimed at improving respiratory muscle function (41).

2.3.4. Neural processing of dyspnea

The sensation of dyspnea is multidimensional. In addition to the preceived sensory intensity of different qualities of dyspnea (i.e. increased work/effort of breathing, air hunger, or chest tightness) (2), the emotional impact or unpleasantness of the sensation (affective dimension) plays also an important role (Figure 2.3) (30, 42). For the same degree of neuromechanical uncoupling and related increases in RND, the sensory intensity of different dyspnea qualities should be similar. The unpleasantness associated with a given intensity of dyspnea can however be perceived very differently (43). This might be relevant in motivating patient behaviors and might also be linked to their willingness to engage or tolerate activities for a given level of dyspnea sensory intensity. In neurophysiological models of dyspnea perception during activities in patients with lung diseases (Figure 2.3), it is assumed that, as a result of increasing neuroventilatory dissociation the activation of central limbic structures is increased (42). The affective unpleasantness component of respiratory distress is believed to be linked to varying degrees of activations of limbic and closely related brain structures, which play important roles in the neural processing and regulation of emotions (42, 44-47). Prominent areas include the insula, anterior cingulate cortex, amygdala, and prefrontal cortex (45-47). Indeed, several studies have linked activations of the insula, anterior cingulate cortex, and/or amgydala complex with the affective unpleasantness of dyspnea (48-50).

So far, the neural processing mechanisms underlying the perception of dyspnea have mostly been studied in healthy subjects using several neuroimaging techniques including (44-47) Positron Emission Tomography (PET) (51), Functional Magnetic Resonance Imaging (fMRI), Near-Infrared Spectroscopy (NIRS) (52), and even lesion methodology (53). Notably, neural processes related to the relief of acute dyspnea have rarely been studied. The two available studies demonstrated contrasting findings with one fMRI study showing a reversal of dyspnea-induced brain activation during dyspnea relief (54), whereas a PET study showed distinct response patterns for dyspnea relief (55).

Respective neuroimaging studies in patients suffering from dyspnea are still rare. However, first studies have suggested that patients might show some differences in their neural processing of dyspneic stimuli (52, 56, 57). For example, a study in asthma patients using fMRI found that, while dyspnea intensity ratings were comparable to healthy subjects, ratings for dyspnea unpleasantness were reduced, which was associated with reduced insular cortex activity and increased activity in the periaqueductal gray (PAG). The authors suggested that this downregulation of insular cortex activity in patients relative to healthy controls may represent a neuronal habituation mechanism of the brain in response to repeated experiences of dyspnea over the course of the disease (58). In general, future investigations in dyspneic patient groups are clearly needed. Reductions in perceived unpleasantness for a given dyspnea intensity are likely contributing in variable degrees to the relief in symptoms observed after treatments aimed at restoring load–capacity balance of the respiratory muscles during exercise such as IMT or breathing exercises. One important research question would be to study the activation patterns of the aforementioned brain areas in response to interventions aimed at relieving dyspnea.

Another noninvasive method to study the neural processing of respiratory stimuli is respiratory-related evoked potentials (RREPs) recorded from the electroencephalogram (EEG) (59). This technique can differentiate between early, sensory-related processing (RREP components with a latency < 100 ms) and later, cognitive-emotional processing (RREP components with a latency > 100 ms). This allows the examination of the impact of emotional states and traits on the neural processing of respiratory sensations (60). For example, studies using RREPs in low anxious healthy volunteers have shown that unpleasant emotional states can reduce the neural processing of respiratory sensations, whereas high anxious individuals demonstrate increased neural processing of respiratory sensations in unpleasant relative to neutral emotional contexts (61, 62) and reduced neural filter capacities for repeatedly presented respiratory stimuli (61, 62). Moreover, in healthy subjects, when breathing itself becomes more difficult and unpleasant, RREPs can reflect an increase in the neural processing of respiratory sensations (63). This might be especially relevant as anxiety and depression are highly prevalent comorbidities in patients with COPD and are associated with worse disease outcomes (64). These

psychological comorbidities can substantially affect the perception of respiratory sensations beyond respiratory limitations, usually by increasing perceived dyspnea unpleasantness levels (65), which is most likely moderated by limbic and closely related brain areas (48, 50, 56). Again, respective RREP studies in patients suffering from dyspnea are still rare, necessitating future investigations. However, first studies have suggested that patients might show some differences in their RREP patterns as well as in other EEG derived measures of respiratory-related cortical activity such as pre inspiratory potentials (52, 56, 57). An important question for future research would be to study changes in these measures in response to interventions aimed at relieving dyspnea.

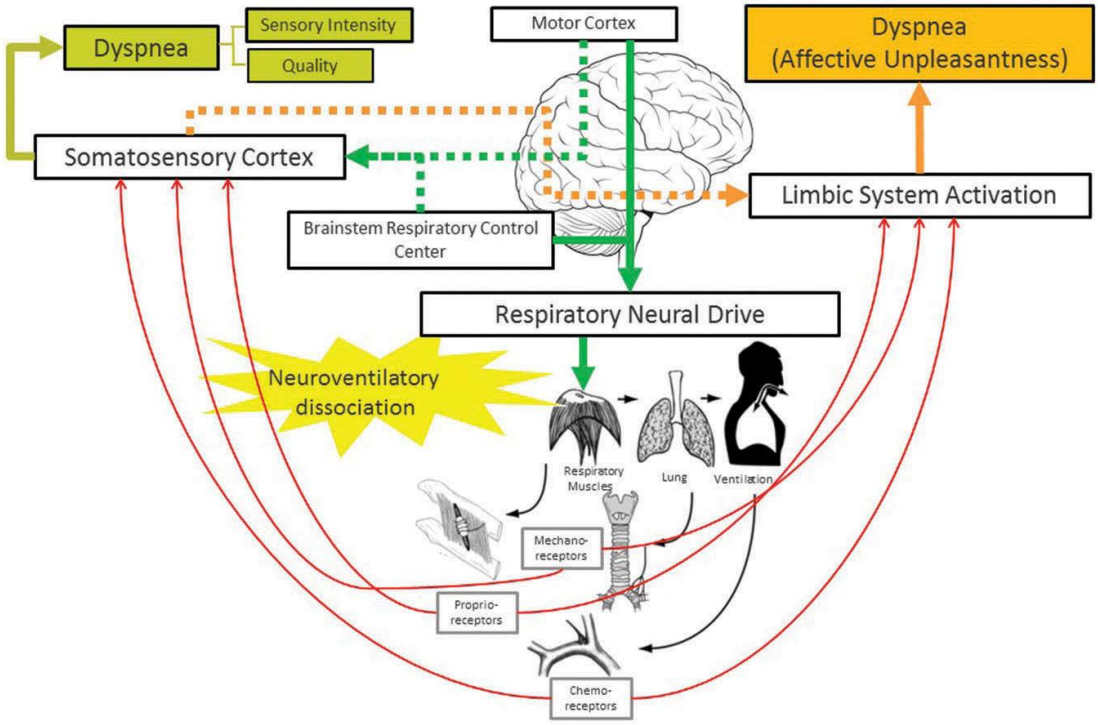

Figure 2.3 Neurobiological model of perceived dyspnea during exercise in patients with COPD. The somatosensory cortex calibrates and interprets the appropriateness of the mechanical/muscular response of the respiratory system (conveyed via afferent signals from different receptors indicated with red arrows) to a copy of the efferent respiratory neural drive that is centrally generated (indicated with green arrows). The sensory intensity of dyspnea during exercise increases in proportion to a widening disparity between increasing drive and the limited muscular-mechanical response of the respiratory system (neuroventilatory dissociation). Afferent sensory input, including input from respiratory muscle afferents (indicated with red arrows), combined with central activation of limbic structures probably contributes to the perception of respiratory distress (affective unpleasantness of dyspnea). Full color available online. (Adapted from O'Donnell DE et al. (42) and Gosselink R, Decramer M. Revalidatie bij chronisch obstructieve longziekten. Bohn Stafleu van Loghum. 2016:2;318. ISBN 978-90-368-1544-4; Fig 2–2:40.)

2.3.5. Respiratory muscle-induced metaboreflex

All the aforementioned mechanical abnormalities of the respiratory system result in an increase of respiratory muscle oxygen consumption both during quiet breathing and especially during exercise in COPD patients in comparison to healthy subjects (66). This higher respiratory muscle oxygen consumption reflects the increased load on, and reduced efficiency of, the respiratory muscles (66). Locomotor muscles are usually also impaired and also contribute to the exercise limitation in COPD

patients (7). The locomotor muscle fatigue developing in the majority of patients after exercise might partly be attributable to insufficient oxygen transport as a consequence of excessive respiratory muscle work (67). In patients with COPD, exercise capacity is limited by constraints on ventilation and on the mechanisms regulating metabolism and blood supply at the level of the working (respiratory and locomotor) muscles (68). Whether and to which extent respiratory muscle fatigue (both in inspiratory and expiratory muscles) can occur following exhaustive exercise in patients with COPD is subject to discussion (69). It is clear however that exercise-induced dynamic lung hyperinflation can result in a fatiguing contraction pattern especially in patients with low PImax by increasing the tension time index ([PI/PImax] × [Ti/Ttot]). Previous studies have proposed that an exercise induced-fatiguing contraction pattern may elicit a sympathetically mediated vasoconstrictor response in locomotor muscles; the so-called 'respiratory muscle metaboreflex' (Figure 2.4) (70). In theory, this fatiguing contraction pattern could reflexively decrease locomotor muscle blood flow, thus allowing blood flow to be redistributed in favor of the respiratory muscles (Figure 2.4) (71).

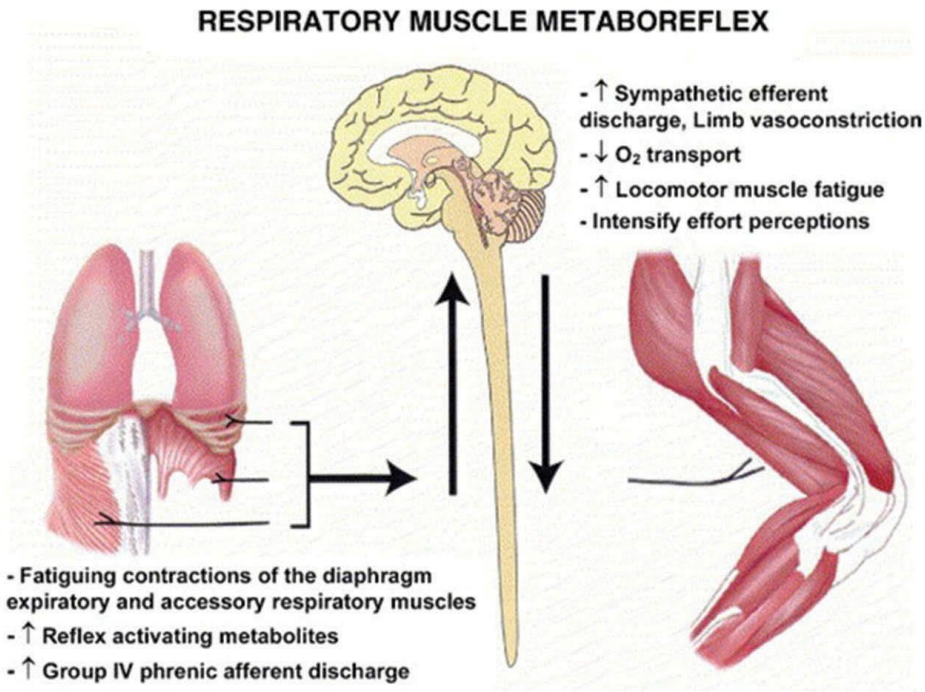

Figure 2.4 Respiratory muscle fatigue-induced metaboreflex, which increases sympathetic vasoconstrictor outflow, causing reduced blood flow to locomotor muscles and locomotor muscle fatigue. In turn, the limb fatigue comprises an important dual contribution to both peripheral and central fatigue mechanisms, which contribute to limiting exercise performance. Reprinted from Dempsey JA, Romer L, Rodman J, Miller J and Smith C. Consequences of exercise-induced respiratory muscle work. Respir Physiol Neurobiol. 2006;151:242–250. Copyright (2006), with permission from Elsevier.

In summary, it is clear that COPD patients not only face increased loads on their respiratory muscles, but the capacity of their respiratory muscles to generate pressure is also frequently decreased. This mismatch easily disturbs the load–capacity balance of the respiratory muscles, especially during periods of tachypnea induced DH resulting in further functional weakening of and increases in elastic and resistive loads on the inspiratory muscles. This dysbalance is also closely related to the intensity and quality of dyspnea and might also be related to the affective dimension (i.e. unpleasantness) of dyspnea perception. As a result, a potentially fatiguing respiratory muscle contraction pattern develops during exercise in many of these patients. This might contribute to the early development of leg muscle fatigue via the so-called respiratory muscle metaboreflex in selected patients. Treatments that either decrease load on the respiratory muscles and/or improve the capacity and dynamic functioning of respiratory muscles should be effective in reducing dyspnea and might also result in improved exercise capacity (22).

2.4. Therapeutic approaches for improving load– capacity balance by decreasing the load on the respiratory muscles

2.4.1. Bronchodilators

Bronchodilation reduces resting and/or dynamic lung hyperinflation (deflation) and has the potential to improve dyspnea and exercise capacity. The physiological effects of pharmacological lung volume deflation are (1) reduced elastic loads on inspiratory muscles at rest and during exercise by breathing at lower lung volumes (2) increased pressure-generating capacity of inspiratory muscles during exercise tidal breathing by breathing at lower lung volumes, and (3) breathing with larger tidal volumes during exercise by breathing at lower lung volumes. These three aspects together will result in enhanced neuroventilatory coupling, delayed ventilatory limitation to exercise, and reductions in exertional dyspnea. A more detailed overview on bronchodilation as part of the pharmacological management of breathlessness is provided in a recent review manuscript on this topic (72).

2.4.2. Exercise training

Aerobic exercise training improves exercise capacity and reduces symptoms of dyspnea in patients with COPD (73). This training primarily improves the utilization of oxygen by the exercising muscles (by delaying anaerobic metabolism, lactate threshold) and thus reduces ventilatory needs for a given work rate. This will in turn reduce the degree of DH at a given work rate (74). Reduced ventilation will allow patients to reduce their breathing frequency, increase VT, and reduce EELV for a given work rate, and thereby result in reduced symptoms of dyspnea and improved exercise endurance (75, 76).

2.4.3. Oxygen supplementation

Similarly, supplemental oxygen during exercise has been consistently shown to reduce ventilation and breathlessness at comparable work rates during endurance exercise testing in COPD patients with and without resting hypoxemia (77). This supplementation delayed ventilatory limitation and accompanying dyspnea by reducing ventilatory demand during exercise for a given work rate (78). This will result in an unloading of the respiratory muscles.

2.4.4. Noninvasive ventilatory support

Improvements in dyspnea during exercise in response to noninvasive ventilation (NIV) are probably mostly related to adjustments in the load/capacity balance of the respiratory muscles (79). This is achieved by unloading the inspiratory muscles during exercise (79, 80). Several small RCTs have demonstrated that the addition of NIV to an exercise training program in COPD results in higher training intensities being tolerated and larger improvements in exercise capacity (81, 82). Using a continuous positive airway pressure (CPAP) during exercise in COPD patients resulted in reduced tidal excursions of esophageal pressure. This decreased inspiratory effort during CPAP was significantly correlated with reductions in perceived dyspnea intensity (83). Pressure support ventilation was also found to be able to reduce inspiratory effort and dyspnea during exercise in patients with COPD (80).

2.4.5. Heliox supplementation

Heliox is a low density gas mixture (79% helium, 21% oxygen) that has been used in patients with COPD to reduce airflow resistance during the increasing ventilatory needs of exercise (84). Heliox breathing increases the size of a resting maximal flow-volume loop and seems to slow down the increase in EELV during exercise (84). This will simultaneaously reduce functional weakening and load on the respiratory muscles during exercise.

2.4.6. Breathing exercises

Slow and deep breathing during exercise has been hypothesized to decrease hyperinflation, work of breathing, and improve symptoms and exercise capacity (85). Spahija et al. found that some patients can achieve reductions in EELV by using pursed lips breathing (PLB) during exercise and breathing deeper and slower. Inspiratory effort decreased and symptoms improved in these selected patients (86).

2.5. Therapeutic approaches for improving load– capacity balance by improving the respiratory muscle capacity

2.5.1. Pharmacological treatment

Only few pharmacological agents that aim to improve respiratory muscle function are available for clinical use. A recent review has summarized the available options (87). Two different strategies are possible. The first approach aims at improving respiratory muscle protein content by either inhibiting proteolysis (e.g. bortezomib) or stimulating muscle growth (e.g. anabolic hormones). Schols et al. found for example that the exogeneous anabolic steroid nandrolone together with high caloric feeding significantly improved inspiratory muscle strength in patients with stable COPD during an 8-week pulmonary rehabilitation (PR) program (88). Another way to improve respiratory muscle function is by optimizing contractile function of the available muscle mass for example by using positive inotropes (e.g. β-adrenoreceptor agonists calcium sensitizers). Levosimendan is the only calcium sensitizer approved for use in humans. Improved calcium sensitivity of diaphragm muscle fibers in COPD patients might enhance their force-generating capacity (89).

2.5.2. Breathing exercises

Contraction of abdominal muscles during active expiration increases abdominal pressure (90). This increase in abdominal pressure results in a lengthening of the diaphragm, which can help to optimize its end-expiratory length tension characteristics in COPD patients with severe static hyperinflation (91). The advantages of optimizing positioning of the diaphragm during active expiration need to be balanced against the risk of premature airway collapse, especially in patients with severe expiratory flow limitation due to emphysema. Active expiration against a positive pressure by using PLB can help to counteract premature airway collapse during active expiration. This technique is therefore oftentimes spontaneously applied especially by patients with severe expiratory flow limitation. PLB in combination with active expiration can moreover also potentially combine the beneficial effects of these two interventions by simultaneously increasing diaphragm capacity and unloading the respiratory muscles by reducing DH.

2.5.3. Body positioning techniques

Adopting a forward leaning position is associated with a significant reduction in electromyographic activity of scalene and sternocleidomastoid muscles, an increase in pressure-generating capacity of the inspiratory muscles, improved diaphragm function (92), and significant improvements in thoracoabdominal movements (93). Moreover, forward leaning position with arm support (e.g. by

using a rollator) might further improve efficiency of accessory inspiratory muscles. This approach has previously been shown to result in reduced dyspnea and improved walking capacity (94).

2.5.4. Inspiratory muscle training

IMT has been extensively studied in the past decades in COPD patients. IMT has been shown to improve inspiratory muscle function and to reduce dyspnea and improve exercise capacity when applied as a stand lone intervention with controlled training loads (16). In patients with COPD, IMT has the potential to reduce the demand/capacity imbalance imposed on the compromised respiratory muscles especially during exercise (16, 34, 95). The three most commonly used types of training are flow-resistive loading, mechanical threshold loading (MTL) and normocapnic hyperpnoea (low pressure-high flow loading). More recently, an electronic flow-resistive breathing device has been developed with a dynamically controlled tapered flow resistive load (TFRL-IMT). This type of loading combines characteristics of threshold loading and flow-resistive loading.

Data obtained from meta-analysis in COPD patients indicate that IMT as a stand-alone therapy improves inspiratory muscle strength and endurance, and reduces symptoms of dyspnea (16). However, exercise performance and quality of life were not significantly improved when IMT was added to general exercise training program (16). The value of IMT as an adjunct to exercise training in COPD patients is therefore still under debate (96-99). Most IMT interventions in patients with COPD have been implemented as fully or partially supervised daily training for 30 min with controlled training loads using MTLIMT. It has also been shown that short and largely home-based IMT protocols (around 6–10 min of daily training) significantly improve inspiratory muscle function with both MTL-IMT and TFRL-IMT protocols (100). A recent study also found additional benefits of this IMT protocol as an adjunct to a PR program in COPD patients with inspiratory muscle weakness. Patients could achieve significantly higher peak work rate and exercise ventilation without increasing dyspnea sensation (101).

2.6. Candidate mechanisms connecting improvements in respiratory muscle function to changes in symptoms and exercise capacity

Several mechanisms might be responsible for improvements in dyspnea and exercise capacity after improvements in respiratory muscle function: (1) reductions in dyspnea intensity and sense of excessive work of breathing by changes in neuromechanical coupling (by reduction in neural drive and improvement in breathing pattern), (2) changes in the neural processing of dyspnea, (3) changes in the respiratory muscle recruitment pattern, and (4) reductions in respiratory muscle energy demands and increased energy supplies to peripheral muscles.

2.6.1. Improving neuroventilatory coupling by reducing neural drive and modifying breathing pattern

Electromyographic activity of the diaphragm (EMGdi) has been studied as a surrogate for RND in patients with COPD (102). A pilot study reported reductions in respiratory effort and EMGdi during iso work rate exercise in response to IMT in these patients (103). Wanke et al. previously investigated the effects of IMT in addition to general exercise training and observed additional improvements in exercise capacity and larger VT expansion at peak exercise in the IMT group (104). More recently, the attenuation of dyspnea after resistive loading in patients with COPD has been related to possible reductions in DH, secondary to alterations in breathing pattern, during whole-body exercise (95). In line with these findings, the addition of IMT to a PR program in COPD patients with inspiratory muscle weakness resulted in a deeper and slower breathing pattern at iso-ventilation during exercise (Figure 2.5) (101). However, timing components of breathing (duty cycle) did not change after IMT. Taken together these data indicate that IMT has the potential to reduce the so-called 'length–tension inappropriateness', thereby improving neuroventilatory coupling and reducing the perception of excessive respiratory effort. The potential effects of combined IMT/breathing retraining interventions on DH by stimulating higher inspiratory flow rates in order to leave more time for expiration during exercise warrant further study.

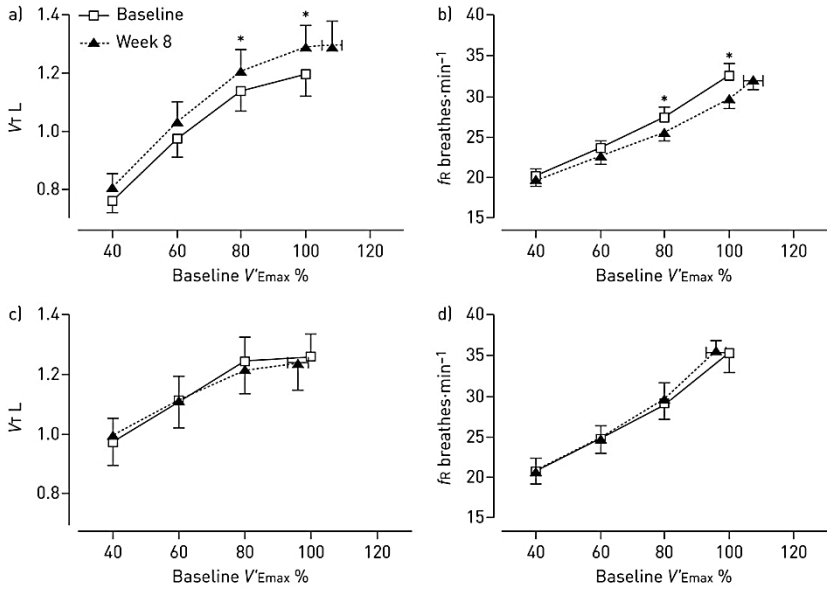

Figure 2.5 Changes in tidal volume (VT) and respiratory frequency (fR) at comparable percentages of baseline maximal ventilation (V'Emax) (40, 60, 80, 100 and peak ventilation) at baseline and after training in (a) and (b) the inspiratory muscle training group and (c) and (d) the control group. *p < 0.05 (baseline versus week 8) based on post hoc tests from mixed model analysis, values represented as mean ± SEM. (Charususin et al. (101).

2.6.2. Changes in respiratory muscle recruitment pattern

It is unclear whether (specific types of) IMT primarily improve diaphragm function or mainly promote rib cage and accessory muscle function in patients with COPD. It has been suggested that in severe COPD, improving rib cage and accessory inspiratory muscle function should be a priority (40). Ramírez-Sarmiento and colleagues found that MTL-IMT in COPD resulted in structural changes (muscle hypertrophy with increases in proportion of type I fibers and size of type II fibers) in external intercostal muscles (105). Dekhuijzen and colleagues showed that adding IMT to PR in COPD improved EMG-fatigability of the diaphragm (106). The recruitment pattern of respiratory muscles during IMT can be manipulated by specific instructions given in healthy subjects. Simple instructions to promote diaphragmatic breathing in these volunteers did increase the recruitment of the diaphragm during training (107). However, training resistance was relatively low (40% PImax) and the tidal volume was not controlled between the different breathing strategies. It is also unclear whether these findings are reproducible in patients with moderate-to-severe expiratory flow limitation and varying degrees of static hyperinflation. It is also unknown whether this will lead to larger improvements in diaphragm strength and whether more recruitment of the diaphragm is desirable in these patients. The differences in the proportion of RND to the diaphragm, the inspiratory muscles of the rib cage, and accessory respiratory muscles as well as changes in the respiratory muscle recruitment patterns both during exercise as during training sessions in response to IMT in patients with COPD should be investigated. This will help to elucidate whether different muscle groups can be specifically targeted during training. It will also help to study which specific improvements in diaphragmatic and non-diaphragmatic muscles will be most beneficial for patients during exercise breathing.

2.6.3. Changes in the neural processing of dyspnea

In healthy subjects, measurement of cortical neuronal activation before and after IMT (75% of PImax, four sets of six breaths per day, 5 days per week for 6 weeks) showed that neither the amplitude nor latency of early peak components of the RREP changed (108). However, it is interesting to investigate the response of RREPs related to perceived dyspnea intensity and dyspnea unpleasantness and the effect of IMT in COPD patients, specifically responses of the cognitive-emotional, later RREP components. Moreover, the number of neural processing studies in relation to dyspnea sensation is still limited, especially in cardiopulmonary patients (44, 45, 47). Therefore, it is currently unknown whether sensory and/or affective aspects of dyspnea and related brain activation patterns might be modifiable by IMT. Further investigations using measurements of the neural processing of respiratory sensations in response to interventions are therefore required. In addition to studying such functional brain activation patterns, examining additional changes in the brain structure of patients due to

interventions such as IMT might be highly informative, also in relation to sensory and affective aspects of dyspnea (109).

2.6.4. Reduction in respiratory muscle energy demands and increased energy supplies to peripheral muscles

Several reports suggest that unloading the respiratory muscles in patients with COPD either by pharmacological (i.e. bronchodilators) or non-pharmacological strategies (i.e. pure oxygen or heliox supplementation, proportional assist ventilation) can significantly improve exercise tolerance (110-115). These effects have been related to improvements in blood flow, oxygen delivery, and oxygen availability at the level of peripheral muscles. Only one study so far measured the response to unloading respiratory muscles on locomotor muscle fatigue in patients with COPD (67). The authors found that the locomotor muscle fatigue is partly attributable to insufficient oxygen transport as a consequence of excessive respiratory muscle work (67). However, the improvement of oxygen availability at the level of peripheral muscles might have occurred due to larger central hemodynamic responses (imposed by the aforementioned strategies) and not as a result of redistribution of blood flow and oxygen from the respiratory to peripheral muscles (112-114, 116). Indeed, Louvaris et al. simultaneously measured inspiratory, expiratory, and locomotor muscle blood flow and oxygen delivery using NIRS along with central hemodynamic responses during cycling exercise in COPD patients (111). They demonstrated that improvement in exercise tolerance, peripheral muscle perfusion, and oxygen delivery following heliox and oxygen supplementation did not occur on the basis of blood flow redistribution from the respiratory to locomotor muscles. In contrast, the effects were due to greater central hemodynamic responses namely greater cardiac output with heliox and higher systemic oxygen concentration with oxygen breathing (111).

2.7. Conclusion

In conclusion, respiratory muscle dysfunction in COPD is known to contribute to dyspnea and exercise limitation. Dyspnea intensity and unpleasantness will increase when the respiratory muscle load–capacity balance is disturbed. Several therapeutic approaches are available to help patients to improve neuroventilatory coupling during exercise. Underlying mechanisms of these interventions on relieving symptoms and increasing capacity deserve further study in the years to come.

2.8. Expert commentary

In order to better identify patients who are likely to achieve functional and symptomatic benefits and to better individualize treatment approaches, the following emerging research areas should be explored in more detail in the coming years. An important and largely unanswered research question

is whether simple breathing exercises, either alone or in combination with IMT, can be used to train patients with COPD to breathe slower and deeper during exercise. It is also unclear whether patients can be trained to combine these modifications in exercise breathing pattern with additional changes in the timing components of their breathing. Increases in inspiratory flow resulting in shortening of inspiratory time should change the duty cycle by leaving more time for expiration during exercise tachypnea. Taken together, these changes in breathing pattern during exercise should reduce DH and improve neuroventilatory coupling, symptoms, and exercise performance. With the advance of novel portable technologies, it should become more feasible to implement breathing retraining strategies with real-time feedback on breathing pattern characteristics into PR programs in the coming years.

Several specific issues are also worth addressing concerning the application of IMT in these patients. It should be investigated whether the diaphragm and non-diaphragmatic muscles of inspiration can be selectively targeted during training interventions. Since diaphragm contribution is impaired in patients with severe lung hyperinflation, both at rest and specifically during exercise, it has long been hypothesized that it might be most useful to specifically provide a training stimulus to non-diaphragmatic respiratory muscles. Exactly how this can be achieved and whether it will result in larger functional and symptomatic benefits has so far however not been elucidated. Evolutions in semiautomatic processing and analyzing of simultaneously recorded analog signals will make it easier to study these mechanisms in the years to come. To provide answers to these unresolved hypotheses, data on changes in respiratory muscle activation and energy demands need to be combined with thoracic and abdominal pressure signals during training interventions as well as during exercise tidal breathing. These data will contribute to provide answers to the important question as to how one should optimally train the respiratory muscles of patients with COPD.

The more widespread use of minimally invasive techniques such as NIRS has furthermore made it feasible to study the competition for energy supplies between respiratory and peripheral muscles during exercise. The impact of this competition on exercise capacity is another important research area that should be further explored in the coming years. It will also allow to study whether interventions aimed at restoring load– capacity balance of the respiratory muscles during exercise might be able to modify these processes.

A particularly interesting and largely unexplored area of research centers on the use of noninvasive techniques (e.g. neuroimaging and EEG methods) to study the neural processing of dyspnea. Reductions in perceived unpleasantness for a given dyspnea intensity are likely contributing in variable degrees to the relief in symptoms after treatments aimed at restoring load–capacity balance of the respiratory muscles. So far, the neural processing mechanisms underlying the perception of dyspnea

have mostly been studied in healthy subjects using several neuroimaging techniques including PET, fMRI, and NIRS. Respective neuroimaging studies in patients suffering from dyspnea are still rare. Neural processes related to the relief of acute dyspnea in response to interventions have also rarely been studied. Based on preliminary findings it has been hypothesized that reductions in ratings for dyspnea unpleasantness in response to interventions will be associated with reduced insular cortex activity and increased activity in the PAG. One important research question would be to study the activation patterns of specific brain areas (e.g. insular cortex activity and PAG) in response to interventions aimed at relieving dyspnea (e.g. breathing exercises or IMT). Another emerging noninvasive method to study the neural processing of respiratory stimuli is RREPs recorded from the EEG. The technique allows the examination of the impact of emotional states and traits on the neural processing of respiratory sensations. This might be especially relevant as anxiety and depression are highly prevalent comorbidities and can substantially affect the perception of respiratory sensations in patients with COPD. RREP studies in patients suffering from dyspnea are still rare, necessitating future investigations. Another important question for future research would be to study changes in these measures in response to interventions aimed at relieving dyspnea. Some of the aforementioned techniques (e.g. fMRI and RREPs from the EEG) might even have the potential to be used during real-life dyspnea inducing situations such as exercise. Even though these studies will be challenging to conduct, they would offer valuable insights into the effects of interventions on modifying dyspnea processing during 'real-life' situations.

2.9. Five-year view

Several key mechanisms which are likely contributing in variable degrees to symptomatic and functional improvements in response to interventions aimed at ameliorating dynamic respiratory muscle function have remain understudied in patients with COPD. The more widespread use of minimally invasive techniques such as NIRS, neuroimaging, and EEG methods is expected to result in exciting novel insights in the coming years. Neural processes related to the relief of acute dyspnea in response to interventions are expected to be discovered. Some of these techniques also have the potential to be used during real-life dyspnea inducing situations such as exercise. These studies will offer valuable insights into the effects of interventions targeting the respiratory muscles on modifying dyspnea processing during 'real-life' situations.

Key issues

- Respiratory muscle dysfunction in COPD is known to contribute to dyspnea and exercise limitation.
- Dyspnea intensity and unpleasantness will increase when the respiratory muscle load-capacity balance is disturbed.

- Several therapeutic approaches are available to help patients to improve neuroventilatory coupling during exercise.
- Underlying mechanisms of these interventions on relieving symptoms and increasing capacity deserve further study in the years to come.
- Examples of underexplored areas include the study of neural processes related to the relief of acute dyspnea and the competition between respiratory and peripheral muscles for limited energy supplies during exercise.

Funding

This project has been supported by the following specific funding sources: Research Foundation – Flanders (FWO) grant GOA4516N and KU Leuven - University of Leuven grant C22/15/035).

Declaration of interests

A von Leupoldt is supported by the 'Asthenes' long-term structural funding grant (#METH/15/011) by the Flemish Government, Belgium, and by a research grant (STRT/13/002) from the Research Fund KU Leuven, Belgium. T Reijnders is supported by the Flemish Government, Belgium, and by a research grant (STRT/13/002) from the Research Fund KU Leuven, Belgium. Z Louvaris is the recipient of a European Respiratory Society Long-Term Research fellowship number LTRF 2016-6686 and a postdoctoral fellow of Research Foundation - Flanders (FWO). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

References

1. Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017 Report. GOLD Executive Summary. Am J Respir Crit Care Med. 2017;195(5):557-82.
2. Parshall MB, Schwartzstein RM, Adams L, Banzett RB, Manning HL, Bourbeau J, et al. An official American Thoracic Society statement: update on the mechanisms, assessment, and management of dyspnea. Am J Respir Crit Care Med. 2012;185(4):435-52.
3. O'Donnell DE. Hyperinflation, dyspnea, and exercise intolerance in chronic obstructive pulmonary disease. Proc Am Thorac Soc. 2006;3(2):180-4.
4. Faisal A, Alghamdi BJ, Ciavaglia CE, Elbehairy AF, Webb KA, Ora J, et al. Common Mechanisms of Dyspnea in Chronic Interstitial and Obstructive Lung Disorders. Am J Respir Crit Care Med. 2016;193(3):299-309.
5. Killian KJ, Jones NL. Respiratory muscles and dyspnea. Clin Chest Med. 1988;9(2):237-48.
6. Decramer M, Demedts M, Rochette F, Billiet L. Maximal transrespiratory pressures in obstructive lung disease. Bull Eur Physiopathol Respir. 1980;16(4):479-90.
7. Gosselink R, Troosters T, Decramer M. Peripheral muscle weakness contributes to exercise limitation in COPD. Am J Respir Crit Care Med. 1996;153(3):976-80.
8. Laghi F, Tobin MJ. Disorders of the respiratory muscles. Am J Respir Crit Care Med. 2003;168(1):10-48.
9. Levine S, Kaiser L, Leferovich J, Tikunov B. Cellular Adaptations in the Diaphragm in Chronic Obstructive Pulmonary Disease. New Engl J Med. 1997;337(25):1799-806.
10. Testelmans D, Crul T, Maes K, Agten A, Crombach M, Decramer M, et al. Atrophy and hypertrophy signalling in the diaphragm of patients with COPD. Eur Respir J. 2010;35(3):549-56.
11. Similowski T, Yan S, Gauthier AP, Macklem PT, Bellemare F. Contractile properties of the human diaphragm during chronic hyperinflation. N Engl J Med. 1991;325(13):917-23.
12. Gorman RB, McKenzie DK, Pride NB, Tolman JF, Gandevia SC. Diaphragm length during tidal breathing in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2002;166(11):1461-9.
13. Rochester DF. The diaphragm in COPD. Better than expected, but not good enough. N Engl J Med. 1991;325(13):961-2.
14. Langer D, Ciavaglia CE, Neder JA, Webb KA, O'Donnell DE. Lung hyperinflation in chronic obstructive pulmonary disease: mechanisms, clinical implications and treatment. Expert Rev Respir Med. 2014;8(6):731-49.
15. Marchand E, Decramer M. Respiratory muscle function and drive in chronic obstructive pulmonary disease. Clin Chest Med. 2000;21(4):679-92.
16. Gosselink R, De Vos J, van den Heuvel SP, Segers J, Decramer M, Kwakkel G. Impact of inspiratory muscle training in patients with COPD: what is the evidence? Eur Respir J. 2011;37(2):416-25.
17. Gea J, Agustí A, Roca J. Pathophysiology of muscle dysfunction in COPD. J Appl Physiol. 2013;114(9):1222-34.
18. ATS/ERS Statement on respiratory muscle testing. Am J Respir Crit Care Med. 2002;166(4):518-624.
19. Gea J, Casadevall C, Pascual S, Orozco-Levi M, Barreiro E. Respiratory diseases and muscle dysfunction. Expert Rev Respir Med. 2012;6(1):75-90.
20. Decramer M, Lacquet LM, Fagard R, Rogiers P. Corticosteroids contribute to muscle weakness in chronic airflow obstruction. Am J Respir Crit Care Med. 1994;150(1):11-6.
21. Rochester DF. Effects of COPD on the respiratory muscles. Chronic Obstructive Pulmonary Disease. Cherniack M ed. Philadelphia: W.B. Saunders and Company; 1991. p. 134-57.
22. Moxham J, Jolley C. Breathlessness, fatigue and the respiratory muscles. Clin Med. 2009;9(5):448-52.

23. Bellemare F, Cordeau MP, Couture J, Lafontaine E, Leblanc P, Passerini L. Effects of emphysema and lung volume reduction surgery on transdiaphragmatic pressure and diaphragm length. Chest. 2002;121(6):1898-910.
24. Dos Santos Yamaguti WP, Paulin E, Shibao S, Chammas MC, Salge JM, Ribeiro M, et al. Air trapping: The major factor limiting diaphragm mobility in chronic obstructive pulmonary disease patients. Respirology. 2008;13(1):138-44.
25. Epstein SK. An overview of respiratory muscle function. Clin Chest Med. 1994;15(4):619-39.
26. O'Donnell DE, Webb KA. Exertional breathlessness in patients with chronic airflow limitation. The role of lung hyperinflation. Am Rev Respir Dis. 1993;148(5):1351-7.
27. McCool FD. Inspiratory pump performance: a pressureflow-volume framework. In: Roussos C, editor. The thorax. 85. New York: Marcel Dekker; 1995. p. 1463-81.
28. O'Donnell DE, D'Arsigny C, Fitzpatrick M, Webb KA. Exercise hypercapnia in advanced chronic obstructive pulmonary disease: the role of lung hyperinflation. Am J Respir Crit Care Med. 2002;166(5):663-8.
29. O'Donnell DE, Laveneziana P. The clinical importance of dynamic lung hyperinflation in COPD. Copd. 2006;3(4):219-32.
30. Lansing RW, Gracely RH, Banzett RB. The multiple dimensions of dyspnea: review and hypotheses. Respir Physiol Neurobiol. 2009;167(1):53-60.
31. Laveneziana P, Similowski T, Morelot-Panzini C. Multidimensional approach to dyspnea. Curr Opin Pulm Med. 2015;21(2):127-32.
32. Laviolette L, Laveneziana P. Dyspnoea: a multidimensional and multidisciplinary approach. Eur Respir J. 2014;43(6):1750-62.
33. Jolley CJ, Moxham J. Dyspnea Intensity: A Patient-reported Measure of Respiratory Drive and Disease Severity. Am J Respir Crit Care Med. 2016;193(3):236-8.
34. Leblanc P, Bowie DM, Summers E, Jones NL, Killian KJ. Breathlessness and exercise in patients with cardiorespiratory disease. Am Rev Respir Dis. 1986;133(1):21-5.
35. Marin JM, Carrizo SJ, Gascon M, Sanchez A, Gallego B, Celli BR. Inspiratory capacity, dynamic hyperinflation, breathlessness, and exercise performance during the 6-minute-walk test in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2001;163(6):1395-9.
36. Jolley CJ, Moxham J. A physiological model of patient-reported breathlessness during daily activities in COPD. Eur Respir Rev. 2009;18(112):66-79.
37. Hill K, Jenkins SC, Hillman DR, Eastwood PR. Dyspnoea in COPD: can inspiratory muscle training help? Aust J Physiother. 2004;50(3):169-80.
38. Killian KJ, Bucens DD, Campbell EJ. Effect of breathing patterns on the perceived magnitude of added loads to breathing. J Appl Physiol Respir Environ Exerc Physiol. 1982;52(3):578-84.
39. Decramer M. Hyperinflation and respiratory muscle interaction. Eur Respir J. 1997;10(4):934-41.
40. Martinez FJ, Couser JI, Celli BR. Factors influencing ventilatory muscle recruitment in patients with chronic airflow obstruction. Am Rev Respir Dis. 1990;142(2):276-82.
41. Benditt JO, Wood DE, McCool FD, Lewis S, Albert RK. Changes in breathing and ventilatory muscle recruitment patterns induced by lung volume reduction surgery. Am J Respir Crit Care Med. 1997;155(1):279-84.
42. O'Donnell DE, Ora J, Webb KA, Laveneziana P, Jensen D. Mechanisms of activity-related dyspnea in pulmonary diseases. Respir Physiol Neurobiol. 2009;167(1):116-32.
43. Carrieri-Kohlman V, Gormley JM, Eiser S, Demir-Deviren S, Nguyen H, Paul SM, et al. Dyspnea and the affective response during exercise training in obstructive pulmonary disease. Nurs Res. 2001;50(3):136-46.
44. von Leupoldt A, Dahme B. Cortical substrates for the perception of dyspnea. Chest. 2005;128(1):345-54.
45. Davenport PW, Vovk A. Cortical and subcortical central neural pathways in respiratory sensations. Respir Physiol Neurobiol. 2009;167(1):72-86.

46. Evans KC. Cortico-limbic circuitry and the airways: insights from functional neuroimaging of respiratory afferents and efferents. Biol Psychol. 2010;84(1):13-25.
47. Herigstad M, Hayen A, Wiech K, Pattinson KT. Dyspnoea and the brain. Respir Med. 2011;105(6):809-17.
48. Stoeckel MC, Esser RW, Gamer M, Buchel C, von Leupoldt A. Brain mechanisms of short-term habituation and sensitization toward dyspnea. Front Psychol. 2015;6:748.
49. von Leupoldt A, Sommer T, Kegat S, Baumann HJ, Klose H, Dahme B, et al. The unpleasantness of perceived dyspnea is processed in the anterior insula and amygdala. Am J Respir Crit Care Med. 2008;177(9):1026-32.
50. Hayen A, Wanigasekera V, Faull OK, Campbell SF, Garry PS, Raby SJM, et al. Opioid suppression of conditioned anticipatory brain responses to breathlessness. Neuroimage. 2017;150:383-94.
51. Banzett RB, Mulnier HE, Murphy K, Rosen SD, Wise RJ, Adams L. Breathlessness in humans activates insular cortex. Neuroreport. 2000;11(10):2117-20.
52. Higashimoto Y, Honda N, Yamagata T, Sano A, Nishiyama O, Sano H, et al. Exertional dyspnoea and cortical oxygenation in patients with COPD. Eur Respir J. 2015;46(6):1615-24.
53. Schon D, Rosenkranz M, Regelsberger J, Dahme B, Buchel C, von Leupoldt A. Reduced perception of dyspnea and pain after right insular cortex lesions. Am J Respir Crit Care Med. 2008;178(11):1173-9.
54. Binks AP, Evans KC, Reed JD, Moosavi SH, Banzett RB. The time-course of cortico-limbic neural responses to air hunger. Respir Physiol Neurobiol. 2014;204:78-85.
55. Peiffer C, Costes N, Herve P, Garcia-Larrea L. Relief of dyspnea involves a characteristic brain activation and a specific quality of sensation. Am J Respir Crit Care Med. 2008;177(4):440-9.
56. Herigstad M, Hayen A, Evans E, Hardinge FM, Davies RJ, Wiech K, et al. Dyspnea-related cues engage the prefrontal cortex: evidence from functional brain imaging in COPD. Chest. 2015;148(4):953-61.
57. Yu L, De Mazancourt M, Hess A, Ashadi FR, Klein I, Mal H, et al. Functional connectivity and information flow of the respiratory neural network in chronic obstructive pulmonary disease. Hum Brain Mapp. 2016;37(8):2736-54.
58. von Leupoldt A, Sommer T, Kegat S, Eippert F, Baumann HJ, Klose H, et al. Down-regulation of insular cortex responses to dyspnea and pain in asthma. Am J Respir Crit Care Med. 2009;180(3):232-8.
59. Chan PY, Davenport PW. Respiratory related evoked potential measures of cerebral cortical respiratory information processing. Biol Psychol. 2010;84(1):4-12.
60. von Leupoldt A, Chan PY, Esser RW, Davenport PW. Emotions and neural processing of respiratory sensations investigated with respiratory-related evoked potentials. Psychosom Med. 2013;75(3):244-52.
61. von Leupoldt A, Chan P-YS, Bradley MM, Lang PJ, Davenport PW. The impact of anxiety on the neural processing of respiratory sensations. Neuroimage. 2011;55(1):247-52.
62. Chan P-YS, Cheng C-H, Hsu S-C, Liu C-Y, Davenport PW, von Leupoldt A. Respiratory sensory gating measured by respiratory-related evoked potentials in generalized anxiety disorder. Front Psychol. 2015;6:957-.
63. von Leupoldt A, Bradley MM, Lang PJ, Davenport PW. Neural processing of respiratory sensations when breathing becomes more difficult and unpleasant. Front Physiol. 2010;1:144.
64. von Leupoldt A, Taube K, Lehmann K, Fritzsche A, Magnussen H. The impact of anxiety and depression on outcomes of pulmonary rehabilitation in patients with COPD. Chest. 2011;140(3):730-6.
65. von Leupoldt A, Mertz C, Kegat S, Burmester S, Dahme B. The impact of emotions on the sensory and affective dimension of perceived dyspnea. Psychophysiology. 2006;43(4):382-6.
66. Field S, Kelly SM, Macklem PT. The oxygen cost of breathing in patients with cardiorespiratory disease. Am Rev Respir Dis. 1982;126(1):9-13.

67. Amann M, Regan MS, Kobitary M, Eldridge MW, Boutellier U, Pegelow DF, et al. Impact of pulmonary system limitations on locomotor muscle fatigue in patients with COPD. Am J Physiol Regul Integr Comp Physiol. 2010;299(1):R314-24.
68. Vogiatzis I, Zakynthinos S. Factors limiting exercise tolerance in chronic lung diseases. Compr Physiol. 2012;2(3):1779-817.
69. Bachasson D, Wuyam B, Pepin JL, Tamisier R, Levy P, Verges S. Quadriceps and respiratory muscle fatigue following high-intensity cycling in COPD patients. PLoS One. 2013;8(12):e83432.
70. Sheel AW, Derchak PA, Morgan BJ, Pegelow DF, Jacques AJ, Dempsey JA. Fatiguing inspiratory muscle work causes reflex reduction in resting leg blood flow in humans. The Journal of physiology. 2001;537(Pt 1):277-89.
71. Derchak PA, Sheel AW, Morgan BJ, Dempsey JA. Effects of expiratory muscle work on muscle sympathetic nerve activity. J Appl Physiol. 2002;92(4):1539-52.
72. O'Donnell DE, Webb KA, Harle I, Neder JA. Pharmacological management of breathlessness in COPD: recent advances and hopes for the future. Expert Rev Respir Med. 2016;10(7):823-34.
73. Lacasse Y, Guyatt GH, Goldstein RS. The components of a respiratory rehabilitation program: a systematic overview. Chest. 1997;111(4):1077-88.
74. Porszasz J, Emtner M, Goto S, Somfay A, Whipp BJ, Casaburi R. Exercise training decreases ventilatory requirements and exercise-induced hyperinflation at submaximal intensities in patients with COPD. Chest. 2005;128(4):2025-34.
75. Casaburi R, ZuWallack R. Pulmonary rehabilitation for management of chronic obstructive pulmonary disease. N Engl J Med. 2009;360(13):1329-35.
76. Troosters T, Gosselink R, Decramer M. Revalidatie bij chronisch obstructieve longziekten: Bohn Stafleu van Loghum; 2016.
77. Eves ND, Petersen SR, Haykowsky MJ, Wong EY, Jones RL. Helium-hyperoxia, exercise, and respiratory mechanics in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2006;174(7):763-71.
78. O'Donnell DE, D'Arsigny C, Webb KA. Effects of hyperoxia on ventilatory limitation during exercise in advanced chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2001;163(4):892-8.
79. van 't Hul A, Gosselink R, Hollander P, Postmus P, Kwakkel G. Acute effects of inspiratory pressure support during exercise in patients with COPD. Eur Respir J. 2004;23(1):34-40.
80. Maltais F, Reissmann H, Gottfried SB. Pressure support reduces inspiratory effort and dyspnea during exercise in chronic airflow obstruction. Am J Respir Crit Care Med. 1995;151(4):1027-33.
81. Johnson JE, Gavin DJ, Adams-Dramiga S. Effects of training with heliox and noninvasive positive pressure ventilation on exercise ability in patients with severe COPD. Chest. 2002;122(2):464-72.
82. van 't Hul A, Gosselink R, Hollander P, Postmus P, Kwakkel G. Training with inspiratory pressure support in patients with severe COPD. Eur Respir J. 2006;27(1):65-72.
83. Petrof BJ, Calderini E, Gottfried SB. Effect of CPAP on respiratory effort and dyspnea during exercise in severe COPD. J Appl Physiol (1985). 1990;69(1):179-88.
84. Palange P, Valli G, Onorati P, Antonucci R, Paoletti P, Rosato A, et al. Effect of heliox on lung dynamic hyperinflation, dyspnea, and exercise endurance capacity in COPD patients. J Appl Physiol (1985). 2004;97(5):1637-42.
85. Macklem PT. Therapeutic implications of the pathophysiology of COPD. Eur Respir J. 2010;35(3):676-80.
86. Spahija J, Marchie M, Ghezzo H, Grassino A. Factors discriminating spontaneous pursed-lips breathing use in patients with COPD. Copd. 2010;7(4):254-61.
87. Schellekens WJ, van Hees HW, Doorduin J, Roesthuis LH, Scheffer GJ, van der Hoeven JG, et al. Strategies to optimize respiratory muscle function in ICU patients. Crit Care. 2016;20(1):103.
88. Schols AM, Soeters PB, Mostert R, Pluymers RJ, Wouters EF. Physiologic effects of nutritional support and anabolic steroids in patients with chronic obstructive pulmonary disease. A placebo-controlled randomized trial. Am J Respir Crit Care Med. 1995;152(4 Pt 1):1268-74.

89. van Hees HW, Dekhuijzen PN, Heunks LM. Levosimendan enhances force generation of diaphragm muscle from patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2009;179(1):41-7.
90. Gosselink R. Controlled breathing and dyspnea in patients with chronic obstructive pulmonary disease (COPD). J Rehabil Res Dev. 2003;40(5 Suppl 2):25-33.
91. Dodd DS, Brancatisano T, Engel LA. Chest wall mechanics during exercise in patients with severe chronic air-flow obstruction. Am Rev Respir Dis. 1984;129(1):33-8.
92. O'Neill S, McCarthy DS. Postural relief of dyspnoea in severe chronic airflow limitation: relationship to respiratory muscle strength. Thorax. 1983;38(8):595-600.
93. Delgado HR, Braun SR, Skatrud JB, Reddan WG, Pegelow DF. Chest wall and abdominal motion during exercise in patients with chronic obstructive pulmonary disease. Am Rev Respir Dis. 1982;126(2):200-5.
94. Probst VS, Troosters T, Coosemans I, Spruit MA, Pitta Fde O, Decramer M, et al. Mechanisms of improvement in exercise capacity using a rollator in patients with COPD. Chest. 2004;126(4):1102-7.
95. Petrovic M, Reiter M, Zipko H, Pohl W, Wanke T. Effects of inspiratory muscle training on dynamic hyperinflation in patients with COPD. Int J Chron Obstruct Pulmon Dis. 2012;7:797-805.
96. Ambrosino N. The case for inspiratory muscle training in COPD. Eur Respir J. 2011;37(2):233-5.
97. McConnell AK. CrossTalk opposing view: respiratory muscle training does improve exercise tolerance. The Journal of physiology. 2012;590(15):3397-400.
98. Polkey MI, Moxham J, Green M. The case against inspiratory muscle training in COPD. Eur Respir J. 2011;37(2):236-7.
99. Patel MS, Hart N, Polkey MI. CrossTalk proposal: training the respiratory muscles does not improve exercise tolerance. The Journal of physiology. 2012;590(15):3393-401.
100. Langer D, Charususin N, Jacome C, Hoffman M, McConnell A, Decramer M, et al. Efficacy of a Novel Method for Inspiratory Muscle Training in People With Chronic Obstructive Pulmonary Disease. Phys Ther. 2015;95(9):1264-73.
101. Charususin N, Gosselink R, McConnell A, Demeyer H, Topalovic M, Decramer M, et al. Inspiratory muscle training improves breathing pattern during exercise in COPD patients. Eur Respir J. 2016.
102. Jolley CJ, Luo YM, Steier J, Reilly C, Seymour J, Lunt A, et al. Neural respiratory drive in healthy subjects and in COPD. Eur Respir J. 2009;33(2):289-97.
103. Langer D, Ciavaglia C, Webb K, Preston M, Neder JA, Gosselink R, et al. Inspiratory muscle training reduces respiratory neural drive (RND) during exercise in patients with COPD. Eur Respir J. 2014;44(Suppl 58).
104. Wanke T, Formanek D, Lahrmann H, Brath H, Wild M, Wagner C, et al. Effects of combined inspiratory muscle and cycle ergometer training on exercise performance in patients with COPD. Eur Respir J. 1994;7(12):2205-11.
105. Ramirez-Sarmiento A, Orozco-Levi M, Guell R, Barreiro E, Hernandez N, Mota S, et al. Inspiratory muscle training in patients with chronic obstructive pulmonary disease: structural adaptation and physiologic outcomes. Am J Respir Crit Care Med. 2002;166(11):1491-7.
106. Dekhuijzen PN, Folgering HT, van Herwaarden CL. Target-flow inspiratory muscle training during pulmonary rehabilitation in patients with COPD. Chest. 1991;99(1):128-33.
107. Ramsook AH, Koo R, Molgat-Seon Y, Dominelli PB, Syed N, Ryerson CJ, et al. Diaphragm Recruitment Increases during a Bout of Targeted Inspiratory Muscle Training. Med Sci Sports Exerc. 2016;48(6):1179-86.
108. Huang CH, Martin AD, Davenport PW. Effect of inspiratory muscle strength training on inspiratory motor drive and RREP early peak components. J Appl Physiol (1985). 2003;94(2):462-8.
109. Esser RW, Stoeckel MC, Kirsten A, Watz H, Taube K, Lehmann K, et al. Structural Brain Changes in Patients With COPD. Chest. 2016;149(2):426-34.

110. Maltais F, Hamilton A, Marciniuk D, Hernandez P, Sciurba FC, Richter K, et al. Improvements in symptom-limited exercise performance over 8 h with once-daily tiotropium in patients with COPD. Chest. 2005;128(3):1168-78.
111. Louvaris Z, Vogiatzis I, Aliverti A, Habazettl H, Wagner H, Wagner P, et al. Blood flow does not redistribute from respiratory to leg muscles during exercise breathing heliox or oxygen in COPD. J Appl Physiol (1985). 2014;117(3):267-76.
112. Louvaris Z, Zakynthinos S, Aliverti A, Habazettl H, Vasilopoulou M, Andrianopoulos V, et al. Heliox increases quadriceps muscle oxygen delivery during exercise in COPD patients with and without dynamic hyperinflation. J Appl Physiol (1985). 2012;113(7):1012-23.
113. Maltais F, Simon M, Jobin J, Desmeules M, Sullivan MJ, Belanger M, et al. Effects of oxygen on lower limb blood flow and O2 uptake during exercise in COPD. Med Sci Sports Exerc. 2001;33(6):916-22.
114. Vogiatzis I, Habazettl H, Aliverti A, Athanasopoulos D, Louvaris Z, LoMauro A, et al. Effect of helium breathing on intercostal and quadriceps muscle blood flow during exercise in COPD patients. Am J Physiol Regul Integr Comp Physiol. 2011;300(6):R1549-59.
115. Borghi-Silva A, Oliveira CC, Carrascosa C, Maia J, Berton DC, Queiroga F, Jr., et al. Respiratory muscle unloading improves leg muscle oxygenation during exercise in patients with COPD. Thorax. 2008;63(10):910-5.
116. Laveneziana P, Valli G, Onorati P, Paoletti P, Ferrazza AM, Palange P. Effect of heliox on heart rate kinetics and dynamic hyperinflation during high-intensity exercise in COPD. Eur J Appl Physiol. 2011;111(2):225-34.

Chapter 3

General methodology

3.1 Overview of the study

According to the introduction and background presented in chapters 1 and 2, the general aim of this thesis is to investigate potential mechanisms that could explain the effect of IMT on dyspnea and exercise tolerance namely neuro-ventilatory, muscle metaboreflex and neural processing mechanism. The overview of the study is presented in figure 3.1 below. In short, an introduction to the available mechanisms linking respiratory muscle dysfunction to dyspnea and exercise limitation are presented in **chapter 2**. Then, **chapter 4** offers a methodological study in which a time efficient analysis method was developed and compared with the previously available manual method. This new method was used in the analysis of respiratory muscle activation in chapter 5. For **chapter 5, 6 and 7**, a randomized control trial (RCT) was performed in order to investigate three different mechanisms regarding dyspnea and exercise limitation in response to IMT in patients with COPD (i.e., neuro-ventilatory, muscle metaboreflex and neural processing mechanism). **Chapter 8** elaborates on the findings of the three mechanisms that has been investigated. The roles of IMT in clinical practice, future perspective of IMT and possible future research questions were discussed.

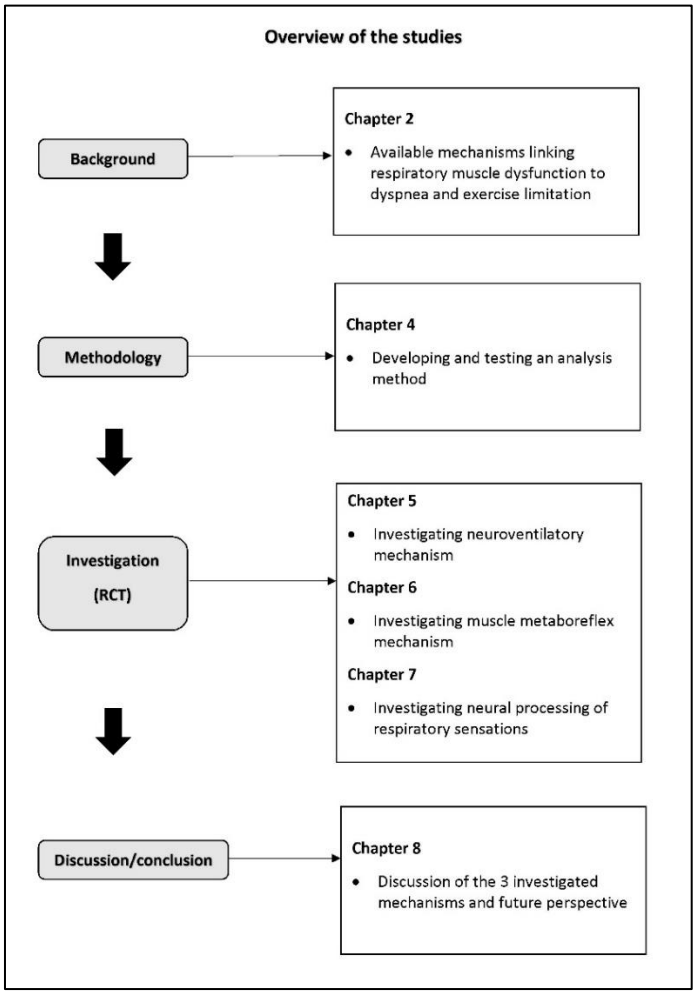

Figure 3.1 Overview of how the chapters are organized in this thesis

3.2 Rationale and specific aims of individual chapters

Chapter 4: Comparison between manual and (semi-) automated analyses of esophageal diaphragm electromyography during endurance cycling in patients with COPD

As mentioned in chapter 2, neural respiratory drive (NRD) can be indirectly assessed by measuring the EMG of the diaphragm (EMGdi) (1-3). In this research project, EMGdi was measured using a multipair-esophageal electrode catheter (Yinghui Medical Equipment Technology Co. Ltd., Guangzhou, China). Because of the proximity of the EMG electrodes to the heart, the ECG artifact is commonly found in the EMG signals. It is known that manual ECG removal methods, which were mostly used in previous studies, is time-consuming and prone to inter-rater variability (3-5). Therefore, in chapter 3, we developed an algorithm to semi-automatically remove the ECG artifact using commercially available software (LABVIEW) to make the analysis more transparent and freely accessible to other researchers. This chapter aims to investigate the inter-rater reliability of the manual method of EMGdi amplitude analysis and to explore the agreement between the manual and the proposed semi-automated analysis method of EMGdi amplitude signals both cross-sectionally (to evaluate validity) and changes in response to intervention (to evaluate responsiveness). We hypothesized that the EMGdi analyzed with the semi-automated method would agree well with values obtained by the manual method while requiring less analyzing time.

Chapter 5: Effects of inspiratory muscle training on dyspnea and respiratory muscle function at rest and during exercise in patients with COPD

Previous studies found that IMT can improve inspiratory muscle function, the sensory intensity of dyspnea, reduce neural respiratory drive, (diaphragmatic EMG) and improve exercise tolerance in COPD patients with inspiratory muscle weakness (6). It has been argued that diaphragm EMG might not reflect the overall NRD and the extra-diaphragmatic inspiratory muscles of the neck and rib cage might also contribute importantly to the NRD (7-9). The distribution of the overall NRD and the muscle activation patterns between the diaphragm and extra-diaphragmatic respiratory muscles in COPD patients and potential changes in response to IMT, have not yet been studied. Therefore, in chapter 5, our aim was to investigate the effects of either IMT or sham-IMT on a complete neural respiratory drive (EMG activity of the diaphragm, scalene, sternocleidomastoid, and rectus abdominis) in relation to dyspnea sensation during CWR cycling and to study how these muscles are recruited and activated during IMT sessions in patients with COPD. We hypothesized that eight weeks of IMT would improve dyspnea sensation during exercise and reduce the neural respiratory drive to the diaphragm and extra diaphragmatic inspiratory muscles. Furthermore, in an explorative fashion, we tested whether during

an IMT session, the activation of the extra-diaphragmatic inspiratory muscles would be higher than the activation of the diaphragm during cycling.

Chapter 6: Impact of inspiratory muscle training on respiratory and locomotor muscle perfusion, oxygenation and locomotor muscle fatigue during exercise in patients with COPD

This study investigates potential changes in the 'muscle metaboreflex' in response to IMT in patients with COPD. In this patient population, during exercise, the work of the respiratory and locomotor muscles can be increased to such an extent that muscle fatigue can be developed (10, 11). This, in turn, may activate the 'muscle metaboreflex' which can induce sympathetically mediated vasoconstriction of locomotor muscles leading to premature termination of exercise secondary to the restriction in blood flow and oxygenation to respiratory and locomotor muscles, and development of respiratory and locomotor muscle fatigue (10, 11). We compared blood flow (BFI) and tissue oxygenation ($StiO_2$) of respiratory and locomotor muscles during the CWR cycling test. The aim was to evaluate the effects of IMT in comparison with sham training on changes in respiratory and locomotor muscle blood flow and locomotor muscle fatigue during cycling exercise. The hypothesis was that after eight weeks of IMT, the relative effort of the inspiratory muscles would be decreased (by increasing their capacity) at iso-load during exercise and this would attenuate the respiratory muscle metaboreflex, which would improve respiratory and locomotor muscle blood flow, and reduced locomotor muscle fatigue.

Chapter 7: Impact of inspiratory muscle training on the perception and neural processing of respiratory sensations in COPD

Chapter 7 is focuses on the perception of the respiratory distress component of dyspnea. The non-invasive technique of measuring respiratory-related evoked potentials (RREPs) recorded from the electroencephalogram (EEG) was used to assess the neural processing of respiratory sensations (12). RREP is a measurement of cerebral cortical activity, which is elicited by the activation of lung and muscle mechanoreceptors in response to short inspiratory occlusions or inspiratory resistive loads (12). Several RREP peaks in the averaged EEG signal have been shown to be related to the onset of the respiratory stimulus. The early peaks (Nf, P1, and N1) reflect the initial arrival and the first-order processing of afferent respiratory signals in sensorimotor regions (13). The later peaks (P2, and P3) characterize subsequent higher-order cognitive processing and are vulnerable to cognitive and affective processes (13). N1, however, can also be affected by attention/affect-related processes (14). In healthy subjects, significant increases in magnitude of N1, P2, and P3 peaks were found when breathing became more difficult and unpleasant indicating an increase in the neural processing of respiratory sensations reflective of higher cognitive processing (15). A previous study found no changes

in early RREP components in response to IMT in healthy subjects (16). The effect of IMT on later components of RREP (P2, and P3) has not yet been studied in patients with COPD. Therefore, in this study, we are focusing on evaluating the effects of IMT on the later components of RREP (P2, and P3).

The aim of this chapter is 1) to investigate the effects following 8 weeks of IMT on changes in the unpleasantness component of dyspnea in relation to the neural processing of respiratory sensations, specifically in the higher order cognitive-affective processing related to the later components of the RREP in response to short inspiratory occlusions during unloaded breathing. 2) to assess the impact of IMT on general anxiety and dyspnea specific fear. In addition, the correlations between changes in later peak components of the RREP in response to IMT and changes in the perceived intensity and unpleasantness of respiratory sensations during unloaded breathing, dyspnea-specific fear and general anxiety are investigated. The hypotheses are 1) after eight weeks of IMT, the unpleasantness of respiratory sensations would be reduced, which would be paralleled by a reduction in the amplitudes of the later RREP components. 2) IMT could reduce levels of general anxiety and dyspnea-specific fears and these might be associated with changes in later peak components of the RREP.

Chapter 8: **General discussion**

In chapter 7, the elaboration on the findings of the methodology chapter and the three mechanisms that had been investigated (in chapter 5, 6 and 7), the role of IMT in clinical practice, future perspective of IMT and possible future research questions are summarized.

3.3 General methodology and experimental set up for the RCT

Clinically stable COPD patients with reduced inspiratory muscle strength were included in this RCT (inclusion and exclusion criteria are presented in chapter 5). Patients were allocated into an IMT group or a sham training group and underwent 8 weeks of IMT or sham training. According to the sample size calculation, 16 patients were required in the intervention group and 8 patients were needed in the control group (detail of group allocation and sample size calculation can be found in chapter 5).

The primary outcome (Borg dyspnea score) and other outcomes were measured during a constant work rate (CWR) cycling test (cycling at 75% of maximal work rate) until symptom limitation. After the training, the test was repeated at the same work rate, comparisons of all outcomes were made at the iso-time (the end time of the shortest cycling test). An additional CWR cycling test was performed to measure the locomotor muscle fatigue using magnetic stimulation. The test was stopped at identical time at post-measurement to measure the fatigue at the iso-time. Additional unloaded breathing trials with inspiratory occlusions were performed during the measurement of electroencephalogram (EEG)

to investigate the affective perception of dyspnea. The specific methods and measurements used in the different studies are described in detail in each study. The ethical committee of the University Hospital Leuven, Belgium approved the study (S58513).

All measurements were performed at baseline and after 8 weeks of training. Six visits (3 pre /3 post) to the Respiratory Division of the University Hospital Leuven were required for each patient for outcome measurements. **Visit 1** was an initial screening for pre-measurement. This visit aimed to assess eligibility and familiarize the patients with testing protocols. The measurements included pulmonary function testing, cardiopulmonary exercise testing (CPET), inspiratory and expiratory muscle strength testing, endurance breathing task, and answering questionnaires regarding dyspnea sensation. An arterial blood gas measurement was also performed, values obtained from the blood gas results were used in the calculation of the central hemodynamic values in chapter 6. **Visit 2** can be separated into two parts. First, the unloaded breathing trial with inspiratory occlusions during the EEG measurement was performed. The results were used in chapter 6 regarding the neural processing of respiratory sensations. The second part of visit 2, locomotor exercise-induced quadriceps contractile fatigue was measured by supra-maximal stimulation of the femoral nerve with a double twitch stimulation technique. This twitch stimulation technique was performed before and after a CWR endurance cycling test. The locomotor muscle fatigue results were presented in chapter 6.

Visit 3 a considerable number of outcomes were measured during two tasks (i.e., one session of IMT and CWR cycling). A multipair-esophageal electrode catheter was used to assess EMGdi and transdiaphragmatic pressure (Pdi). A surface EMG wireless transmission system was used to measure the activation of extra-diaphragmatic inspiratory muscles (sternocleidomastoid, and scalene) and one expiratory muscle (rectus abdominis). The results of the activation of all muscles measured were used in chapter 4. Cardiac output, heart rate, and stroke volume were continuously measured by an impedance cardiography device. Respiratory and locomotor muscle blood flow was assessed by near-infrared spectroscopy (NIRS) using the light-absorbing tracer indocyanine green (ICG) dye. Muscle oxygenation ($StiO_2$) was also continuously assessed by NIRS. These hemodynamic parameters, muscle blood flow, and muscle oxygenation results were used in chapter 6. At post-measurement visits, all the tests were repeated except the CPET, and arterial blood gas measurement. The CWR cycling test for twitch measurements was stopped concurrently as the time limit of pre-measurement rather than at symptom limitation. Sequences of measurements performed in each visit are presented in Table 3.1.

Table 3.1 measurement sequences

	Pre-measurement		Post-measurement
Visit 1 **(3 hours)**	*Initial screening* • pulmonary function assessments • inspiratory muscle strength and endurance tests • questionnaires regarding dyspnea perception (BDI/TDI, MRC) • arterial blood gas measurement • cardiopulmonary exercise testing (CPET)	**8 weeks IMT**	• pulmonary function assessments • inspiratory muscle strength and endurance tests • questionnaires regarding dyspnea perception (BDI/TDI, MRC)
Visit 2 **(3 hours)**	• unloaded breathing trial during EEG measurement • quadriceps contractile fatigue (measured using magnetic stimulation pre and post CWR cycling test)		• unloaded breathing trial during EEG measurement • quadriceps contractile fatigue (measured using magnetic stimulation pre and post CWR cycling test)
Visit 3 **(3 hours)**	• inspiratory muscle training trial • CWR cycling test		• Inspiratory muscle training trial • CWR cycling test

Inspiratory muscle training (IMT)/sham training protocol

According to a previously established method (17), patients performed home-based daily inspiratory muscle training consisting of two training sessions of 30 breaths (4-5 minutes per session). The intensity of the training starting at approximately 40% of maximal inspiratory pressure (MIP) measured at baseline. In the training group, the intensity would be increased weekly to the highest tolerable intensity (approximately 40-50% of MIP achieved on that week). In the sham group, the training intensity remained the same for the entire 8 weeks at approximately 10% of the patient's initial MIP. Weekly measurements of MIP were performed in both groups. One training session per week was performed while supervised at the research center. IMT was performed using an electronic tapered flow-resistive loading (TFRL) device (POWERbreath®KH1, HaB International Ltd., Southam, UK) for 8 weeks.

References

1. Moxham J, Jolley C. Breathlessness, fatigue and the respiratory muscles. Clin Med. 2009;9(5):448-52.
2. Jolley CJ, Luo YM, Steier J, Rafferty GF, Polkey MI, Moxham J. Neural respiratory drive and breathlessness in COPD. Eur Respir J. 2015;45(2):355-64.
3. Luo YM, Moxham J. Measurement of neural respiratory drive in patients with COPD. Respir Physiol Neurobiol. 2005;146(2-3):165-74.
4. Luo YM, Moxham J, Polkey MI. Diaphragm electromyography using an oesophageal catheter: current concepts. Clin Sci (Lond). 2008;115(8):233-44.
5. Jolley CJ, Luo YM, Steier J, Reilly C, Seymour J, Lunt A, et al. Neural respiratory drive in healthy subjects and in COPD. Eur Respir J. 2009;33(2):289-97.
6. Langer D, Ciavaglia C, Faisal A, Webb KA, Neder JA, Gosselink R, et al. Inspiratory muscle training reduces diaphragm activation and dyspnea during exercise in COPD. J Appl Physiol (1985). 2018;125(2):381-92.
7. Martinez FJ, Couser JI, Celli BR. Factors influencing ventilatory muscle recruitment in patients with chronic airflow obstruction. Am Rev Respir Dis. 1990;142(2):276-82.
8. Montes de Oca M, Celli BR. Respiratory muscle recruitment and exercise performance in eucapnic and hypercapnic severe chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2000;161(3 Pt 1):880-5.
9. Ora J, Laveneziana P, Wadell K, Preston M, Webb KA, O'Donnell DE. Effect of obesity on respiratory mechanics during rest and exercise in COPD. J Appl Physiol (1985). 2011;111(1):10-9.
10. Sheel AW, Boushel R, Dempsey JA. Competition for blood flow distribution between respiratory and locomotor muscles: implications for muscle fatigue. J Appl Physiol. 2018;125(3):820-31.
11. Dempsey JA, Romer L, Rodman J, Miller J, Smith C. Consequences of exercise-induced respiratory muscle work. Respir Physiol Neurobiol. 2006;151(2-3):242-50.
12. von Leupoldt A, Chan PY, Esser RW, Davenport PW. Emotions and neural processing of respiratory sensations investigated with respiratory-related evoked potentials. Psychosom Med. 2013;75(3):244-52.
13. Chan PY, Davenport PW. Respiratory related evoked potential measures of cerebral cortical respiratory information processing. Biol Psychol. 2010;84(1):4-12.
14. Davenport PW, Chan PY, Zhang W, Chou YL. Detection threshold for inspiratory resistive loads and respiratory-related evoked potentials. J Appl Physiol (1985). 2007;102(1):276-85.
15. von Leupoldt A, Bradley MM, Lang PJ, Davenport PW. Neural processing of respiratory sensations when breathing becomes more difficult and unpleasant. Front Physiol. 2010;1:144.
16. Huang CH, Martin AD, Davenport PW. Effect of inspiratory muscle strength training on inspiratory motor drive and RREP early peak components. J Appl Physiol (1985). 2003;94(2):462-8.
17. Langer D, Charususin N, Jacome C, Hoffman M, McConnell A, Decramer M, et al. Efficacy of a Novel Method for Inspiratory Muscle Training in People With Chronic Obstructive Pulmonary Disease. Phys Ther. 2015;95(9):1264-73.

Chapter 4

Comparison between Manual and (Semi-) Automated Analyses of Esophageal Diaphragm Electromyography during Endurance Cycling in Patients with COPD

Sauwaluk Dacha, Luc Janssens, Antenor Rodrigues,

Zafeiris Louvaris, Lotte Janssens, Rik Gosselink, & Daniel Langer

Front Physiol. 2019;10(885)

4.1 Abstract

Background: Electrocardiogram (ECG) contamination is present in diaphragm electromyography (EMGdi) recordings. Obtaining EMGdi without ECG contamination is crucial for EMG amplitude analysis. Manually selecting EMGdi in between QRS complexes has been most commonly applied in recent years (manual method). We developed a semi-automated analysis method based on Least Mean Square Adaptive Filtering combined with a synchronously recorded separate ECG channel to remove ECG artifacts from the EMGdi signals. We hypothesized that this approach would shorten analysis duration and might minimize the potential for inter-rater disagreement.

Aims: We aimed to evaluate agreement between the semi-automated method and the manual method and inter-rater reliability of the manual method.

Methods: EMGdi signals of seven patients with COPD were recorded using an esophageal catheter during an exercise test on a cycle ergometer. Four patients subsequently participated in an inspiratory muscle training (IMT) program for eight weeks. After IMT, the tests were repeated. EMGdi/EMGdiMax as obtained either manually by the two assessors or retrieved from the semi-automated method were compared.

Results: Semi-automated EMGdi/EMGdiMax agreed well with values obtained by one of the two manual assessors (assessor 1) both at pre-intervention measurements (mean difference -0.5%, 95% CI -19.6% to 18.6%) and for the pre/post IMT differences (mean difference 1.2%, 95% CI -16.8% to 19.2%). Intra-class correlation coefficients between methods were 0.96 (95% CI: 0.94 to 0.97) at pre-intervention measurements and 0.78 (95% CI: 0.58 to 0.89) for pre/post IMT differences (both $p<0.001$). EMGdi/EMGdiMax from assessor 2 was systematically lower than from assessor 1 and agreed less well with the semi-automated method both at pre-intervention measurements (mean difference: 9.3%, 95% CI:-11.4% to 29.9%) and for pre/post IMT differences (mean difference 7.0%, 95% CI: -20.4% to 34.4%). Analysis duration of the semi-automated method was significantly shorter (29±9 minutes) than the manual method (82±20 minutes, $p<0.001$).

Conclusion: The developed semi-automated method is more time efficient and will be less prone to inter-rater variability that was observed when applying the manual analysis method. It is, therefore, proposed as a new standard for objective EMGdi amplitude analyses in future studies.

Keywords: electromyography, electrocardiography, diaphragm electromyography, chronic obstructive pulmonary disease, respiratory muscle training

4.2 Introduction

Electromyography (EMG) is an assessment of muscle activation by recording the electrical activity of the muscle tissue. Assessments of diaphragm EMG (EMGdi) amplitude are frequently applied in both clinical and research settings, where they can serve as an indirect measure of neural respiratory drive (NRD) during different conditions such as resting breathing, exercise breathing, or during sleep (1-4). EMGdi can be recorded either via surface electrodes placed on the chest wall, with needle electrodes inserted into the costal diaphragm, or with an esophageal catheter equipped with EMG electrodes (5-7). The EMGdi recording contains artifacts from the power line, from movement, and from cardiac activity. Movement artifacts are associated with very low frequencies and can be easily removed by applying high pass filtering at 20Hz. However, the cardiac activity artifacts, as detected by electrocardiogram (ECG), is more difficult to remove because of the overlapping bandwidth spectrum between ECG and EMGdi. The majority of the EMGdi signal is concentrated in the bandwidth between 20-250Hz, while the bandwidth of the ECG frequency spectrum lies between 0-100Hz (8). It is crucial to obtain the EMGdi signal without the ECG contamination, to ensure the accuracy of the EMGdi signal, (7, 9, 10). Separating ECG from EMGdi is particularly challenging in EMG amplitude analyses, especially during exercise, since the EMGdi amplitude can be larger than the ECG. This makes it more difficult to identify ECG artifacts within the EMG signal.

One widely used method to obtain the EMGdi signal without ECG contamination is to manually select EMGdi data in between QRS complexes (2, 7, 11-14). By placing a separated time-synchronized ECG channel next to the EMG channel, the ECG channel is visually identifiable, thereby allowing to retrieve the EMGdi in between QRS complexes. However, there are some limitations to this method. First, this method is time-consuming, especially for recordings that contain many breathing cycles such as during exercise. Second, based on the experience in our research group, it might be subjective to inter-rater variability since the retrieved data can vary depending on the judgment of the assessor. Inter-rater variability could arise from the fact that several EMGdi parts are available to choose from in between QRS complexes during every inspiration. No specific instructions are currently available as to which interval should be preferably selected under these circumstances while selection of either ascending, descending, or peak intervals of the uncontaminated signal might result in vast differences in the recorded EMG amplitude. Selection width of the chosen interval while avoiding artifact to either side of the selected interval might be another factor that could explains the inter-rater variability of the obtained EMG amplitude for a given breath. A final limitation of the manual method is that EMGdi activity “buried” in the ECG signal cannot be selected. Depending on the location of the QRS complexes the data outside of contaminated area might not be the best representation of the actual EMG

amplitude (e.g., the part containing the highest amplitude of the signal might not be available to select). This might be especially problematic during exercise, when several heartbeats typically occur during a single inspiration.

Several methods have been previously applied to automatically deduct or remove ECG artifacts from EMGdi signals. However, the majority of methods does not rely on ECG data from a separately collected ECG channel. These methods typically suffer from problems with frequency-overlapping, difficulties in waveform identification, and processing difficulties due to the sometimes smaller amplitude of the ECG signal in comparison to the EMG signal (e.g. during near maximal diaphragm activation throughout exercise hyperpnea) (8, 10, 15, 16). Bloch suggested using a separate and simultaneous recording of a time-synchronized ECG channel to avoid these problems (17). For analysis, he proposed to initially use the amplitude threshold to identify the QRS complex of the ECG, followed by applying a least squares subtraction on the time domain to remove ECG artifacts (17). This method introduced by Bloch has not been extensively evaluated or validated especially not for EMGdi recordings of resting and exercise breathing obtained with an esophageal catheter.

Up to now, there is no gold standard method available for removing ECG artifacts while analyzing EMGdi amplitude data. From the reviewed methods above, manually selecting EMGdi in between QRS complexes has so far been the most applied method. This method will be mentioned onward as the "manual" method. Because of the shortcomings of the manual method, we were interested in developing and evaluating an alternative method that could potentially shorten the duration of the analysis and overcome several problems related to the expected inter-rater ambiguity that seems inherent to the somewhat subjective judgements that have to be made while applying the manual analysis method. Therefore, we developed a custom "semi-automated method" based on a Least Mean Square (LMS) Adaptive Filtering method (17) combined with a synchronously recorded, separated ECG channel. We aimed to compare this "semi-automated method" with results obtained from the manual method. In addition, we also aimed to formally study the degree of inter-rater variability that can be expected when applying the manual analysis method. Responsiveness (i.e. the ability of a measure to detect change) is an important feature of assessment methods that needs to be evaluated separately from reliability and validity (18).The degree of agreement between methods was therefore evaluated both cross-sectionally (i.e. of data obtained at a single point in time) to evaluate validity and reliability as well as by comparing changes in activation observed after an intervention period between methods to evaluate and compare responsiveness.

Accordingly, the aims of this study were the following: (1) to investigate the inter-rater reliability of the manual method of EMGdi amplitude analysis and (2) to explore the agreement between the

manual and the proposed semi-automated analysis method of EMGdi amplitude signals both cross-sectionally (to evaluate validity) and of changes in response to an intervention (to evaluate responsiveness).

4.3 Materials and methods

4.3.1 Study design and subjects

Clinically stable patients with moderate to severe COPD were included in this study. Data were retrieved from patients who had been enrolled in a clinical study (ClinicalTrials.gov Identifier: NCT03240640). The Ethical Committee Research of KU Leuven/ UZ Leuven, Belgium approved the study (S58513). All participants signed written informed consent. EMGdi was recorded during a constant work rate cycle ergometer (CWR) test before and after eight weeks of inspiratory muscle training (IMT). The EMGdi data were first analyzed using the manual method by two independent assessors. The same data were then analyzed again using the semi-automated method. Comparisons were made both between the results obtained by the two assessors using the manual analyzing method as well as between results obtained by both manual assessors and from the semi-automated method. The details of each analysis method are described below. Interim analysis of these data has been presented at ERS International Congress 2018 (19).

4.3.2 Pulmonary function and respiratory muscle function measurements

Pulmonary function testing (MasterScreen Body, CareFusion, Hoechberg, Germany) was performed according to ERS guidelines (20-22). Maximal inspiratory, expiratory mouth pressures (MIP and MEP) and transdiaphragmatic pressure (PdiMax) during sniff maneuver were assessed according to international guidelines (23).

4.3.3 EMG recording and analysis

Esophageal catheter and positioning

A multipair-esophageal electrode catheter (Yinghui Medical Equipment Technology Co. Ltd., Guangzhou, China) was used to assess EMGdi. The catheter is approximately 60 centimeters long, two millimeters in diameter, and is equipped with five EMG electrode pairs feeding five EMGdi channels. The catheter was inserted nasally and then swallowed by the patient. The positioning of the catheter was performed according to procedures established in previous studies (1, 2). In short, the patient was asked to perform several slow maximal inspiratory capacity (IC) maneuvers (an inspiration through the open mouth from the functional residual capacity to total lung capacity). The best position was determined as the location which the largest EMGdi amplitudes were recorded from the outer

electrode pairs and the smallest from the middle pairs (Figure 4.1). (1, 2, 24). After positioning, the catheter was secured by taping one end onto the patient's nose.

EMGdi sampling and processing

The EMGdi signals were first amplified (Biomedical amplifier, Guangzhou, China), sampled at 2000Hz by a data acquisition system (Micro1401-3, Cambridge Electronic Design Limited, Cambridge, UK) and then processed with a specific software package (Spike 2, Cambridge Electronic Design Limited, Cambridge, UK). During processing the raw EMGdi data were first high pass filtered at 20Hz to minimize motion artifacts and then transformed into "Root Mean Square" (RMS). The EMGdi signals recorded during breathing were then normalized by presenting the recorded value relative to the signal obtained during maximal activation; EMGdi/EMGdiMax%. The highest EMGdi signal obtained from any of the five channels during each subsequent breath was retrieved for analyses. Maximal activation of the diaphragm was obtained during typical (i.e. fast) exercise IC maneuvers, either during resting breathing or during exercise breathing (25). The patients were asked to perform these IC maneuvers every minute during the three resting minutes preceding the cycling test, and every other minute during the cycling test. The largest RMS amplitude obtained during any of the recorded IC maneuvers was selected as EMGdiMax.

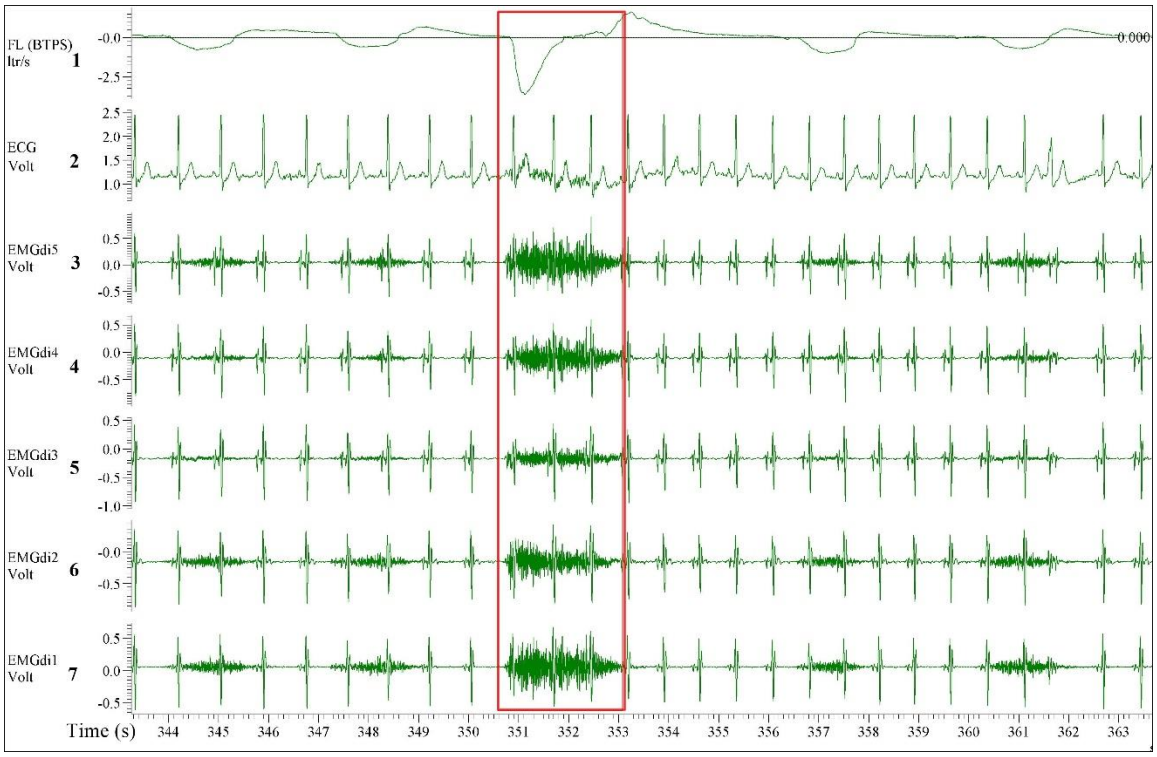

Figure 4.1 A five diaphragm EMG (EMGdi) channel recording contains electrocardiogram (ECG) artifacts during resting breathing. From top to bottom the channels are as follow; Channel 1: Respiratory flow (l/sec; negative flow indicating the inspiratory cycle), Channel 2: ECG recording (volt), Channel 3-7: EMGdi recordings. The correct positioning of the catheter is shown when the largest EMGdi amplitudes are in the outer EMGdi channels (3 and 7), and the smallest amplitude is in the middle channel (5). The inspiratory capacity (IC) maneuver is highlighted in the red box indicated by the higher flow, which accompanies the maximal activation of the diaphragm (EMGdiMax).

4.3.4 ECG removal with the manual method

From the processed data, both assessors were instructed to perform the manual analysis of the EMGdi signals in agreement with previously published methods. They were instructed to extract the EMGdi signals from segments of inspiratory cycles between QRS complexes (1, 2, 13, 24); however, reflecting previously-published methods, no instructions were given with regards to handling possible residual interference by P, T, or U waves (Figure 4.2). Thus, we cannot exclude the possibility of such interference within the manually-derived EMGdi signal. Noteworthy, the values that have been extracted between QRS complexes in most previous literature is the peak RMS EMGdi signal of a given breath (2, 13, 26-28). However, as we were interested in measuring an estimate of the integral (i.e., mean) respiratory neural drive of the inspiratory cycle of a given breath, the mean value between QRS complexes that would represent the integral activation was used for analysis instead of the peak value (29). The time-synchronized flow and ECG channels were used as a guide for EMGdi selection. Five representative (preferably consecutive) breaths towards the end of each minute were selected. The choice of using five breaths towards the end of a given minute is based on in-house previous analysis that shows the mean value obtained from the last five breaths of a given minute being similar to the

average of the values obtained from the last 30 seconds of the same minute. Breaths were disregarded in case they represented short sighs or included visible noise (e.g. from coughing) or if they were visibly different compared to surrounding breaths. The average of EMGdi of these five breaths was used as representative of diaphragm activation of each minute of the cycling test.

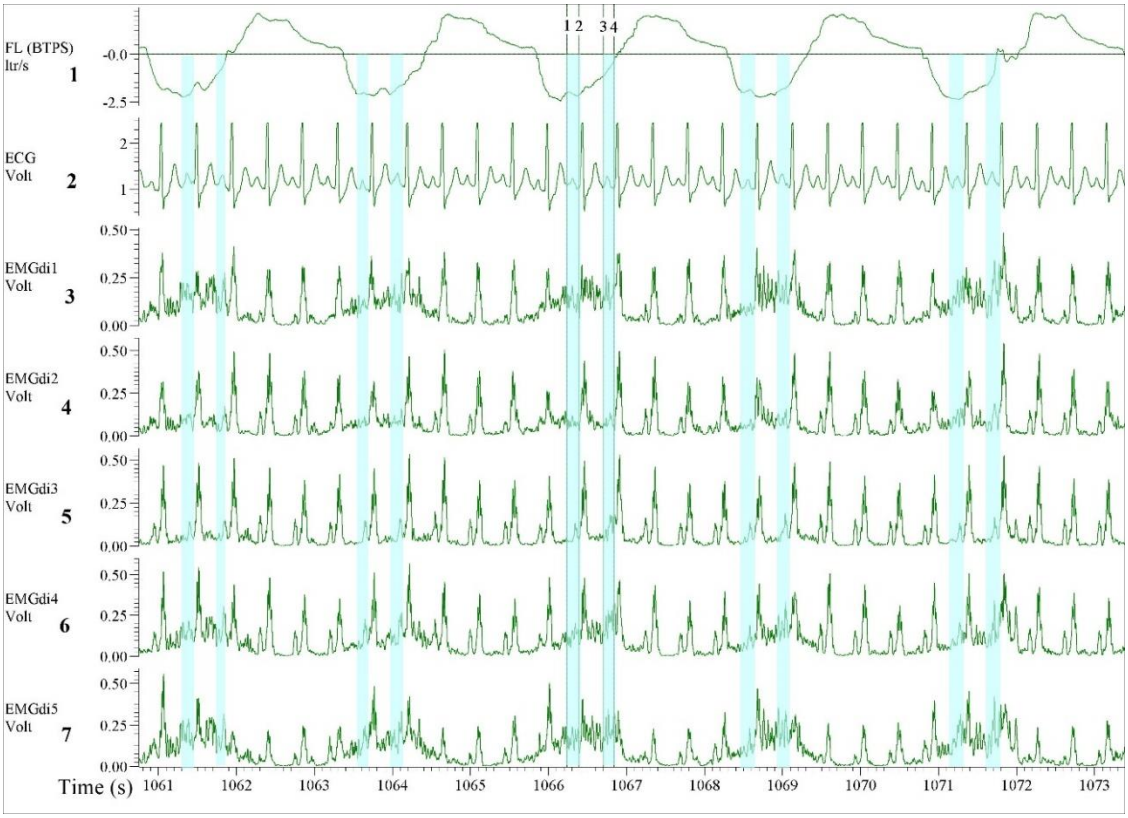

Figure 4.2 Illustration of the diaphragm EMG (EMGdi) recordings towards the end of the (symptom limited) cycling test. Channel 1: respiratory flow (l/sec; negative flow indicating the inspiratory cycle), Channel 2: electrocardiogram (ECG) recording (volt), Channels 3-7: RMS EMGdi recordings. For the data analysis using the manual method, the mean values of EMGdi in between QRS complexes during the inspiratory cycle were selected. The periods highlighted in light blue are the possible periods that could be chosen without ECG contamination in each inspiratory cycle. The average of EMGdi of five consecutive breaths was used as a representative value of EMGdi of that minute. Vertical cursors 1, 2, 3, and 4 were used as a tool during manual analysis to retrieve mean values of EMGdi in the selected period.

4.3.5 ECG removal with the semi-automated method

To perform semi-automated ECG exclusion using the newly developed algorithm several steps had to be executed. First, in the data acquisition software (Spike 2, Cambridge Electronic Design Limited, Cambridge, UK), the following recording channels were selected and exported at 1000 Hz into a text file using the export option from the data acquisition software: ECG, EMGdi (5 channels),respiratory flow and volume, and a channel including event markers. These markers were manually inserted during the test to spot the transition from one condition to another during the test. The entire length of the data file, including the resting period before cycling, one minute of unloaded cycling and all minutes of loaded cycling until symptom limitation were exported. The exported file was then imported into

LABVIEW (National Instruments, Austin, TX) software. The waveforms of the recorded channels was also visible in LABVIEW for inspection.

To reduce the ECG content of the diaphragm EMG channels we used a method called “adapted filtering”. The Least Mean Square (LMS) Adaptive Filter is a pattern recognition algorithm, which is available in the LABVIEW software. This method is a filtering method in the frequency domain that aims to remove the ECG frequency content out of the total signal. The filter was tuned to comply with the minimum error and consequently delivered the best results to remove the ECG component from the recorded signal. We used a filter length of 70 and a step size of 0.01 as the most optimal coefficients for this analysis. A separate channel was used to record the ECG synchronously to tune the coefficients of the Finite Impulse Response (FIR) filter continuously. In this way, the removal was very precise, even though the heart rhythm was changing throughout the test. More detail concerning the Least Mean Square (LMS) Adaptive Filtering can be found in this link http://www.ni.com/example/31220/en/.

The ECG filtering algorithm was pre-set in the LABVIEW software, the ECG channel was recognized automatically by the algorithm. After importing the data, the assessor selected the ECG exclusion option on all EMGdi channels. The algorithm then automatically ran and the assessor was notified when the “cleaned” EMGdi data were ready to be retrieved. These results were then saved in a separate text file. This text file containing the cleaned EMGdi data was then re-imported into the data acquisition software (Spike 2). The assessor then used a respiratory script application (commercially available upon purchase of the software) available in the data acquisition software (spike 2) to further process the data. The respiratory script automatically marks the inspiratory and the expiratory cycle of each breath throughout the selected recording interval based on the respiratory flow signal (i.e. based on zero-flow points). The mean of the integrated EMGdi signal (RMS) during every inspiratory cycle (marked periods) throughout the cycling test was then automatically calculated and exported to an excel sheet. The values of these mean integrated EMGdi signals of every breath could not be manipulated by the assessor. The assessor then identified the resting and exercise period of each test and each minute of the test was manually marked. The average of the mean of integrated RMS from every inspiratory cycle in each minute was then manually calculated and used as a representative diaphragm activation of each minute of the cycling test (Figure 4.3). In a similar way, IC maneuvers were manually identified and activation data retrieved. In summary, while the method involves some manual steps it is not possible to manually manipulate EMGdi amplitude signals within separate breaths. The method is therefore (in contrast to the manual method) not prone to inter rater variability. Differences in outcomes could only occur in case of not selecting appropriate minute intervals or IC maneuvers.”

The reported results used for analysis were taken from one of the five channels that (on average) contained the largest EMGdi signals during IC maneuvers.

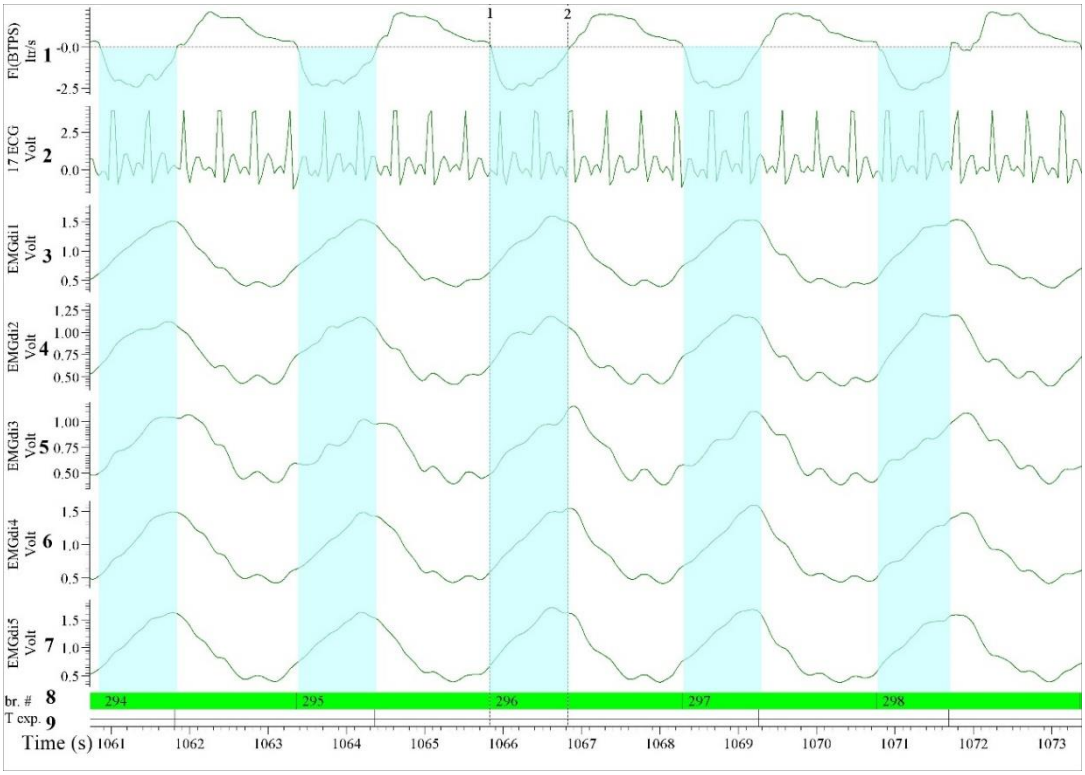

Figure 4.3 Illustration of the diaphragm EMG (EMGdi) recordings towards the end of the (symptom limited) cycling test (period comparable to Figure 4.2). For the data analysis using the semi-automated method. Channel 1: respiratory flow (l/sec; negative flow indicating the inspiratory cycle), Channel 2: ECG recording (volt) ; The absolute ECG values (volt) from this re-imported data after having been processed in LabVIEW using the semi-automatic algorithm were transformed into an abstract unit. Therefore, the signal appeared to be distorted and cannot be compared directly to the pre-processed ECG signal, Channels 3-7: the processed EMGdi data from our customized algorithm without ECG contamination in the diaphragmatic EMG signal (EMGdi). Channel 8: br.# = beginning of the inspiratory cycle and Channel 9: T exp. = beginning of the expiratory cycle indicates the inspiratory and expiratory cycle of each breath which was marked automatically by the program. The EMGdi during a full inspiratory cycle can be selected (highlighted in light blue). The average of the mean EMGdi from every inspiratory cycle in each minute was analyzed. Vertical cursors 1 and 2 indicate a longer period that the value of EMGdi could be retrieved compare to the same breath in figure 4.2 that only shorter periods were available.

4.3.6 Exercise testing

All patients underwent constant work rate (CWR) cycling tests consisting of three minutes of resting, one minute of unloaded cycling and immediately followed by cycling against 75% of the patient's peak work rate achieved during a maximal incremental cardiopulmonary exercise test (CPET)(30) until symptom limitation. The tests were conducted on an electrically-braked cycle ergometer (Ergometrics 900, Ergoline, Blitz, Germany) with detailed metabolic (SensorMedics Vs229d, Acertys Healthcare, Aartselaar, Belgium) and cardiopulmonary measurements (Cardiosoft, Acertys Healthcare, Aartselaar, Belgium). The respiratory flow signal was recorded during the exercise to be able to identify the

respiratory cycle. ECG recordings were obtained via an impedance cardiography device (PhysioFlow, Manatec Biomedical, Folschviller, France) validated for COPD patients and recorded as a separate channel (31). Analog outputs of all variables (i.e., respiratory flow, 5 EMGdi and ECG) were collected in separate channels with a data acquisition unit (Micro1401-3, Cambridge Electronic Design Limited, Cambridge, UK). Data channels were synchronously collected by the same system and processed with the same acquisition software (Spike 2, Cambridge Electronic Design Limited, Cambridge, UK). The system was a priori checked for potential time delays between the different systems providing the signals of the different channels (e.g., ECG and EMGdi). No time delays were present and therefore, no additional post collection synchronization of data had to be performed.

4.3.7 Inspiratory muscle training (IMT)

Inspiratory muscle training was performed daily by four subjects using an electronic POWERbreathe®KH2 device (HaB International Ltd., Southam, UK) for eight weeks, according to a previously published protocol (32). In short, the patients trained at the highest tolerable intensity, 30 breaths per session and two sessions per day. Progression of training intensity and MIP measurements were performed weekly.

4.3.8 Statistical analyses

Comparisons of diaphragm activation at pre-measurement and pre/post IMT differences obtained from two assessors using the manual signal processing method and with the semi-automated signal processing method were made. Pearson's correlation coefficient (r) was used to establish associations between measurements. The intra-class correlation coefficient (ICC) based on a mean-rating, absolute-agreement, 2-way mixed-effects model was used to quantify agreement between two assessors (inter-rater reliability) and between the two methods. Agreement of the results from two assessors and between the two methods was assessed by plotting mean differences between assessors or methods against average values (Bland-Altman plots) (33). Limits of agreement were defined as ±1.96 × standard deviation of the difference between the two methods, corresponding to 95% confidence intervals (CI). The interaction over time between the two assessors and the two methods was assessed using repeated measures ANOVA. Within rater Coefficient of Variation (CV) for the two raters was calculated from 5 representative breaths during resting and at the end of exercise. For the semi-automated method, the CV was also calculated at the same time points. Statistical analyses were performed using GraphPad Prism version 8 for Windows (GraphPad Software, La Jolla, California, USA) and IBM SPSS Statistics 25.0 Desktop (IBM Corp., Armonk, NY, USA). Statistical significance was considered at $p<0.05$. Data are presented as means ± SD

4.4 Results

Datasets supporting the conclusions of this manuscript are available on request. Characteristics of included patients are presented in table 4.1. Patients exhibited moderate to severe airway obstruction with static hyperinflation, reduced exercise capacity, and inspiratory muscle strength. There are approximately four heartbeats during one inspiratory cycle both at rest and during exercise (table 4.1).

	All subjects (n=7)
General characteristic	
Male : Female	4:3
Age, years	66±5
BMI, kg/m^2	25±7
Pulmonary function	
FEV_1, L (%pred)	1.37±0.57 (56±31)
FEV_1/FVC,%	42±15
IC, L (%pred)	1.96±0.44 (76±27)
FRC, L (%pred)	5.14±1.85 (161±42)
RV, L (%pred)	3.64±1.48 (149±57)
TLC, L (%pred)	7.10±1.73 (122±19)
D_LCO, mmol/min/Kpa (%pred)	4.63±2.01 (59±25)
Respiratory muscle strength	
MIP at RV, cmH_2O (%pred)	-77±11(85±18)
Pdimax, cmH_2O	89±19
MEP at TLC, cmH_2O (%pred)	167±55(99±34)
Symptom-limited peak incremental cycling ergometer exercise test	
Cycling duration (minutes)	7.7±1.5
Peak Work rate, W (% pred)	82±27 (64±24)
VO_2, L/min (%pred)	1.40±0.60 (74±33)
HR, bpm (%pred HRmax)	118±16 (76±9)
Ventilation, L/min (%MVV)	44.6±7.6 (88±13)
Constant work rate cycling test (CWR cycling)	
Cycling work rate, W (%Wmax)	59±20 (72±3)
Cycling duration, min	8.0±3.7
Resting HR, bpm (%pred HRmax)	80±10 (52±16)
HR at end exercise, bpm (%pred HRmax)	124±17 (80±10)
Resting BF, bpm	22±8
BF at end exercise, bpm	32±8
HR:BF at rest, per min	3.9±1.1
HR:BF at end exercise, per min	4.1±1.2
Ventilation, L/min (%MVV)	38.3±8.4 (80±11)
Resting Ti, s	1.1±0.5
Ti at end exercise, s	0.8±0.2
Resting Te, s	2.1±0.7
%change from rest Ti	-25±22
%change from rest Te	-36±23

Table 4.1 Patient characteristics: pulmonary function, respiratory muscle strength, maximal and endurance exercise capacity. Abbreviations: "FEV1, forced expiratory volume in one second; FVC, forced vital capacity; IC, inspiratory capacity; FRC, functional residual capacity; RV, residual volume; TLC, total lung capacity; DLCO, diffusing capacity of the lung for carbon monoxide; MIP at RV, maximal inspiratory mouth pressure at residual volume; PdiMax, maximal transdiaphragmatic pressure during sniff maneuver; MEP at TLC, maximal expiratory mouth pressure at total lung capacity; VO2, oxygen consumption; HR, heart rate; MVV, maximal voluntary ventilation; BF, breathing frequency; Ti, inspiratory time; Te, expiratory time; %pred, %predicted."

Comparisons of EMGdi/EMGdiMax% obtained by either the two assessors or as processed with the semi-automated method from data collected during a constant work rate cycling task

The intra-class correlation coefficients (ICC) between diaphragm activation signals obtained with the manual methods by two assessors at pre-measurement was 0.94, p<0.0001, 95% CI 0.17 to 0.98 (Figure 4.4A). The ICC between EMGdi signals from the semi-automated method and the results obtained by using the manual method from assessor 1 and 2 at pre-measurement were 0.96, p<0.0001, 95% CI 0.94 to 0.97(Figure 4.4B) and 0.91, p<0.0001, 95% CI 0.60 to 0.97(Figure 4.4C), respectively).

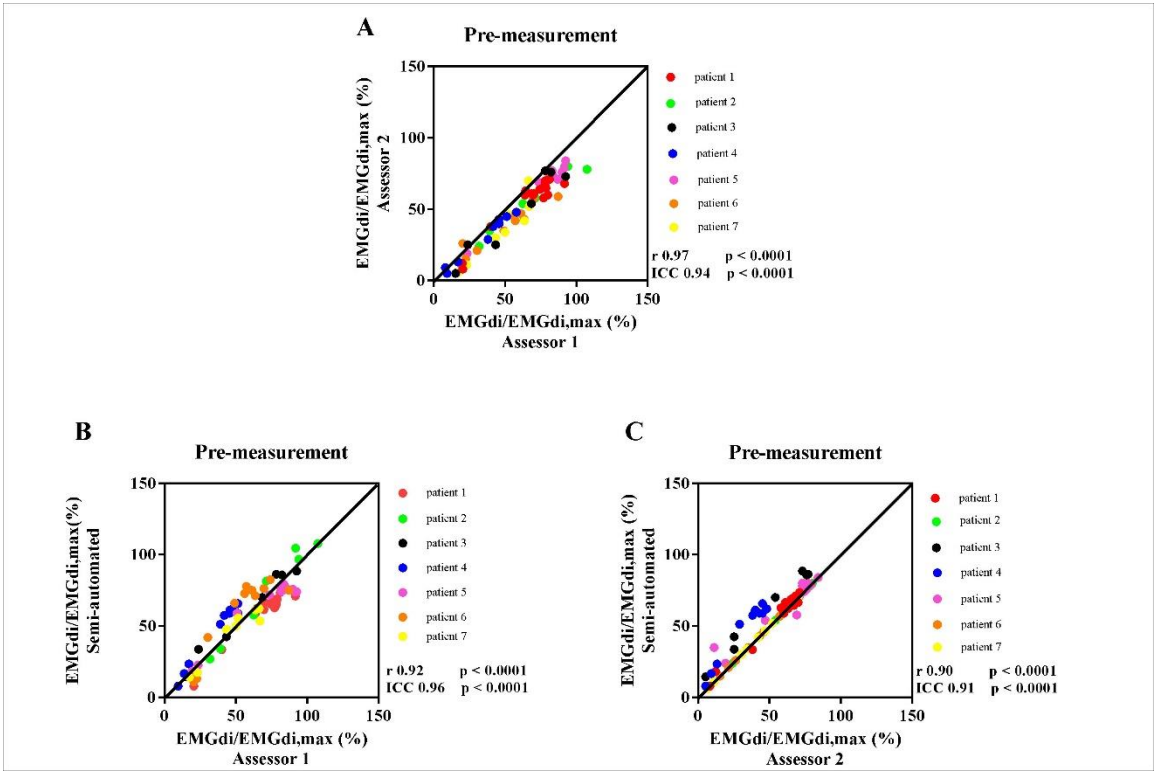

Figure 4.4 The correlation between EMGdi/EMGdiMax% calculated from the manual method by two assessors during the pre-measurement CWR cycling test (A); the semi-automated method and the manual method by assessor 1 (B); and the semi-automated method and the manual method by assessor 2 (C). Line of identity, linear regression coefficients, intra-class correlation coefficients (ICC) and significances are presented in each figure. Each of the data points represents the activation of diaphragm EMG (EMGdi/EMGdiMax%) of each patient in every minute during the pre-measurement CWR cycling test.

Bland - Altman plots for the agreement of EMGdi/EMGdiMax% for the above-mentioned comparisons are presented in Figures 4.5A, 5B, and 5C. On average, the EMGdi/EMGdimax% obtained from the manual method by assessor 2 resulted in lower values than those obtained from assessor 1 (average bias of the differences: -9.9%; CI: -22.9% to 3.0%, Figure 4.5A). The plot of agreement between EMGdi/EMGdimax% values from the semi-automated method and results of the manual method obtained by assessor 1 showed that on average the values from the semi-automated method were very similar with the values obtained from the manual method by assessor 1 (average bias of the differences: -0.5%; CI: -19.6% to 18.6%, Figure 4.5B). The plot of agreement between the values from

the semi-automated method and those obtained by the manual method from assessor 2 showed higher values from the semi-automated method (average bias of the differences: 9.3%; CI: -11.4% to 29.9% Figure 4.5C).

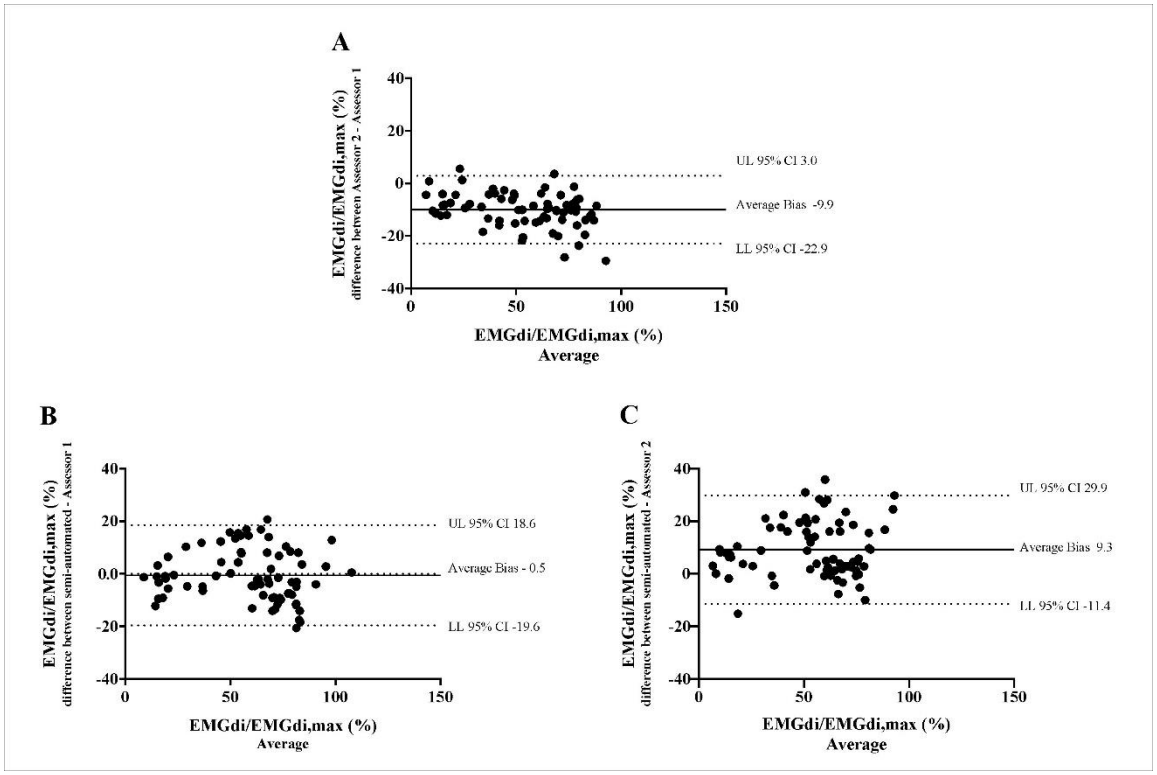

Figure 4.5 The results from the manual method by two assessors and the semi-automated method are compared in seven patients in each minute during the pre-measurement CWR cycling test. Bland-Altman plots of EMGdi/EMGdiMax% calculated from the manual method by two assessors (A); the semi-automated method and the manual method by assessor 1 (B); the semi-automated method and the manual method by assessor 2 (C).

Average EMGdi/EMGdiMax% obtained from the manual method by two assessors and values obtained with the semi-automated method were plotted against time for each minute during CWR cycling are presented in Figures 4.6A, 6B, and 6C. There were no significant method by time interactions observed neither between the values from two assessors (Figure 4.6A; P=0.24), nor between values from the semi-automated method and assessor 1 (Figure 4.6B; P=0.30), or the semi-automated method and assessor 2 (Figure 4.6C; p=0.11).

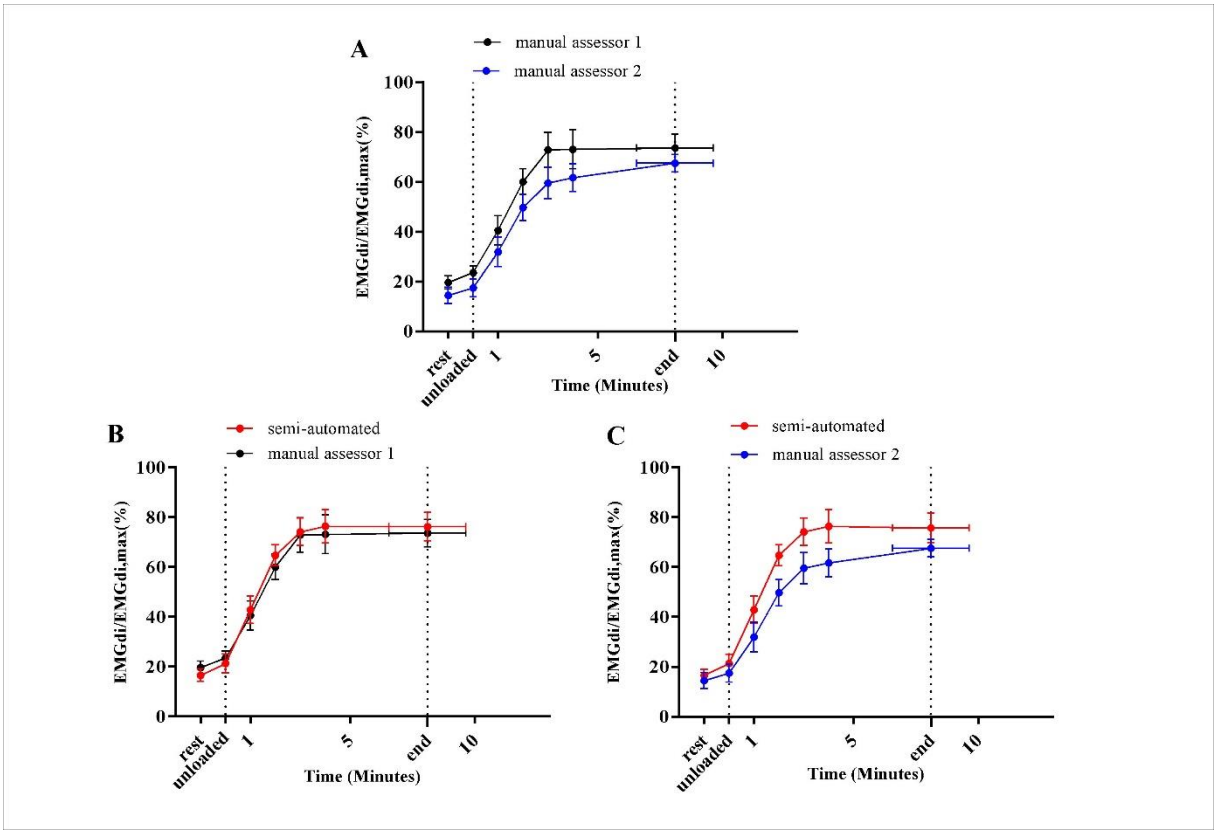

Figure 4.6 Average diaphragm activation (EMGdi/EMGdiMax%) of seven patients during CWR cycling test at pre-measurement calculated from the manual method by two assessors (A); the semi-automated method and the manual method by assessor 1 (B); the semi-automated method and the manual method by assessor 2 (C).

Average absolute maximal activation values (obtained during IC maneuvers) obtained by assessor 1 and 2 with the manual analysis method of were 0.146±0.062 volt and 0.150±0.060 volt, respectively. No significant differences were found between the maximal activation values obtained by assessor 1 and 2 (p=0.25).

The Coefficient of Variation (CV) of assessor 1 was 22% at rest and 13% at the end of exercise at pre-measurement. At post-measurement, the CV was 21% at rest and 26% at end exercise. For assessor 2 the CV was 54% at rest and 11% at the end of exercise. At post-measurement, the CV was 28% and 20% at rest and end exercise respectively. The CV calculated from the semi-automated were 13% and 11% at rest and end exercise respectively at pre-measurement. At post-measurement, the CV was 10% and 12% at rest and end exercise respectively.

Comparisons of the pre/post intervention differences in EMGdi/EMGdiMax% obtained by either the two assessors or as processed with the semi-automated method from data collected during a constant work rate cycling task

After eight weeks of IMT, inspiratory muscle function was improved in four patients that had completed the IMT protocol (two men and two women, age 64±4 years, BMI 25±7 kg/m^2, FEV_1

1.56±0.69 L (63±41 %predicted)) who participated in the IMT intervention. Maximal inspiratory pressure (MIP) improved from -77±15 cmH_2O (84±16 %predicted) to -91±25 cmH_2O (100±30 %predicted). Maximal transdiaphragmatic pressure (Pdi) measured during maximal inspiratory sniff maneuvers improved from 93±21cmH_2O to 105±24 cmH_2O.The average cycling duration was 8.4±2.5 minutes at pre-measurement and 16.4±7.8 minutes at post measurement. The pre/post IMT differences in EMGdi/EMGdiMax% during cycling before and after IMT, were calculated. The correlations between the pre/post IMT differences in EMGdi/EMGdiMax% calculated from the manual method by two assessors and the semi-automated method during the CWR cycling test before and after the intervention are presented in Figures 4.7A, 7B and, 7C.

The ICC between the values of pre/post differences from assessor 1 and assessor 2 was 0.40, p=0.02, 95% CI -0.09 to 0.68 (Figure 4.7A). The ICC between the pre/post IMT differences from the semi-automated method and assessor 1 was 0.78, p<0.0001, 95% CI 0.58 to 0.89 (Figure 4.7B), while the ICC between the pre/post IMT differences from the semi-automated method assessor 2 was 0.04, p=0.44, 95% CI -0.58 to 0.46 (Figure 4.7C).

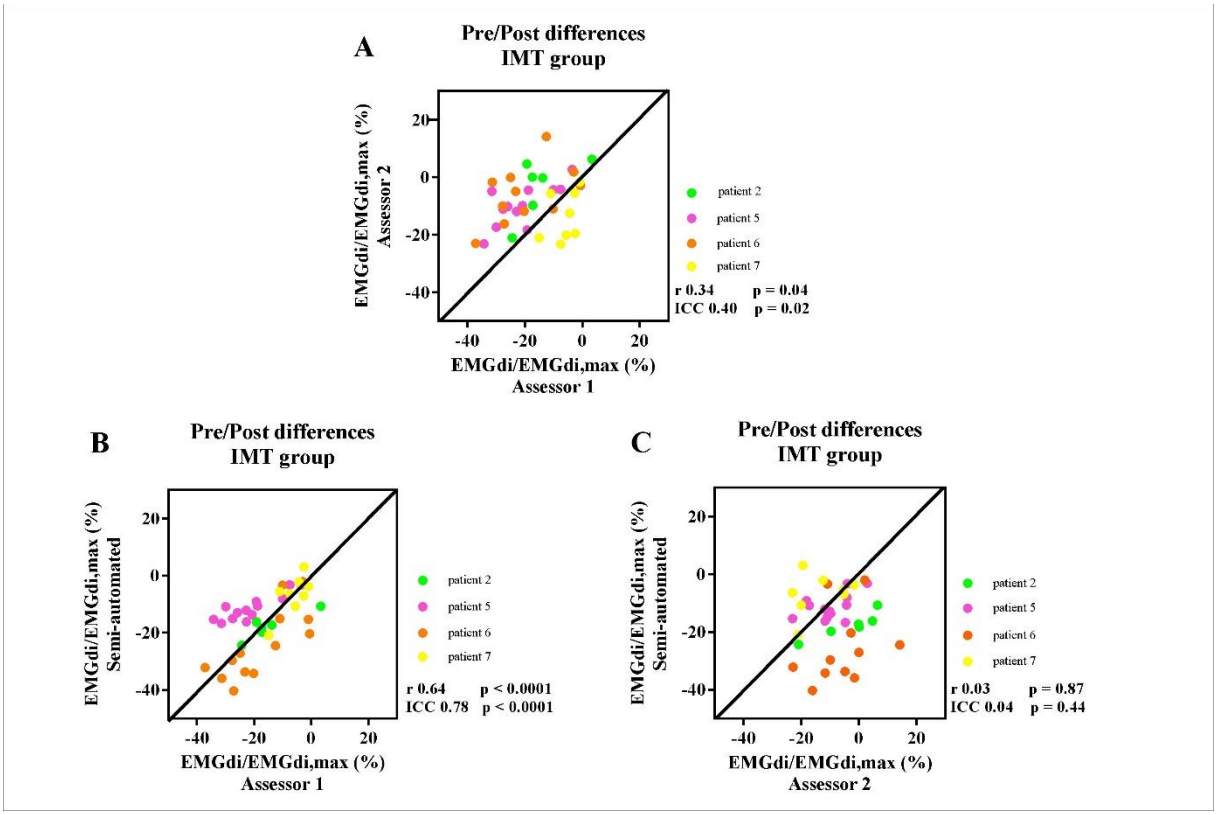

Figure 4.7 The correlation between the pre/post difference in four participants EMGdi/EMGdiMax% calculated from the manual method by two assessors (A); the semi-automated method and the manual method by assessor 1 (B); the semi-automated method and the manual method by assessor 2 (C). Line of identity, linear regression coefficients and intra-class correlation coefficients (ICC) and significances are presented in each figure. Each of the data points represents the pre/post differences of diaphragm EMG activation (EMGdi/EMGdiMax%) pre and post the intervention of each patient in every minute during CWR cycling test.

Bland - Altman plots for the agreements of pre/post IMT differences in EMGdi/EMGdiMax% calculated from two analyzing methods are presented in Figure 4.8A, 8B, and 8C. On average, the pre/post IMT differences in EMGdi/EMGdiMax% obtained from the manual method from assessor 2 was lower than assessor 1 (average bias of differences: -8.2%; CI:-30.9% to 14.5%, Figure 4.8A). The pre/post IMT differences in EMGdi/EMGdiMax% obtained from the semi-automated method was on average similar to values obtained with the manual method by assessor 1 (average bias of differences: 1.2%;-16.8% to 19.2%, Figure 4.8B). The pre/post differences values from the semi-automated method are higher than the values from the manual method by assessor 2 (average bias of differences: 7.0%; CI: -20.4% to 34.4%, Figure 4.8C).

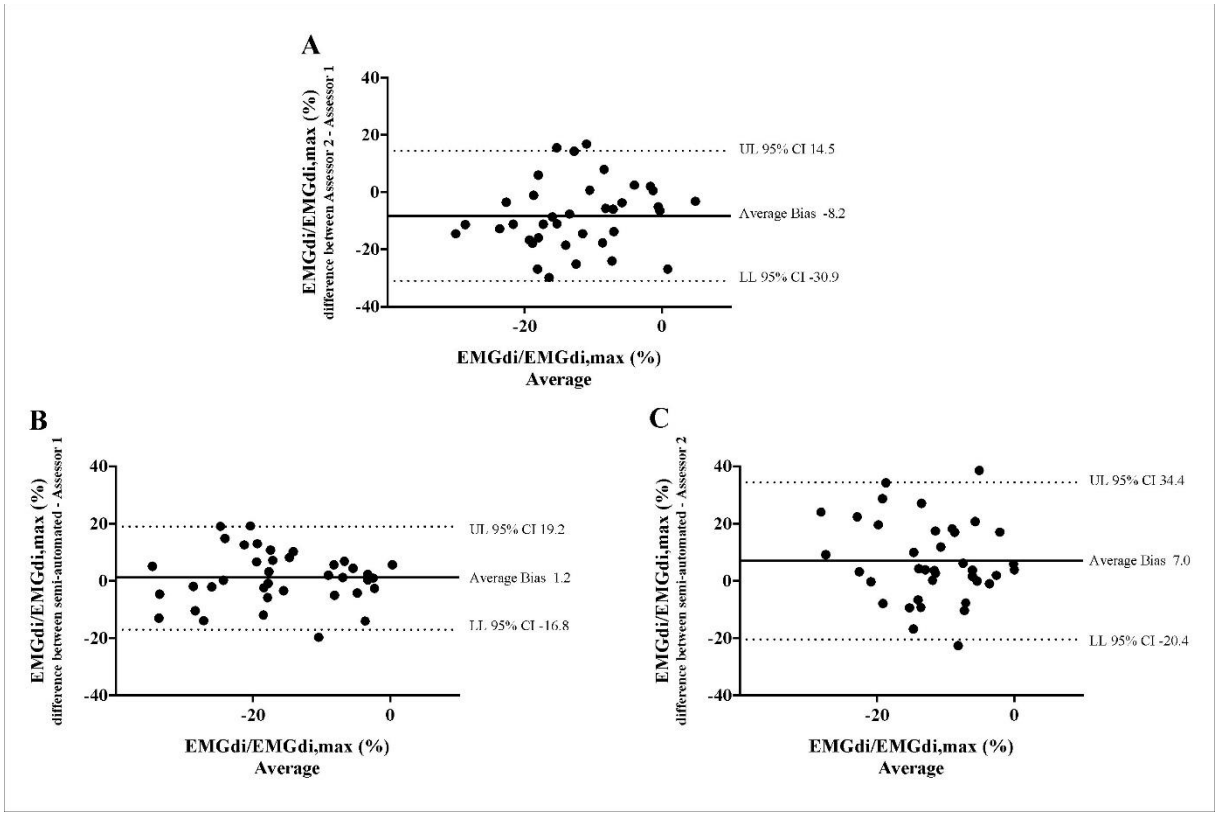

Figure 4.8 The results from the manual method by two assessors and the semi-automated method are compared in four patients in each minute during CWR cycling test pre and post the intervention. Bland-Altman plots of the pre/post differences in EMGdi/EMGdiMax% calculated from the manual method by two assessors (A); the semi-automated method and the manual method by assessor 1 (B); the semi-automated method and the manual method by assessor 2 (C).

Average EMGdi/EMGdiMax% values obtained from the manual method by the two assessors and the semi-automated method were plotted against time for each minute during CWR cycling performed pre and post the IMT intervention period (Figures 4.9A, 9B and 9C). There were no significant method by time interactions observed between the values from two assessors (figure 4.9A; P=0.29), the semi-automated method and assessor 1 (Figure 4.9B p=0.55) and the semi-automated method and assessor 2 (Figure 4.9C; P=0.50).

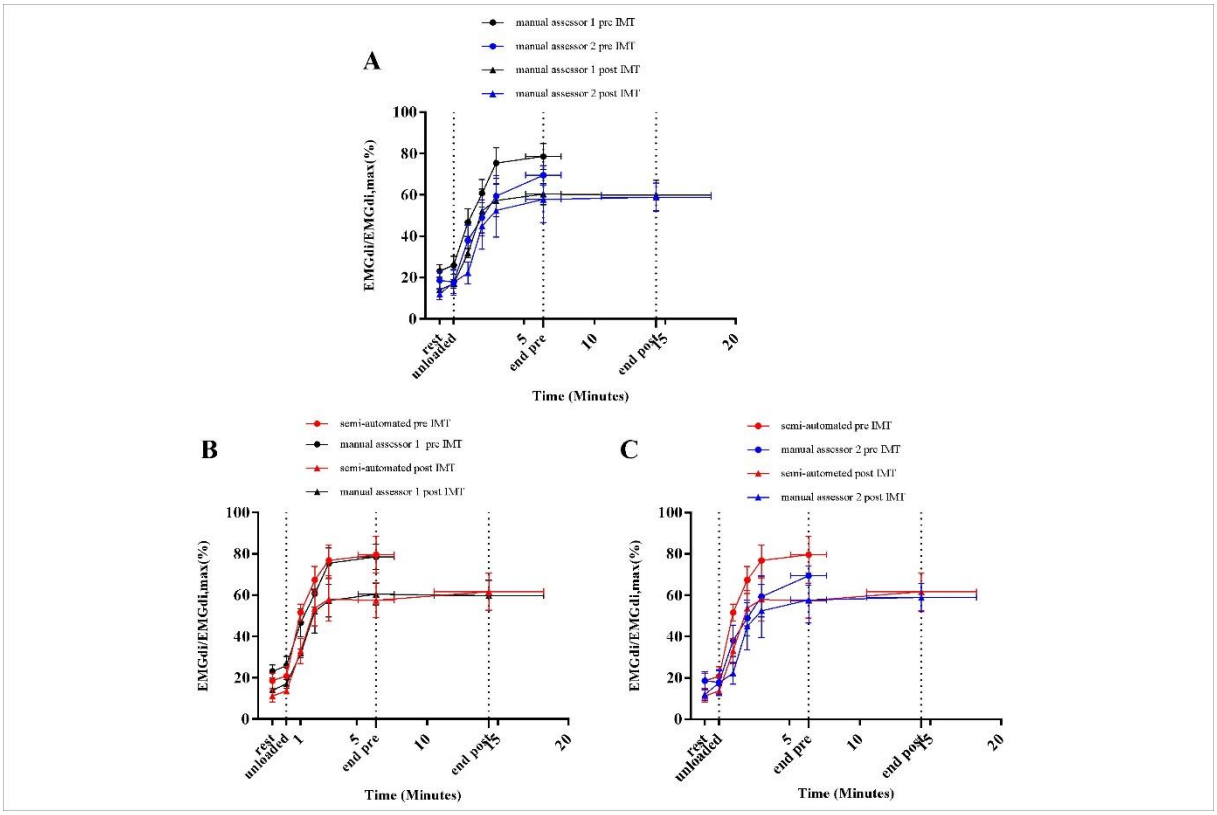

Figure 4.9 Average diaphragm activation (EMGdi/EMGdiMax%) of four patients during CWR cycling test pre and post the intervention calculated from the manual method by two assessors (A); the semi-automated method and the manual method by assessor 1 (B); the semi-automated method and the manual method by assessor 2 (C).

Average absolute maximal activation values (during IC maneuver) obtained with the manual analysis method by assessor 1 and 2 were 0.121±0.075 volt and 0.124±0.072 volt respectively at pre-measurement, and 0.158±0.101 volt and 0.142±0.92 volt respectively at post-measurement (p=0.62 and P=0.20 respectively).

Duration of analysis between manual and semi-automated method

The average duration of 11 CWR cycling tests (seven at pre-measurement and four at post measurement), including resting and unloaded cycling, was 13±6 minutes (range 4.2 to 22.0 minutes). The analyzing time using the manual method was 82±20 minutes (range 63 to 115 minutes) for assessor 1 and for the semi-automated method was 29±9 minutes (range 18 to 49 minutes). Difference between methods 53±15 minutes (p <0.0001)

4.5 Discussion

Main findings

We validated a custom developed ECG removal method for EMG amplitude analysis against a commonly used manual approach. The main findings of this study are that the newly developed semi-automated EMGdi analysis method is more time efficient and that it will be less prone to the inter-rater variability that was observed when the manual method was applied by two independent assessors. EMGdi amplitudes obtained with the semi-automated method agreed well with values obtained by one of the two manual assessors. The findings suggest that EMGdi analysis using the proposed semi-automated method can be used to evaluate changes in EMG amplitudes over a wide range of minute ventilations recorded at rest and during exercise in patients with COPD.

Inter-rater reliability of the manual method

Resting diaphragm activation (EMGdi/EMGdiMax%) values obtained by both assessors using the manual method ranged from 10% to 20% (Figure 4.6 A, B, and 4.9 A, B). During the CWR cycling exercise, this activation increased steeply at the beginning of the exercise and reached a plateau until the exercise was terminated by patients' symptom limitation (Figure 4.6 A, B, and 4.9 A, B). Similar patterns were observed in previous studies (1, 2, 12). The observed differences of 8% to 9% in EMGdi amplitudes between raters are however substantial and might impact on the ability to detect differences in EMG amplitudes after interventions (Figures 4.5A and 4.8A). Along these lines the pre/post IMT differences manually obtained by assessor 1 resulted in a reduction of approximately 20% of EMGdi/EMGdiMax at iso-time (Figure 4.9A, B), whereas analyses performed by assessor 2 resulted in a much smaller reduction of approximately only 10% (Figure 4.9A and 4.9C). In an attempt to explain these differences we looked into the manual analyses as performed by the two assessors in more detail.

Since EMGdimax (volt) values obtained by the two assessors were similar, the differences in the EMGdi/EMGdiMax ratio between the two assessors must have originated from the selection of the EMGdi signal between QRS complexes of the tidal breaths. Retrospectively, we observed that in most cases there were several intervals between QRS complexes that assessors could select for their analyses (Figure 4.2). Upon closer inspection we further realized that assessor 1 systematically tended to choose the period that resulted in the "highest" EMGdi value of each inspiratory cycle (frequently occurring towards the very end of an inspiratory cycle), while assessor 2 always chose intervals that contained the "widest" available signal typically located more 'centrally' within each inspiratory phase. This is illustrated in Figure 4.2. While assessor 1 systematically selected the period between cursor 3

to 4, assessor 2 tended to choose the period between cursor 1 to 2. We noticed that especially during pre-IMT assessments the amplitude of EMGdi was higher towards the end of each inspiratory cycle, indicating more pronounced diaphragm activation towards the end of the inspiratory cycle (Figure 4.11A). Since the given illustrative example occurred frequently during the tests the intervals selected by assessor 1 often resulted in higher values than the intervals chosen by assessor 2 (Figure 4.5A and 4.8A).

As stated earlier the values of EMGdimax were not significantly different between two assessors. This initially seems surprising given the different approaches taken by the two assessors as described above. It can be explained however based on the shorter inspiratory period (Ti) during the IC maneuvers (during which the EMGdimax signals were obtained) in comparison to the tidal breaths (during which the EMGdi intervals were selected). As illustrated in Figure 4.10 during the short inspiratory periods of the IC maneuvers there was frequently only a single EMG interval between QRS complexes available to select. This can most likely explain the smaller differences in EMGdimax values between assessors in comparison to EMGdi.

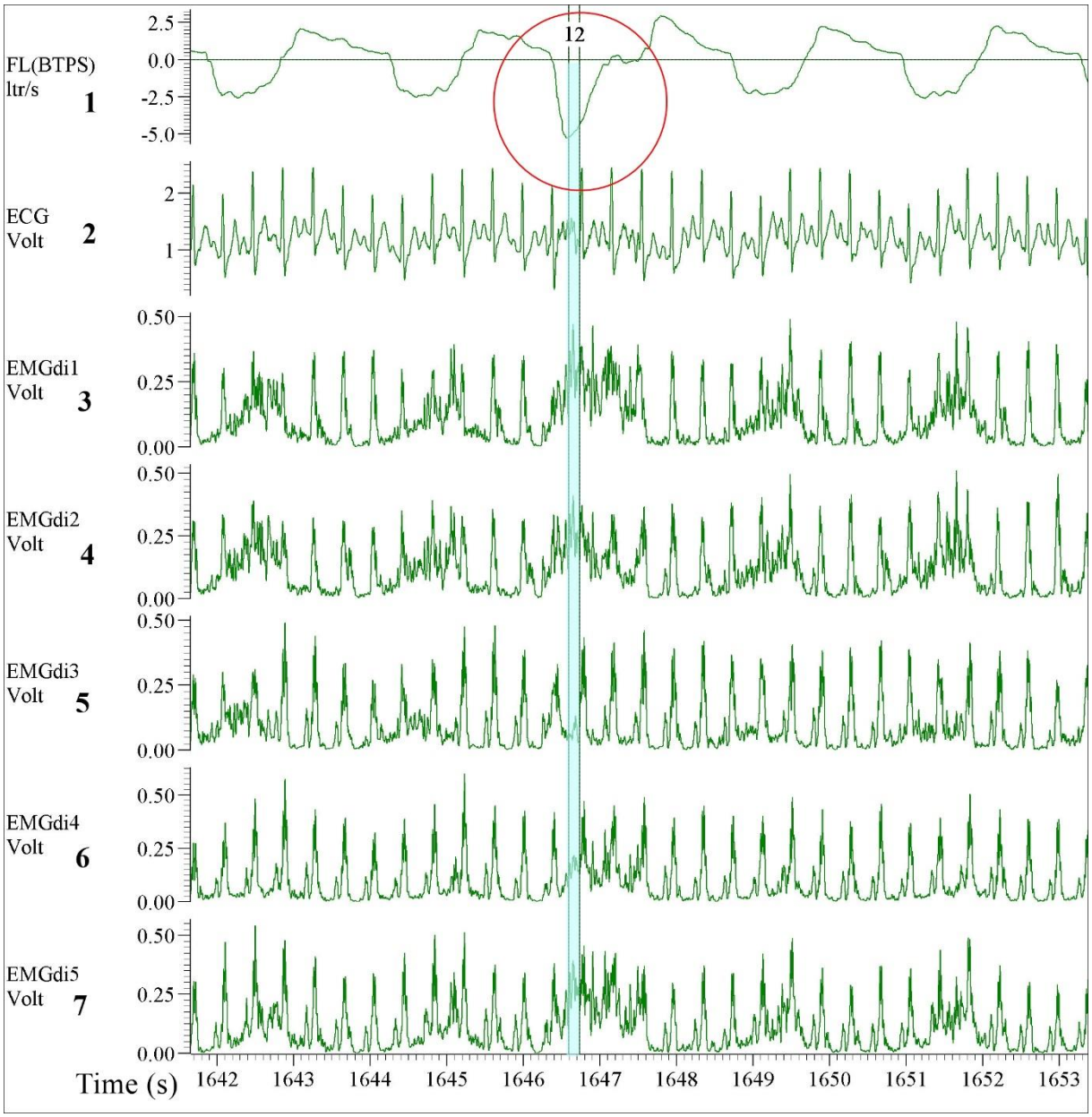

Figure 4.10 Example of EMGdi recordings during a cycling exercise towards the end of the (symptom limited) cycling test for the analysis using the manual method. Channel 1: respiratory flow (l/sec; negative flow indicating the inspiratory cycle), Channel 2: ECG recording (volt), Channels 3-7: Diaphragm EMG (RMS EMGdi) recordings. The IC maneuver is highlighted in the red circle indicated by the higher flow, which accompanies the maximal activation of the EMGdi. The periods highlighted in light blue are the periods that could be chosen without ECG contamination in an inspiratory cycle during IC maneuver. With the short inspiratory time during the IC maneuver, it left only one available (light blue) period that EMGdi could be retrieved.

Interestingly there was also less disagreement between the results from assessor 1 and 2 during the post-intervention analyses in comparison to pre-intervention comparisons (Figure 4.9A). Despite both assessors treating the EMGdi data with the same approach as for the pre-intervention measurements (i.e., one rater looking for the highest while the other looked for the widest available signal interval), the values from assessor 2 were closer to the values from assessor 1. This finding can probably be explained by previously reported pre/post differences in EMG amplitude signal patterns over time,

during muscular activation, in response to muscle training (34). Known effects of muscle training, including inspiratory muscle training, are improvements in force output and motor learning, thereby decreasing muscle activation levels at iso-loads (35, 36). In fact, higher EMGdimax and decreased relative activation of the diaphragm (i.e., lower muscle activity) after training at iso-loads were previously reported by our group (12).

As shown in figure 4.11A and as mentioned earlier, during pre-IMT assessments, the EMGdi signal from the diaphragm increased from the beginning towards the end of the inspiration. The EMGdi values (volt) between cursors 1 to 2 were always lower than those between cursors 3 to 4 (Figure 4.11A). The numbers marked in red are the values taken as a representative mean EMGdi of that breath (Figure 4.11A, B). After inspiratory muscle training, however, EMGdi values earlier during inspiration were less different from those towards the end of the inspiratory phase (Figure 4.11B). It therefore seems like patterns that had previously been observed after resistance training of peripheral muscles (reduced EMG/time slopes after training) (34), were also detected in our diaphragm EMG data. These findings can probably help to explain why inter-rater differences, despite of using similar approaches, were less pronounced after the resistance training period.

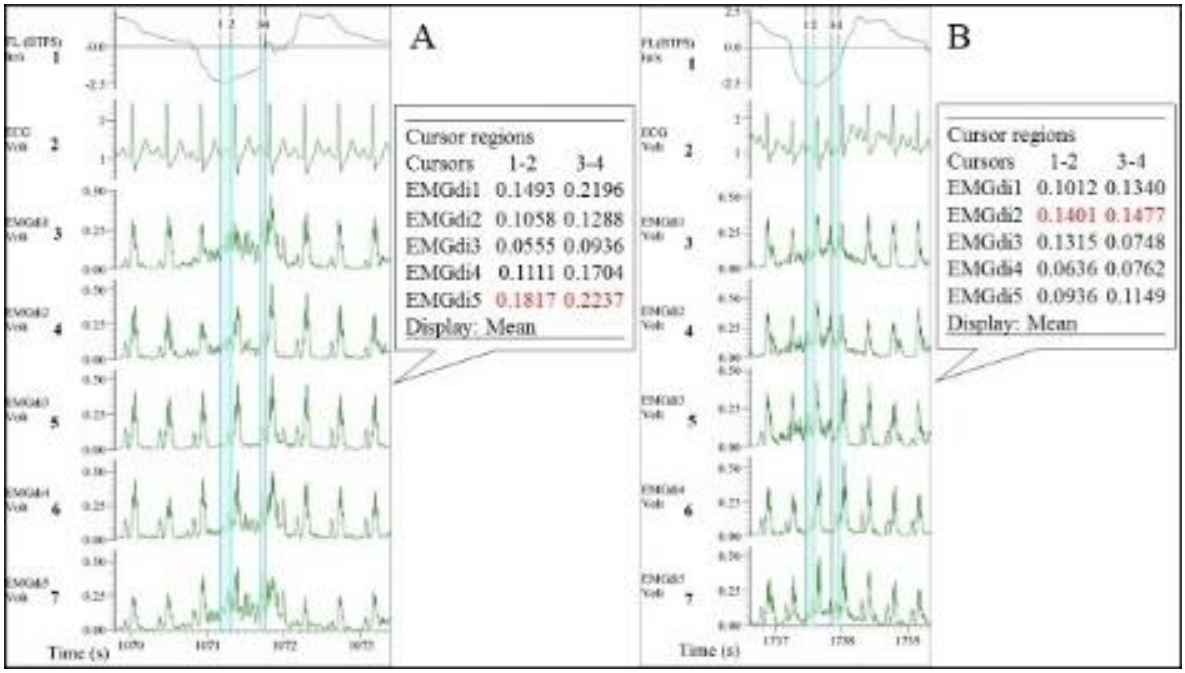

Figure 4.11 Example of EMGdi recordings during cycling exercise towards the end of the (symptom limited) cycling test at the pre- (A) and post- (B) measurement for the analysis using the manual method. Channel 1: respiratory flow (l/sec; negative flow indicating the inspiratory cycle), Channel 2: ECG recording (volt), Channels 3-7: Diaphragm EMG (RMS EMGdi) recordings. The periods highlighted in light blue are the possible periods that could be chosen without ECG contamination in an inspiratory cycle. Numbers in cursors regions boxes show the mean EMGdi values between two vertical cursors. The numbers in red indicate that the differences in the mean values between cursors 1 to 2 are closer to the values between cursor 3 to 4 at post-measurement.

Comparisons between manual and semi-automated EMGdi analyses

Data from the manual analyses of assessor 1 (who searched for the intervals containing the EMGdi signal with highest amplitude for every breath) resulted in good agreement with the results from the semi-automated method. We assume that the semi-automatically processed data are most representative of the "real" EMGdi values since the ECG contamination was eliminated from the signal. Based on these findings the analysis strategy of assessor 1 should probably be favored (i.e. selecting the EMGdi interval in between QRS complexes that provides the "highest" amplitude) should probably be favored above the approach taken by assessor 2. This is further supported by the fact that magnitude of pre-post intervention differences of both assessor 1 and from the semi-automated method are in line with findings from a previous study that assessed diaphragm activation during the CWR cycling test before and after a similar IMT intervention (12).Therefore, if the manual method should be used, we would recommend to manually select EMG parts between QRS complexes that result in the "highest" average EMGdi (i.e. selecting intervals towards the end of each inspiratory period). This strategy of manual analysis showed a good agreement with the semi-automated method on a group level, suggesting that both methods can be used interchangeably. The discussion onward will focus on the comparisons between the results of the semi-automated method and values obtained with the manual method from assessor 1.

Bland - Altman plots of both pre-intervention measurements and pre/post IMT differences in EMGdi/EMGdiMax% showed good overall agreement. Considering the differences, which scattered randomly above and below zero, it did not appear as if there were systematic over- or underestimations present, or that differences between methods became larger when activation was higher (i.e., at higher minute ventilation during cycling) (Figure 4.5B and 4.8B).

Two factors probably contribute to the relatively wide (+/- 20%) limits of agreement that were observed between methods. Firstly, during manual analyses QRS complexes occurring during the inspiratory cycles can cover major parts of the inspiratory EMG signal. These parts (which might contain the highest activation portion during a given inspiration) are consequently not available for analysis (Figure 4.12). This limited availability of EMGdi signal probably contributed to either over- or underestimation of the manual signal in contrast to the semi-automatically processed signal which could always take the full inspiratory period (i.e. from zero flow to zero flow) into consideration.

A second factor that probably contributes to the width of the limits of agreement between methods is the fact that the average EMGdi from the manual method is obtained from a representative sample of 5 consecutive breaths towards the end of each exercise minute. In contrast during the semi-automated method, all breaths performed during each minute are analyzed (2). It might very well be that the

sample of 5 breaths is not always a perfect representation of average EMGdi during a given minute of breathing resulting in between method differences on a minute-by-minute basis. The overall agreement of the EMGdi/EMGdiMax between two methods on a group level was however good, and no significant method by time interaction effects were observed (Figure 4.6B and 4 9B). This suggests that both methods can be used interchangeably on a group level.

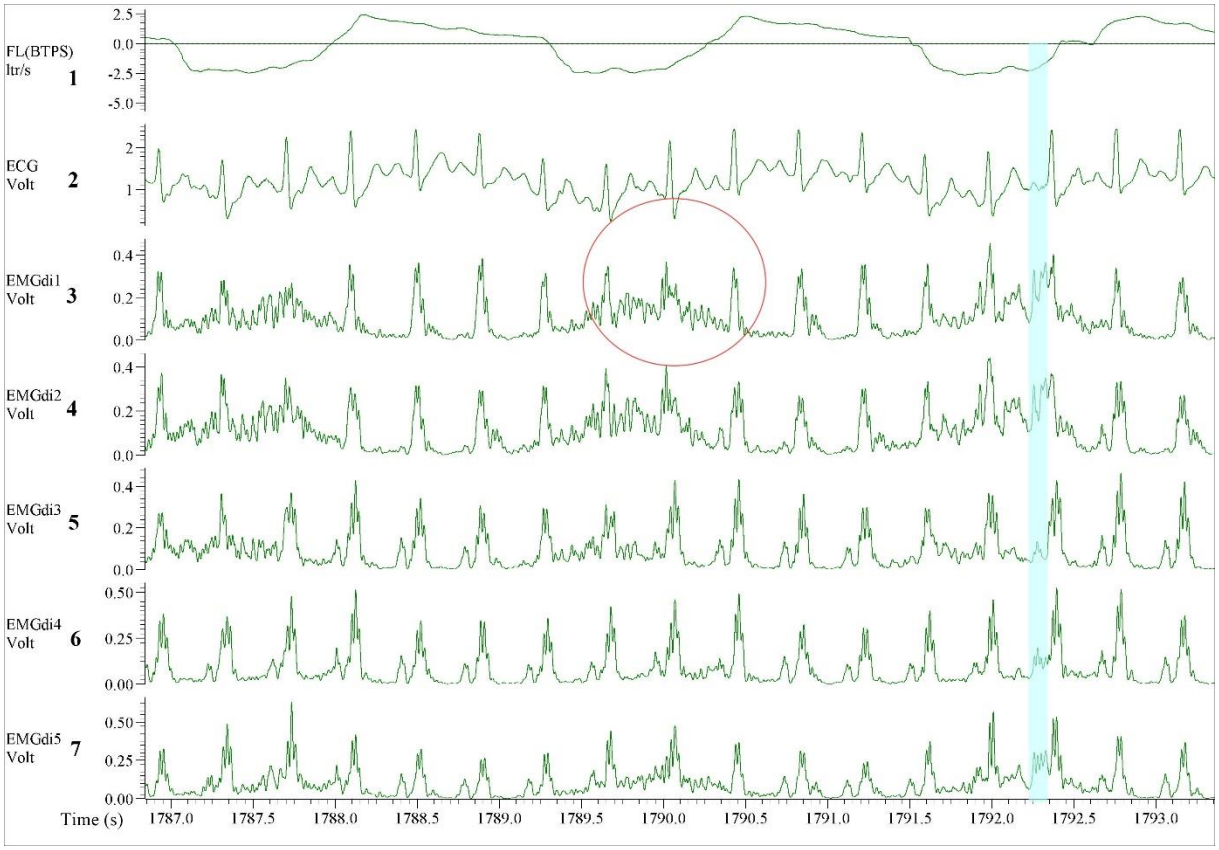

Figure 4.12 Illustration of the EMGdi recordings during cycling exercise towards the end of the (symptom limited) cycling test when the inspiratory time (Ti) is shorter. Channel 1: respiratory flow (l/sec; negative flow indicating the inspiratory cycle), Channel 2: ECG recording (volt), Channel 3-7: Diaphragm EMG (RMS EMGdi) recordings. Several QRS complexes appear during the inspiratory cycle which is already a short period. The QRS complexes take up a large part during the inspiratory cycle resulting in less "clean" EMGdi to be selected. The red circle shows one inspiratory cycle when the QRS complex appears precisely at the peak of the amplitude of the EMGdi. The EMGdi buried under this QRS complex cannot be retrieved. Therefore, the assessor must choose the part outside of the QRS complex, which results in lower mean EMGdi value, thus underestimates the diaphragm activation. The blue highlight shows a small period of peak EMGdi. The mean EMGdi value of this period will be higher (average of high values in the short time) therefore the EMGdi being overestimated.

Degree of variation

The coefficients of variation (CV) were calculated from the manual analyses performed by two raters were mostly higher than the CV calculated from the semi-automated method. This lower degree of variability when using the semi-automated method will probably increase the ability to detect true differences between measurement conditions. The reduction in variability is most likely due to both

the absence of noise within breaths as well as the fact that instead of a representative sample of 5 breaths all breaths of each minute were used for analyses.

Clinical implications

The EMGdi/EMGdiMax ratio is currently being used as a surrogate of neural respiratory drive (NRD) and both magnitude as well as changes in NRD have been shown to be closely related to (changes in) dyspnea sensation (12, 37) which is an important symptom in patients with chronic obstructive and restrictive lung diseases (38). It is essential to obtain correct values of diaphragm activation to be able to interpret the results linked with the patient's symptoms and also to reliably detect changes induced by different interventions. A reliable and objective method to process these data is beneficial for breathlessness management in patients with COPD both in research and clinical routine.

Strengths and limitations

The semi-automated method was designed to overcome several shortcomings of the manual method. By automatically removing ECG artifacts throughout the recording, the analysis time is shortened by more than half. After the ECG artifact was removed the resulting 'clean' EMGdi signal could be integrated over the full inspiratory cycle. This integration of EMG activity over the course of a contraction is a common practice for other skeletal muscles but was not possible with the manual EMGdi analysis methods available so far. In addition it facilitates the performance of breath-by-breath analyses which allows all data points to be considered. This is the first time that the inter-rater reliability of the often used "manual method" has been evaluated. We were for the first time able to identify several sources for inter-rater variability which should be eliminated by the more objective, semi-automated processing of full inspirations that have previously been cleaned of ECG artifacts by our newly developed method.

Limitations of our study are the relatively small sample size and the absence of an age-matched control group. The results would need to be confirmed in a larger sample of subjects performing different types of exercise tests resulting in a large variability of heart rate and ventilation responses. In addition, inclusion of a healthy age-matched control group and comparisons of findings with COPD patients would have allowed further investigations into the validity of the semi-automated method at rest and during exercise. Since our methods were only compared in a specific group of patients future studies might be required to further validate the use of the semi-automated EMGdi analysis in other populations. In our study EMGdi signals were evaluated only at the extremes of activation (i.e. resting breathing and close to maximal activation). The model/approach has not been tested over a range of intensities (and as such diaphragm activity). It would have been preferable to evaluate the responses

over a broader range of minute ventilations and heart rate (e.g. during a stepwise maximal incremental exercise test). Further study is also needed with regard to responsiveness of the signals and reproducibility of findings both before and after different pharmacological and non-pharmacological interventions that are supposed to reduce respiratory effort and symptoms of breathlessness.

4.6 Conclusion

The semi-automated ECG artifact removal method for EMGdi analyses will be helpful to eliminate sources of inter-rater variability that were observed between different raters applying the manual method. Therefore the semi-automated method offers a more objective approach for analyzing EMGdi data while at the same time requiring significantly less analyzing time. We propose this method as a new standard for objective EMGdi amplitude analyses in the future.

4.7 Data availability

The datasets generated for this study are available on request to the corresponding author.

4.8 Ethics statement

The Ethical Committee Research of KU Leuven/UZ Leuven, Belgium approved the study (S58513).

4.9 Author contributions

SD, RG, and DL contributed to the conception and design of the study. SD, ZL, and LoJ contributed to the acquisition. LuJ developed the analysis algorithm. SD and AR performed the data analysis. SD organized the database, performed the statistical analysis, and wrote the first draft of the manuscript. LuJ, AR, RG, and DL wrote sections of the manuscript. All authors contributed to the manuscript revision, and read and approved the submitted version.

4.10 Funding

This research project was supported by the Research Foundation – Flanders (FWO): G0A4516N – ZKC9570 – C22/15/035. ZL is a recipient of an ERS fellowship (LTRF 2016-6686) and a postdoctoral research fellow of the FWO Flanders (12U5618N). AR is supported by the Coordination for the Improvement of Higher Education Personnel (CAPES), Brazil (88881.188754/2018-01). SD is supported by Chiang Mai University's scholarship, Chiang Mai, Thailand (Ref. No. 6392(7)/0807).

4.11 Acknowledgments

We wish to thank Prof. Dr. Wim Janssens and Prof. Dr. Thierry Troosters for their support and constructive criticism. We also thank our colleagues in the Respiratory Rehabilitation and Respiratory Division, University Hospital Leuven, Belgium for their invaluable support. Aspects of this manuscript have been presented at the European Respiratory Society (ERS) International Congress 2018, Paris, France, September 16–19, 2018 and also published as a congress abstract in the supplement material of the European Respiratory Journal 2018 52: Suppl. 62, PA1714. doi: 10.1183/13993003.congress 2018.PA1714; https://erj.ersjournals.com/content/52/suppl_62/PA1714.

References

1. Luo YM, Li RF, Jolley C, Wu HD, Steier J, Moxham J, et al. Neural Respiratory Drive in Patients with COPD during Exercise Tests. Respiration. 2011;81(4):294-301.
2. Luo YM, Moxham J. Measurement of neural respiratory drive in patients with COPD. Respiratory physiology & neurobiology. 2005;146(2-3):165-74.
3. Steier J, Cade N, Walker B, Moxham J, Jolley C. Observational Study of Neural Respiratory Drive During Sleep at High Altitude. High Alt Med Biol. 2017;18(3):242-8.
4. Xiao SC, He BT, Steier J, Moxham J, Polkey MI, Luo YM. Neural Respiratory Drive and Arousal in Patients with Obstructive Sleep Apnea Hypopnea. Sleep. 2015;38(6):941-9.
5. Duiverman ML, van Eykern LA, Vennik PW, Koeter GH, Maarsingh EJ, Wijkstra PJ. Reproducibility and responsiveness of a noninvasive EMG technique of the respiratory muscles in COPD patients and in healthy subjects. Journal of applied physiology. 2004;96(5):1723-9.
6. Sinderby C, Beck J, Spahija J, Weinberg J, Grassino A. Voluntary activation of the human diaphragm in health and disease. J Appl Physiol. 1998;85(6):2146-58.
7. Luo YM, Moxham J, Polkey MI. Diaphragm electromyography using an oesophageal catheter: current concepts. Clin Sci (Lond). 2008;115(8):233-44.
8. Schweitzer TW, Fitzgerald JW, Bowden JA, Lynne-Davies P. Spectral analysis of human inspiratory diaphragmatic electromyograms. Journal of applied physiology: respiratory, environmental and exercise physiology. 1979;46(1):152-65.
9. Levine S, Gillen J, Weiser P, Gillen M, Kwatny E. Description and validation of an ECG removal procedure for EMGdi power spectrum analysis. Journal of applied physiology. 1986;60(3):1073-81.
10. Zhou P, Kuiken TA. Eliminating cardiac contamination from myoelectric control signals developed by targeted muscle reinnervation. Physiol Meas. 2006;27(12):1311-27.
11. Jolley CJ, Luo YM, Steier J, Reilly C, Seymour J, Lunt A, et al. Neural respiratory drive in healthy subjects and in COPD. The European respiratory journal. 2009;33(2):289-97.
12. Langer D, Ciavaglia C, Faisal A, Webb KA, Neder JA, Gosselink R, et al. Inspiratory muscle training reduces diaphragm activation and dyspnea during exercise in COPD. J Appl Physiol. 2018;125(2):381-92.
13. Reilly CC, Jolley CJ, Ward K, MacBean V, Moxham J, Rafferty GF. Neural respiratory drive measured during inspiratory threshold loading and acute hypercapnia in healthy individuals. Exp Physiol. 2013;98(7):1190-8.
14. Schaeffer MR, Mendonca CT, Levangie MC, Andersen RE, Taivassalo T, Jensen D. Physiological mechanisms of sex differences in exertional dyspnoea: role of neural respiratory motor drive. Exp Physiol. 2014;99(2):427-41.
15. Mak JN, Hu Y, Luk KD. An automated ECG-artifact removal method for trunk muscle surface EMG recordings. Med Eng Phys. 2010;32(8):840-8.
16. Willigenburg NW, Daffertshofer A, Kingma I, van Dieën JH. Removing ECG contamination from EMG recordings: A comparison of ICA-based and other filtering procedures. J Electromyogr Kinesiol. 2012;22(3):485-93.
17. Bloch R. Subtraction of electrocardiographic signal from respiratory electromyogram. Journal of applied physiology: respiratory, environmental and exercise physiology. 1983;55(2):619-23.
18. Husted JA, Cook RJ, Farewell VT, Gladman DD. Methods for assessing responsiveness: a critical review and recommendations. J Clin Epidemiol. 2000;53(5):459-68.
19. Dacha S, Janssens L, Louvaris Z, Janssens L, Gosselink R, Langer D. Comparison between manual and automated analyses of esophageal diaphragm electromyography during endurance cycling in patients with COPD. Eur Respir J. 2018;52(suppl 62):PA1714.
20. Wanger J, Clausen JL, Coates A, Pedersen OF, Brusasco V, Burgos F, et al. Standardisation of the measurement of lung volumes. The European respiratory journal. 2005;26(3):511-22.

21. Graham BL, Brusasco V, Burgos F, Cooper BG, Jensen R, Kendrick A, et al. 2017 ERS/ATS standards for single-breath carbon monoxide uptake in the lung. The European respiratory journal. 2017;49(1).
22. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J. 2005;26(2):319-38.
23. ATS/ERS Statement on respiratory muscle testing. American journal of respiratory and critical care medicine. 2002;166(4):518-624.
24. Luo YM, Hart N, Mustfa N, Lyall RA, Polkey MI, Moxham J. Effect of diaphragm fatigue on neural respiratory drive. Journal of applied physiology. 2001;90(5):1691-9.
25. Sinderby C, Beck J, Spahija J, Weinberg J, Grassino A. Voluntary activation of the human diaphragm in health and disease. Journal of applied physiology. 1998;85(6):2146-58.
26. Sinderby C, Friberg S, Comtois N, Grassino A. Chest wall muscle cross talk in canine costal diaphragm electromyogram. J Appl Physiol (1985). 1996;81(5):2312-27.
27. Reilly CC, Ward K, Jolley CJ, Lunt AC, Steier J, Elston C, et al. Neural respiratory drive, pulmonary mechanics and breathlessness in patients with cystic fibrosis. Thorax. 2011;66(3):240-6.
28. Reilly CC, Jolley CJ, Elston C, Moxham J, Rafferty GF. Measurement of parasternal intercostal EMG during an infective exacerbation in patients with Cystic Fibrosis. Eur Respir J. 2012:erj01631-2011.
29. Langer D, Ciavaglia C, Faisal A, Webb KA, Neder JA, Gosselink R, et al. Inspiratory muscle training reduces diaphragm activation and dyspnea during exercise in COPD. Journal of applied physiology. 2018;125(2):381-92.
30. ATS/ACCP Statement on Cardiopulmonary Exercise Testing. Am J Respir Crit Care Med. 2003;167(2):211-77.
31. Louvaris Z, Spetsioti S, Andrianopoulos V, Chynkiamis N, Habazettl H, Wagner H, et al. Cardiac output measurement during exercise in COPD: A comparison of dye dilution and impedance cardiography. Clin Respir J. 2019.
32. Langer D, Charususin N, Jacome C, Hoffman M, McConnell A, Decramer M, et al. Efficacy of a Novel Method for Inspiratory Muscle Training in People With Chronic Obstructive Pulmonary Disease. Phys Ther. 2015;95(9):1264-73.
33. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;1(8476):307-10.
34. Oliveira Ade S, Goncalves M. EMG amplitude and frequency parameters of muscular activity: effect of resistance training based on electromyographic fatigue threshold. J Electromyogr Kinesiol. 2009;19(2):295-303.
35. Campos GE, Luecke TJ, Wendeln HK, Toma K, Hagerman FC, Murray TF, et al. Muscular adaptations in response to three different resistance-training regimens: specificity of repetition maximum training zones. Eur J Appl Physiol. 2002;88(1-2):50-60.
36. Lay BS, Sparrow WA, Hughes KM, O'Dwyer NJ. Practice effects on coordination and control, metabolic energy expenditure, and muscle activation. Human movement science. 2002;21(5-6):807-30.
37. Jolley CJ, Luo YM, Steier J, Rafferty GF, Polkey MI, Moxham J. Neural respiratory drive and breathlessness in COPD. The European respiratory journal. 2015;45(2):355-64.
38. Parshall MB, Schwartzstein RM, Adams L, Banzett RB, Manning HL, Bourbeau J, et al. An official American Thoracic Society statement: update on the mechanisms, assessment, and management of dyspnea. American journal of respiratory and critical care medicine. 2012;185(4):435-52.

Chapter 5

Effects of Inspiratory Muscle Training on Dyspnea and Respiratory Muscle Function at Rest and during Exercise in Patients with COPD

Sauwaluk Dacha, Antenor Rodrigues, Zafeiris Louvaris,
Lotte Janssens, Rik Gosselink, & Daniel Langer

Preliminary results

5.1 Abstract

Background: Patients with COPD experience dyspnea during exercise, leading to the limitation in exercise tolerance and activities in daily life. Patients with low inspiratory muscle strength experience more significant dyspnea during exercise due to the mismatch between load and capacity of the respiratory muscles. It has been demonstrated that inspiratory muscle training (IMT) reduces dyspnea sensation by reducing activation of the diaphragm (EMGdi). However, whether IMT also reduces activation of the extra-diaphragmatic inspiratory muscles is unknown.

Aim: This study evaluated the effect of 8 weeks IMT or sham training on dyspnea, respiratory muscle function and activation during exercise. Also, respiratory muscle activation during an IMT session was investigated.

Method: Patients were randomized into an IMT (n=11) or sham-IMT (n-5) group. The training consisted of two sessions daily, each including 30 breaths, for eight weeks. Training load was 40-50% of the weekly maximal inspiratory pressure (MIP) in the IMT group and 10% of the initial MIP in the sham group. MIP and constant work rate (CWR) cycling test were performed before and after the training. Perception dyspnea was rated every minute during CWR cycling test using a modified Borg scale. An esophageal catheter and surface EMG electrodes were used to assess EMGdi, transdiaphragmatic pressures and extra-diaphragmatic respiratory muscle activation [scalene, sternocleidomastoid (SCM) and rectus abdominis]. All parameters were compared at iso-time (time of the shortest cycling test).

Results: After 8 weeks of IMT, there was a significant improvement in MIP in the IMT group compared to the sham group (mean between-group difference in the pre-post intervention change scores of -16 [95%CI -29, -3] cmH_2O, p=0.02), Cohen's d effect size of 0.86. At iso-time during the CWR cycling test, a trend of improvement in dyspnea measured by Borg score was found in the IMT group compared to the sham group (mean between-group difference in the pre-post intervention change scores of -2.2 [95%CI -4.5, 0.2], p=0.07), , Cohen's d effect size of 0.60. Mean between-group difference in the pre-post intervention change scores of endurance cycling time was 5.0 [95%CI -1.9, 11.9] minutes, p=0.14 in the IMT group compared to the sham group, Cohen's d effect size of 0.50. Mean between-group difference in the pre-post intervention changes of diaphragm, scalene, SCM and rectus abdominis muscle activation reported as ratios of maximal activation (EMG/EMGMax%) were -22 [95%CI -57, 13]%; p=0.17, -8 [95%CI -25, 10]%; p=0.37, -4 [95%CI -27, 20]%; p=0.74, -4 [95%CI -12, 5]%; p=0.33 in the IMT group compared to the sham group, with Cohen's d effect size of 0.64, 0.31, 0.11 and 0.34 respectively. There was a significant within-group reduction in activation of the diaphragm

(EMGdi/EMGdiMax%) in the IMT group at post-measurement compared to pre-measurement (from 78±10% to 57±21%, p=0.046). This reduction was a result of the reduction in the tidal EMGdi (-22%) and increase in EMGdiMax (8%). The unchanged ratio of SCM activation was the result of a combination of increased tidal EMGSCM and increased SCMMax (percent changes of tidal and Max EMG were 21% and 16% respectively). During the IMT session, SCM and scalene muscles were activated more compared to during cycling.

Conclusion: These preliminary results suggest that IMT improves inspiratory muscle strength with large effect size, while a trend of dyspnea improvement during potential longer exercise duration with medium effect size was observed. These improvements after IMT during the exercise were accompanied by a potentially better function of the diaphragm (improvement in Pdi,sniff, and reduction of diaphragm activation during exercise with medium effect sizes) and more reliance on the SCM muscle during exercise breathing. The more reliance on the SCM potentially was contributed by higher SCM stimulation during IMT.

Keywords: Dyspnea, Inspiratory Muscle Training (IMT), Diaphragm activation, Neural Respiratory Drive NRD, COPD, Extra-diaphragmatic muscle activation

5.2 Introduction

Chronic obstructive pulmonary disease (COPD) is one of the leading causes of morbidity, mortality and health care cost worldwide (1-3). A large proportion of patients with COPD experience a limitation in activities in daily life and decreased exercise tolerance because of persistent dyspnea (4-6). Dyspnea is a sensation of respiratory discomfort, which could be described differently in each individual such as air-hunger, work/effort, and tightness (7). Dyspnea was defined as "a subjective experience of breathing discomfort that consists of qualitatively distinct sensations that vary in intensity" (8)[P436]. Due to dyspnea, patients avoid exercise and develop deconditioning which further reduces their exercise capacity and aggravates symptoms. (8, 9).

Respiratory muscle weakness is highly prevalent and contributes to symptoms and functional limitations in patients with COPD (10). Independent of the severity of airflow obstruction patients who have decreased inspiratory muscle strength experience more symptoms of dyspnea during exercise (11). Hyperinflation is an important factor that contributes to an increase in dyspnea in patients with COPD. During quiet breathing, the diaphragm was adapted and remodeled to generate better respiratory work (e.g., increasing in slow-twitch muscle fiber to increase resistance in fatigue) (12, 13). However, during exercise, acute dynamic hyperinflation reduces the pressure generating capacity of the diaphragm, therefore, the adaptation to chronic mechanical loaded became more difficult (14, 15).

Factors that contribute to increasing dyspnea in patients with COPD mainly are the factors that either increase load or decrease capacity of respiratory muscles (11). This mismatch between load and capacity increases Neural Respiratory Drive (NRD) (neural control of respiration descending from the central motor cortex) which is closely related to perceived dyspnea sensation (16, 17). However, since NRD cannot be directly measured, it was suggested to measure this neural output through activation of respiratory muscles or electromyogram of the diaphragm (EMGdi) (17).

Pulmonary rehabilitation is one of the most successful treatments that reduces dyspnea, improves functional exercise capacity, and quality of life in patients with COPD (18, 19). In addition, selective inspiratory muscle training (IMT) effectively improves inspiratory muscle functions (strength and endurance), as measured by EMGdi, dyspnea and exercise capacity (20, 21). However, the diaphragm is not the only muscle responsible for inspiration, the extra-diaphragmatic inspiratory muscles could play an additional role in pressure generation at rest and during exercise breathing (22). For example, it has been found that in the patients with increasing chronic airflow obstruction, ribcage and accessory inspiratory muscles are activated more (22). Abdominal muscles, which are expiratory muscle, also activate more during exercise. The role of abdominal muscles is to decrease the end expiratory volume during expiration and gradually relax during inspiration (to prevent immediate fall in abdominal

pressure) to allow the two compartments of the ribcage to expand synchronously and as a result, permit diaphragm to act as a flow generator (23).

Langer and colleagues found an improvement in dyspnea sensation together with a reduction of diaphragm activation during exercise breathing in response to IMT (24). However, whether the activation of extra-diaphragmatic respiratory muscles contributed to these changes in dyspnea sensation is unclear. Also, whether the changes in the overall respiratory neural drive were mostly related to modifications in the diaphragm or extra-diaphragmatic respiratory muscles still needs to be assessed. Therefore, the aim of this study is to investigate the effects of either IMT or sham-IMT on a more comprehensive measurement of neural respiratory drive (EMG activity of the diaphragm, scalene, sternocleidomastoid and rectus abdominis) in relation to dyspnea sensation during constant work rate (CWR) cycling. We furthermore also aimed to study how these muscles were recruited and activated during IMT sessions in patients with COPD. The hypothesis was that eight weeks of IMT would improve dyspnea sensation during exercise and reduce the neural respiratory drive to the diaphragm and extra-diaphragmatic respiratory muscles.

5.3 Materials and method

5.3.1 Subjects

Clinically stable patients with COPD who had a reduction in inspiratory muscle strength (maximal inspiratory pressure (MIP) below predicted values) and a persistent activity related dyspnea (score fewer than 7 on baseline dyspnea index (BDI) questionnaire) were included in this study. Exclusion criteria were as follow: unable to perform physiological testing, active cardiovascular comorbidity, or other conditions that could affect dyspnea or exercise capacity.

5.3.2 Design

This single-blind, placebo-controlled, randomized clinical trial was approved by The Ethical Committee Research of KU Leuven/ UZ Leuven, Belgium (S58513) and registered with ClinicalTrials.gov (NCT03240640). All patients signed written informed consent. After the inclusion, the patients were randomized into intervention (IMT) or control (sham IMT) group. The allocation was performed by using a previously published method (25); opaque sealed envelopes were prepared and numbered sequentially, IMT and control interventions were distributed in a two to one ratio (see sample size calculation) in randomly ordered block sizes of 3 and 6. Physiotherapists providing the intervention and the outcome assessors were aware of group allocation. However, the subjects were blinded to their group assignment. To enhance adherence and compliance in the sham IMT group, the training was presented as an endurance training in the sham IMT group and strength training in the IMT group.

Three testing visits and eight training follow up visits were required for each patient. The first visit was to assess eligibility and familiarize the patients with the testing protocol. The measurements included pulmonary function testing, cardiopulmonary exercise testing (CPET), inspiratory and expiratory muscle strength testing (MIP, MEP), endurance breathing task, and answering questionnaires regarding dyspnea sensation [Baseline and transitional dyspnea index (BDI/TDI)](26) and, modified Medical Research Council dyspnea scale (mMRC) (27, 28). The second and the third testing visits were identical, but measured at pre and post eight weeks of intervention. The measurements for these visits were constant work rate (CWR) cycling test until symptom limitation with the recording of the diaphragm and extra diaphragm respiratory muscles electromyography (EMG). The outcomes measured were compared at the iso-time (time of the shortest test).

5.3.3 Pulmonary function and respiratory muscle function measurements

Pulmonary function testing (MasterScreen Body, CareFusion, Hoechberg, Germany) was performed according to ERS guidelines (29-31). Global respiratory muscle strength was assessed by measured maximal static inspiratory and expiratory mouth pressures (MIP and MEP) according to the international guidelines (32). MIP was measured at residual volume (RV). Patients were asked to perform a maximum inspiratory (Mueller maneuver) effort for at least 1.5 seconds through a system that allows a small leak to prevent glottis closing. The pressures were recorded via a pressure transducer. MEP were measured at total lung capacity (TLC) with the same system but the patients were asked to perform the maximal expiratory (Valsalva maneuver) effort instead (33). The three values that vary less than 10% were obtained and the maximal value of these three was recorded as a MIP or MEP, respectively.

Maximum sniff and cough pressures were assessed via a multipair-esophageal electrode catheter (Yinghui Medical Equipment Technology Co. Ltd., Guangzhou, China). Sniff esophageal (Pes,max) pressure assesses global inspiratory muscle strength and sniff transdiaphragmatic pressure (Pdi,max) assesses diaphragm strength. Maximal gastric pressure (Pga,max) during coughing represents abdominal strength. The patients were asked to perform maximum effort of sniff through un-occluded nostrils for the maximal sniff maneuver. For the maximal cough maneuver, the patients were asked to cough as forceful as possible. Each maneuver was repeated at least 3 and up to 10 attempts until a plateau was reached then the highest values were recorded (32, 33).

Inspiratory muscle endurance was measured using an electronic POWERbreathe®KH2 device (HaB International Ltd., Southam, UK) as performed in previous studies (34, 35). The patients were asked to breathe against resistance until symptom limitation. The inspiratory load that allows them to breathe for 3-7 minutes (approximately 50%-60% of their initial MIP) was selected for the test. During

the test, the same instructions were given as during the training session (fast, forceful and deep inspiration). At post measurement, the same tests were repeated with the same resistance. Borg dyspnea and breathing effort scores [ranging from 1 (nothing at all) to 10 (maximal)] were asked before the test and at the end of pre measurement test (36). The Borg scores at iso-time were also asked when the post-tests were longer than pretests. The test will be stopped when the patient perform the task for 15 minutes.

5.3.4 Questionnaires

5.3.4.1 Baseline and transitional dyspnea index (BDI/TDI)

Baseline Dyspnea Index (BDI) is a questionnaire which administers rating of dyspnea severity at a single state providing a multidimensional measurement of dyspnea based on 3 components that evoke dyspnea in activities of daily living in symptomatic individuals. There were 24 items in total in the BDI and divided into 3 categories, namely functional impairment, magnitude of task, and magnitude of effort. Rating of each category ranging from 0 (very severe) to 4 (no impairment). A total score ranging from 0 to 12. The lower the score, the worse the severity of dyspnea. There are also three additional options in each category, which do not contribute to the scoring, allow circumstances in which dyspnea cannot be rated (26).

Transition Dyspnea Index (TDI) measures changes in dyspnea severity from the baseline established by the TDI. The TDI consists of 24 items and divided into 3 categories which are change in functional impairment, change in magnitude of task, and change in magnitude of effort. Each category can be rated by seven grades ranging from -3 (major deterioration) to +3 (major improvement). A total score ranging from - 9 to + 9. The lower the score means the more deterioration in the severity of dyspnea. There is one additional option in each category, which does not contribute to the score, allows for circumstances in which impairment is due to reasons other than dyspnea (26).

The BDI and TDI show good reliability and validity in dyspnea measurement in patients with COPD (37). Change in the transition dyspnea index of 1-unit has been reported as a minimal clinically important difference (38).

5.3.4.2 Modified Medical Research Council dyspnea scale (mMRC)

The modified medical research council dyspnea scale (mMRC) is used to establish baseline functional impairment due to dyspnea attributable to respiratory disease. The mMRC is rated on a five scale, ranging from 0 (best) to 4 (worst) based on the patient's perception of dyspnea in daily activities (39). It has been validated in patients with COPD (40).

5.3.5 Measurements and analyses

5.3.5.1 Diaphragm electromyography (EMGdi) and respiratory pressure recording via esophageal catheter

A multipair-esophageal electrode catheter (Yinghui Medical Equipment Technology Co. Ltd., Guangzhou, China) was used to assess EMGdi and transdiaphragmatic pressure (Pdi) (calculated from esophageal and gastric pressures (Pes and Pga). The catheter is approximately 60 centimeters long, two millimeters in diameter, and is equipped with five EMG electrode pairs, esophageal and gastric balloons. After applied topical anesthesia, the catheter was inserted nasally and then swallowed by the patient. The positioning of the catheter was performed according to procedures established in previous studies (41, 42). The patients performed several slow maximal inspiratory capacity (IC) maneuvers (an inspiration through the open mouth from the functional residual capacity to total lung capacity). The best position was determined as the location that the largest EMGdi amplitudes were recorded from the outer electrode pairs and the smallest from the middle pairs (41-43). After positioning, the catheter was secured by taping one end onto the patient's nose then connected to an amplifier (Biomedical amplifier, Guangzhou, China) before being sampled at 2000Hz.

Esophageal and gastric balloons were filled with 0.8 and 1.4 milliliters of air respectively. Pes and Pga were recorded using differential pressure transducers range ±250 cmH_2O and ±50 cmH_2O respectively (Model MP 44 1-871 for Pes and MP 45-28-871 for Pga, Validyne Engineering Corp., Northridge, CA USA), amplified and sampled at a rate of 100Hz. Forced vital capacity (FVC) and IC maneuver were performed to obtain maximal muscle activation (EMG) and dynamic inspiratory and expiratory pressures before and during the exercise tests.

5.3.5.2 Surface electromyography (EMG) recording

EMG of extra-diaphragmatic inspiratory muscles (sternocleidomastoid, and scalene) and one expiratory muscle (rectus abdominis) were recorded using a surface EMG wireless transmission system (Desktop Direct Transmission System (DTS), Noraxon USA, Inc., Scottsdale, AZ USA). The skin was prepared by scrubbing the area with cotton pads soaked with alcohol until the skin turn light red. The specific location of the placement of these surface EMG electrodes were as follow; at midpoint along the long axis of the sternocleidomastoid muscle between the mastoid process and the medial clavicle, posterior triangle of the neck at the level of the cricoid process for scalene muscle, and at muscle belly above umbilicus for rectus abdominis muscle. The EMG sensors were connected and placed next to each electrode for the transmission of the signal.

5.3.5.3 EMG sampling and processing

EMG data (both EMGdi and surface EMG) were sampled by a data acquisition system (Micro1401-3, Cambridge Electronic Design Limited, Cambridge, UK) and then processed with a specific software package (Spike 2, Cambridge Electronic Design Limited, Cambridge, UK). During processing, the raw EMGdi data were first high pass filtered at 20Hz and then transformed into "Root Mean Square" (RMS). ECG artifacts were removed by the method presented in the previous chapter (chapter 4). Then all EMG signals were normalized as a proportion of the volitional maximum and EMG/EMG,Max%. Maximal activation (EMG,Max) of all interested muscles were obtained by selecting the highest activation during IC maneuvers (inspiratory muscle) either during resting breathing or during exercise breathing of the CWR cycling test (44) and FVC maneuvers (expiratory muscle) performed before the cycling test.

5.3.6 Exercise testing

A maximal incremental cardiopulmonary exercise test (CPET) until symptom limitation was conducted on an electrically-braked cycle ergometer (Ergometrics 900, Ergoline, Blitz, Germany) with detailed metabolic (SensorMedics Vs229d, Acertys Healthcare, Aartselaar, Belgium) and cardiopulmonary measurements (Cardiosoft, Acertys Healthcare, Aartselaar, Belgium) according to guidelines (45). ECG recording was obtained via an impedance cardiography device (PhysioFlow, Manatec Biomedical, Folschviller, France) validated for COPD patients and recorded as a separate channel (46). The incremental protocol was a 10 or 20 W/min stepwise protocol. Breath-by-breath measurements were evaluated as 30-s averages, and "peak" was defined as the last 30 s of loaded pedaling.

Constant work rate (CWR) cycling test consists of three minutes of resting, one minute of unloaded cycling and immediately followed by cycling against approximately 75% of the peak work rate achieved during CPET with the same equipment. Analog outputs of all variables (i.e., respiratory flow, 5 EMGdi, surface EMGs and ECG) were collected in separate channels with a data acquisition unit (Micro1401-3, Cambridge Electronic Design Limited, Cambridge, UK). Data channels were synchronized in time and processed with the same acquisition software (Spike 2, Cambridge Electronic Design Limited, Cambridge, UK).

Inspiratory capacity (IC) was measured at rest, every second minute during cycling, and at the end of the cycling test (47). Perceived dyspnea intensity, unpleasantness of breathing and leg discomfort at rest, every minute during cycling, and the end of the cycling were asked using the modified 10-point Borg scale [ranging from 1 (nothing at all) to 10 (maximal)] (36).

5.3.7 Inspiratory muscle training (IMT)

According to a previously established method (34), patients performed home-based daily inspiratory muscle training consisting of two training sessions of 30 breaths (4-5 minutes per session). The intensity of the training starting at approximately 40% of maximal inspiratory pressure (MIP) measured at baseline. In the training group, the intensity was increased weekly to the highest tolerable intensity (approximately 40-50% of MIP achieved on that week). In the sham IMT group, the training intensity remained the same for the entire 8 weeks at approximately 10% of the patient's initial MIP. Weekly measurements of MIP were performed in both groups. One training session per week was performed supervised at the research center. IMT was performed using an electronic tapered flow-resistive loading (TFRL) device (POWERbreath®KH1, HaB International Ltd., Southam, UK) for 8 weeks.

5.3.8 Breathing pattern and respiratory muscle EMG during an IMT session

At baseline, all patient performed one IMT session against approximately 50% of their MIP for 30 breaths using the same training device. This task is a replication of one IMT session that the patient performed at home. During this IMT session, the breathing parameter (flow, volume, and pressure of breathing), diaphragm EMG, and surface EMG were recorded. Borg dyspnea and breathing effort were asked before the IMT session (resting breathing) and at the end of the session.

5.3.9 Physical activity

Physical activity was measured twice, once during the first week after the initial visit and second during the last week of the IMT or sham IMT training. The physical activity was measured using the Dynaport Movemonotor (Mc Roberts BV, The Hague, The Netherlands). The patients were wearing the monitor at their waist during the period of 7 days from the moment they were wakened up until the moment that they went to bed. The days that the patients were wearing the monitor for more than eight hours were defined as valid days, and the results were included in the analysis.

5.3.10 Statistical analyses

The power calculation was performed to detect a between-group difference of one unit in the change in dyspnea intensity rating on the Borg dyspnea scale (minimal clinically important difference), at iso-time limit of CWR cycle test. The pre-specified level of statistical power (β) is 80% and the alpha level (α), or the type I error rate is less than 5%. By assuming a decrease of 1 point in the intervention group and no change in the sham IMT group with an SD of 1 unit in the change in dyspnea between pre- and post- measurements, a sample size of 16 and 8 patients is required in the IMT and the sham IMT group respectively.

Statistical significance was considered at $p<0.05$. Data are presented as means ± SD. Test of normality was performed using Kolmogorov-Smirnov and/or Shapiro-Wilk. The difference between pre and post measurement were calculated for all outcomes measured including the outcomes measured at iso-time during CWR cycling tests and unpaired t-tests were applied to test for between-group differences. Paired t-test was used to test the within-group differences between pre and post-intervention measurements. In case the normality was not met, or the sample size was too small to test for normality, nonparametric tests were performed. Wilcoxon match-pairs signed rank test, and Mann-Whitney test compared ranks were used instead of paired and unpaired t-test respectively. In addition, Cohen's d effect sizes were calculated by dividing mean differences by the standard deviations of the differences. Small effect sizes were defined as scores between 0.2 and 0.5, medium effect sizes as scores between 0.5 and 0.8 and large effect sizes were those greater than 0.8. Statistical analyses were performed using GraphPad Prism version 8 for Windows (GraphPad Software, La Jolla, California, USA) and IBM SPSS Statistics 25.0 Desktop (IBM Corp., Armonk, NY, USA).

5.4 Results

The results presented here in this chapter are preliminary results.

A consort flow diagram is presented in Figure 5.1. During the inclusion period from October 2016 to April 2019, 128 patients who had previously attended the pulmonary rehabilitation program at University Hospital Leuven and who were still in clinical follow-up until at least 2014 were screened. One hundred and eleven patients were excluded because the inclusion criteria were not met. Forty-two patients were contacted and 25 of them declined to participate. Therefore, 16 patients were included, and after randomization 11 were allocated into an intervention group, five were allocated into a sham IMT group. The results presented here is the analysis of 11 patients in the intervention group and five in the sham IMT group (Figure 5.1). Of note, two patients in the sham IMT group and five patients in the IMT group did not agree to swallow the esophageal electrode catheter.

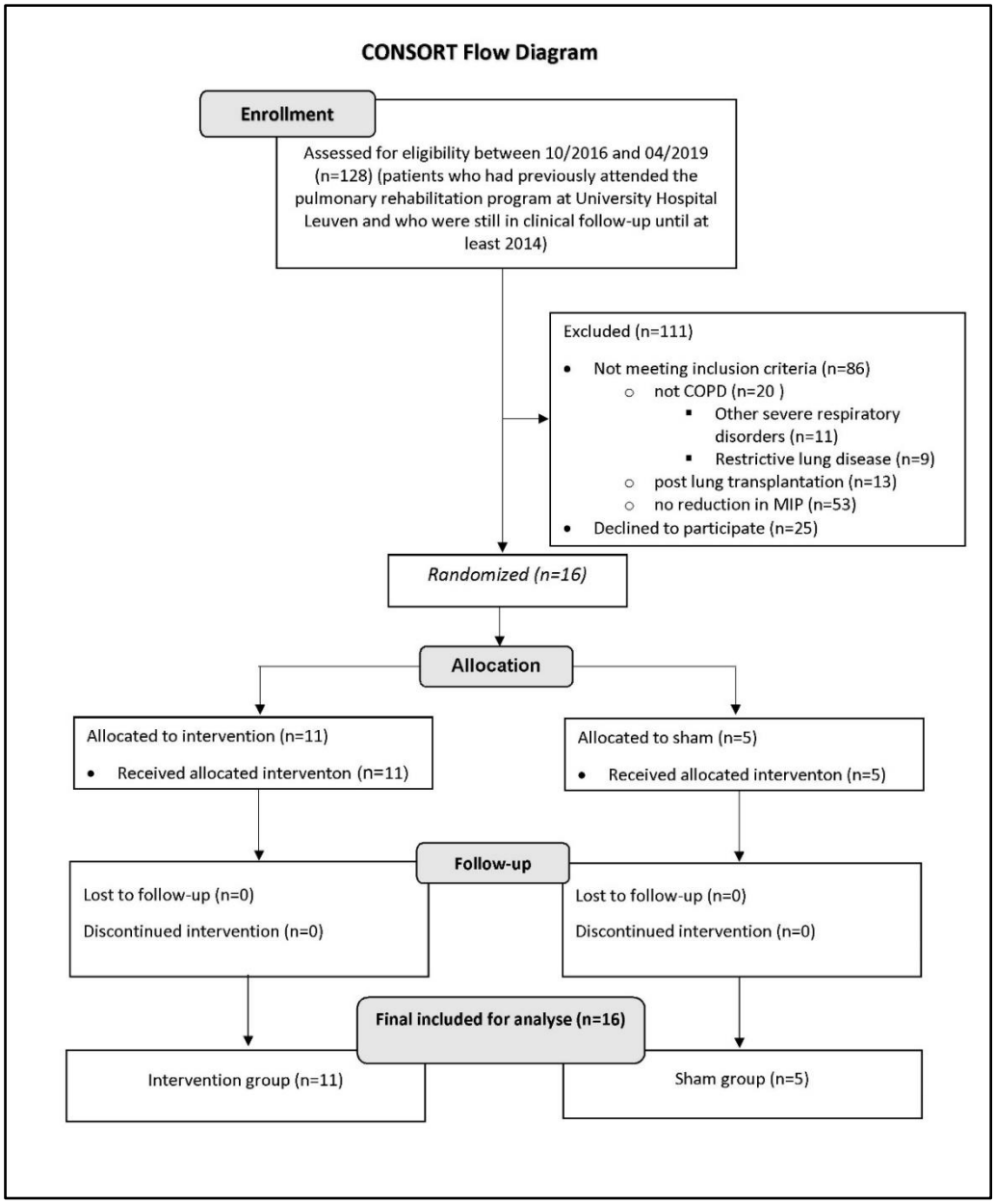

Figure 5.1 Consort flow diagram showing the progress of participants through different phases of the study.

Baseline characteristics of the included patients are summarized in Table 5.1. Participants were patients with moderate to severe COPD. The patients in the sham IMT group compared with the IMT group showed greater severity in expiratory airflow limitation, lower body mass index, reduced TLCO, lower exercise capacity and greater mechanical constraints at the end of exercise (IRV 0.19 L). The age, level of activity related dyspnea, lung hyperinflation, functional capacity, physical activity, and respiratory and locomotor muscles strength were comparable between two groups (Table 5.1). Seven patients indicated that the reason for stopping exercise during symptom-limited peak incremental cycle ergometer exercise was from breathing discomfort, six patients stated that it was a combination

between breathing and leg discomfort and three patients reported legs discomfort as a primary reason for stopping exercise.

The six-minute walking distance was comparable in both groups. The patients in the IMT group achieved slightly higher steps per day compared to the sham group. The quadriceps muscle force was slightly higher in the IMT group (Table 5.1).

Table 5.1 Baseline characteristics at study enrolment

	All Subjects (n=16)	Sham IMT (n=5)	IMT (n=11)
Overall Characteristics			
Male:Female, n	9:7	2:3	7:4
Age, years	66 ± 5	68 ± 9	65 ± 4
Height, cm	164 ± 9	157 ± 3	167 ± 9
Weight, kg	70 ± 21	52 ± 3	77 ± 21
Body Mass index, kg/m²	25.8 ± 6.6	21.6 ± 1.6	27.7 ± 7.1
BDI total score (0-10)	6.5 ± 1.7	6.3 ± 1.0	6.6 ± 1.9
MRC dyspnea scale (0-4)	1.4 ± 0.8	2.0 ± 0.8	1.1 ± 0.6
Lung Function			
FEV_1, L (% predicted)	1.52 ± 0.56 (62 ± 26)	1.32 ± 0.40 (59 ± 17)	1.68 ± 0.66 (64 ± 34)
FEV_1/FVC, % (% predicted)	43.6 ± 11.5	40.0 ± 8.6	45.2 ± 12.7
IC, L (% predicted)	2.52 ± 0.62 (79 ± 24)	2.08 ± 0.31 (74 ± 30)	2.71 ± 0.63 (82 ± 22)
FRC, L (% predicted)	4.89 ± 1.49 (160 ± 32)	4.65 ± 1.00 (168 ± 14)	4.99 ± 1.7 (157 ± 38)
RV, L (% predicted)	3.47 ± 1.15 (153 ± 42)	3.50 ± 0.80 (172 ± 16)	3.45 ± 1.32 (145 ± 48)
RV/TLC, %	52 ± 10	63 ± 5	48 ± 8
TLC, L (% predicted)	6.82 ± 1.57 (123 ± 15)	6.14 ± 0.90 (127 ± 7)	7.13 ± 1.74 (122 ± 18)
sRaw, kPa/L/s (% predicted)	0.51 ± 0.32 (198 ± 130)	0.69 ± 0.46 (265 ± 166)	0.43 ± 0.23 (168 ± 108)
TLCO, mmol/min/Kpa (% predicted)	4.07 ± 1.70 (54 ± 19)	3.24 ± 0.39 (47 ± 7)	4.49 ± 1.98 (57 ± 23)
Symptom-limited peak incremental cycle ergometer exercise test			
Work Rate, W (%predicted)	81 ± 26 (61 ± 18)	67 ± 24 (64 ± 22)	87 ± 26 (60 ± 17)
VO_2, L/min (%predicted)	1.43 ± 0.47 (75 ± 23)	1.21 ± 0.35 (70 ± 18)	1.53 ± 0.50 (77 ± 26)
HR, beats/min (%predicted)	121 ± 18 (78 ± 10)	114 ± 21 (75 ± 12)	124 ± 16 (79 ± 10)
Ventilation, L/min (%MVV)	47 ± 11 (92 ± 15)	45 ± 18 (87 ± 4)	48 ± 8 (95 ± 18)
IRV, L	0.37 ± 0.35	0.19 ± 0.15	0.46 ± 0.39
Dyspnea, Borg Units	6.4 ± 2.5	5.6 ± 1.5	6.9 ± 2.8
Leg Discomfort, Borg Units	6.1 ± 2.0	4.8 ± 1.5	6.8 ± 2.0
Unpleasantness, Borg Units	6.3 ± 2.8	4.8 ± 1.7	6.9 ± 3.0
Functional capacity, physical activity, respiratory and locomotor muscle strength			
6-minute walking test, meters (%predicted)	489 ± 50 (72 ± 16)	489 ± 45 (78 ± 17)	489 ± 55 (69 ± 16)
Quadriceps muscle force, kg	37 ± 10	34 ± 13	38 ± 10
Sniff Pes, cmH_2O#	-62 ± 12	-55 ± 11	-65 ± 12
MIP at RV, cmH_2O (% predicted)	-73 ± 14 (84 ± 21)	-73 ± 7 (98 ± 14)	-73 ± 17 (77 ± 21)
MEP max at TLC, cmH_2O (% predicted)	158 ± 45 (167 ± 46)	143 ± 46 (164 ± 46)	165 ± 45 (168 ± 48)
Physical activity, steps/day	7278 ± 3713	6796 ± 4267	8363 ± 2079

Values are means ± SD. #=Pes, sniff cut-off below -70 cmH_2O in men and -60 cmH_2O in women for significant inspiratory muscle weakness. *Abbreviations:* BDI = Baseline Dyspnea Index, total scores range from 0 (most severe activity related dyspnea) to 12 (no activity-related dyspnea); mMRC dyspnea scale = modified Medical Research Council dyspnea scale, scores range from 0 (best) to 4 (worst); VO_2 = oxygen consumption; HR = heart rate; MVV= maximal voluntary ventilation; FEV_1 = forced expiratory volume in one second; FRC = plethysmographic functional residual capacity; FVC = forced vital capacity; IC = inspiratory capacity; RV= residual volume; TLC = total lung capacity; sRaw = specific airway resistance; DLCO = diffusing capacity of the lung for carbon monoxide; Pes= esophageal pressure; MIP at RV= maximal inspiratory mouth pressure at residual volume; MEP at TLC= maximal expiratory mouth pressure at total lung capacity. Predicted values of Neder et al. were used (48) for MIP and MEP. Predicted peak work rate and peak VO_2 were those of Blackie et al. (49). Predicted peak HR=220-age. Predicted 6MWD Troosters et al. (50).

After eight weeks of IMT or sham IMT training, there were no significant within-group differences in quadriceps strength or steps per day observed. There was a reduction of 735±3514 and 1164±2702 steps per day in the sham and IMT group, respectively.

The compliance and the progression of the training intensity of each week of training are presented in Figure 5.2. The compliance with the prescribed training sessions was 95±9% in the IMT group and 91±11% in the sham IMT group.

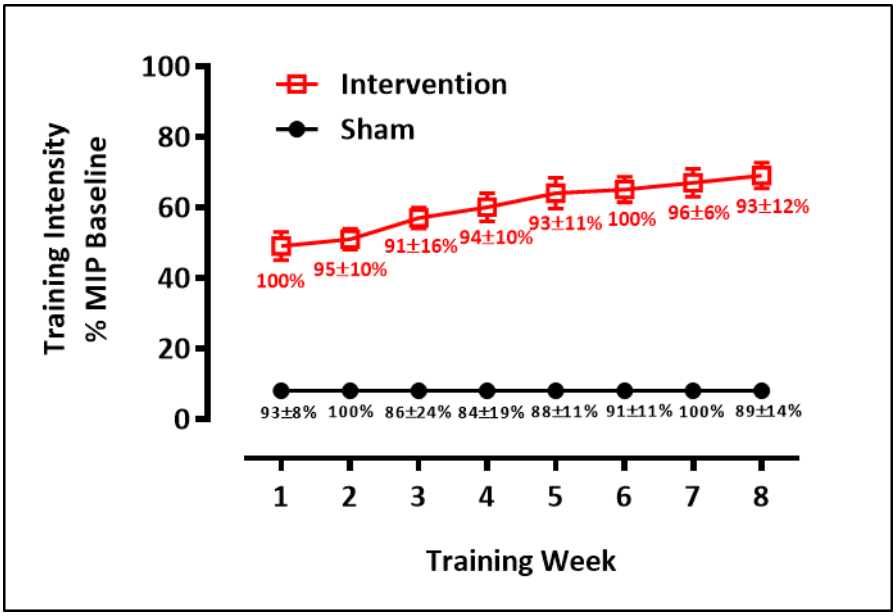

Figure 5.2 Average inspiratory resistance that had to be overcome by the patients during weekly-supervised inspiratory muscle training (IMT) sessions expressed as percentage of baseline maximal inspiratory mouth pressure (MIP) measured from residual volume. Percentages displayed below weekly averages indicate average compliance of participants with prescribed sessions each week. Values are means ±SD.

The responses of IMT or sham IMT on the primary outcome measures are presented in Table 5.2. There was a significant improvement in the pre-post intervention changes of inspiratory muscle strength (MIP) in the IMT group compared to the sham group (Cohen's d effect size of 0.86). The MIP was also significantly increased within the IMT group compared to the pre-measurement scores. There was no significant improvement in the pre-post intervention change scores of maximal esophageal pressures (Pes,sniff), and maximal transdiaphragmatic pressures (Pdi, sniff) in the IMT group compared to the sham group (Table 5.2), (Cohen's d effect size 0.34 and 0.64 respectively). Pes,sniff and Pdi,sniff were not changed within-group after either intervention compared to pre-intervention (Table 5.2).

There was no significant improvement in the pre-post intervention changes of inspiratory power per breath and total work of breathing in the IMT group compared to the sham group during the endurance-breathing test (Cohen's d effect size of 0.39 and 0.14 respectively). The inspiratory power per breath and total work of breathing were significantly improved within-group in the IMT group at

post-intervention compared to pre-intervention. There was no significant improvement in the pre-post intervention changes of endurance breathing time in the IMT group compared to the sham group (Cohen's d effect size 0.29). However, the endurance breathing time was significantly improved within-group at post-intervention compared to pre-intervention in both groups. There was no significant improvement in the pre-post intervention changes of cycling duration in the IMT group compared to the sham group (Cohen's d effect size of 0.50). However, the cycling duration was five minutes longer within-group in the IMT group post-intervention compared to pre-intervention while this longer duration was not observed within-group in the sham group (Table 5.2, Figure 5.3 A-D). There was no significant improvement in the pre-post intervention change scores of TDI and mMRC in the IMT group compared to the sham group (Cohen's d effect size of 0.29 and 0.09 respectively).

Table 5.2 Responses to IMT and sham IMT: main outcome measures

	Sham IMT			IMT			Mean difference of change Sham IMT- IMT (95% CI) or differences between medians	P values Between groups
	Pre	Post	P values within group	Pre	Post	P value within group		
FEV_1, L (% predicted)	1.32 ± 0.40 (59 ± 17)	1.27 ± 0.36 (57±19)	0.6250	1.68 ± 0.66 (64 ± 34)	1.65 ± 0.61 (63 ± 33)	0.4374	-0.25 (-0.87 to 0.38)	0.3765
FRC, L (%predicted)	4.65 ± 1.00 (168 ± 14)	4.80 ± 1.08 (173 ± 16)	0.2939	5.41 ± 1.83 (166 ± 36)	5.34 ± 2.01 (165 ± 43)	0.5904	0.21 (-0.23 to 0.67)	0.2939
IC, (L) (%predicted)	1.49 ± 0.31 (74 ± 30)	1.42 ± 0.66 (70 ± 38)	0.5660	2.28 ± 0.57 (85 ± 29)	2.24 ± 0.43 (85 ± 29)	0.7107	-0.03 (-0.39 to 0.33)	0.8600
IC/TLC, %	25 ± 9	23 ± 11	0.4870	31 ± 9	31 ± 10	0.7209	-20 (-7 to 3)	0.3720
Inspiratory muscle strength								
Pes, sniff, cmH_2O#	-55 ± 11	-58 ± 15	0.5063	-65 ± 12	-74 ± 19	0.1548	7 (-13 to 28)	0.4312
Pdi, sniff, cmH_2O##	72 ± 10	67 ± 23	0.6313	94 ± 18	105 ± 18	0.1111	-16 (-39 to 8)	0.1590
MIP at RV, cmH_2O (%predicted)	-73 ± 7 (98 ± 14)	-77 ± 11 (102 ±5)	0.4624	-73 ± 17 (77 ± 21)	-93 ± 19 (97 ± 24)	***0.0004****	-16 (-29 to -3)	***0.0175***[ǂ]
Inspiratory muscle endurance test								
Inspiratory load, cmH_2O	45 ± 12		-	49 ± 13		-	-	-
Endurance breathing time, min	7.1 ± 4.1	10.3 ± 4.9	***0.0158****	5.9 ± 3.1	10.6 ± 4.4	***0.0310****	-2.2 (-7.8 to 3.4)	0.4059
Inspiratory power/breath, W	3.3 ± 1.2	4.0 ± 1.4	0.2619	3.3 ± 1.7	5.0 ± 3.0	***0.0161****	-1.3 (-3.4 to 0.9)	0.2381
Total external inspiratory work, J	237 ± 153	283 ± 102	0.5408	206 ± 151	483 ± 313	***0.0114****	-52 (-338 to 234)	0.6964
Constant work rate cycle ergometer test								
Work Rate, W (% Max)	53 ± 21 (79 ± 6)		-	65 ± 21 (74 ± 5)		-	-	-
Exercise time, min	6.8 ± 4.7	6.8 ± 5.0	>0.9999	6.6 ± 2.3	11.6 ± 7.2	0.0996	-5.0 (-11.9 to 1.9)	0.1398
Activity-related dyspnea								
mMRC dyspnea scale (1-5)	2.0 ± 0.8	2.0 ± 1.4	>0.9999	1.1 ± 0.6	1.2 ± 0.4	>0.9999	-0.1 (-1.0 to 0.8)	0.7867
TDI total score (-9 to 9)	-	2.3 ± 3.3	-	-	3.9 ± 3.0	-	-1.6 (-5.7 to 2.5)	0.3976

Values are means ± SD. #=Pes,sniff cut-off below -70 cmH_2O in men and -60 cmH_2O in women for significant inspiratory muscle weakness. ##=Pdi,sniff normal value of Pdi sniff range 82-204 cmH_2O.
Abbreviations: FEV_1 = forced expiratory volume in one second; FRC = plethysmographic functional residual capacity; IC = inspiratory capacity; TLC = total lung capacity; Pes = esophageal pressure; Pdi = diaphragmatic pressure; MIP at RV= maximal inspiratory mouth pressure at residual volume; mMRC = modified Medical Research Council, with dyspnea scale scores ranging from 0 (best) to 4 (worst);TDI, transition dyspnea index, with scores ranging from -9 (maximal worsening of symptoms) to 9 (maximal improvement of symptoms); TLC, total lung capacity. *P < 0.05, within-group difference, pre- vs. post-intervention by paired t-test (or Wilcoxon match-pairs signed rank test).). [ǂ]P < 0.05 by unpaired t-test (or Mann-Whitney test compares ranks) comparing treatment differences for IMT vs. sham IMT.

Training responses at iso-time during CWR cycling test

Exercise physiological response

Training responses to IMT at iso-time during CWR cycling test are presented in Table 5.4. There was no significant difference in the pre-post intervention changes of ventilation (VE), breathing pattern (Vt, BF), operating lung volumes (IC, IRV) and oxygen saturation (SpO_2), at iso-time in the IMT group compared to the sham group (Table 5.4 and Figure 5.3 A-D). The sham IMT group seems to have ventilated more at post-measurement cycling test compared to pre-measurement, however, the value

did not reach a significant difference. While the ventilation was more or less the same at pre/post measurement in the IMT group, however, the oxygen consumption (VO_2) was significantly reduced within-group at post-measurement at iso-time (Table 5.4 and Figure 5.3 A-D). The sham IMT group was breathing faster, and with lower tidal volume compared to the IMT group, however, the values were not significantly different (Table 5.4). The IC was significantly lower in the sham IMT group compared to the IMT group at pre-measurement (p=0.0168). However, the IC values were not changed within-group at post-measurement in both groups. At iso-time during CWR cycling test, the IRV in both groups was higher than at the end of maximal cycling test (table 5.1 and 5.4). Heart rate responses were comparable in both groups and were not changed after the intervention (Table 5.4).

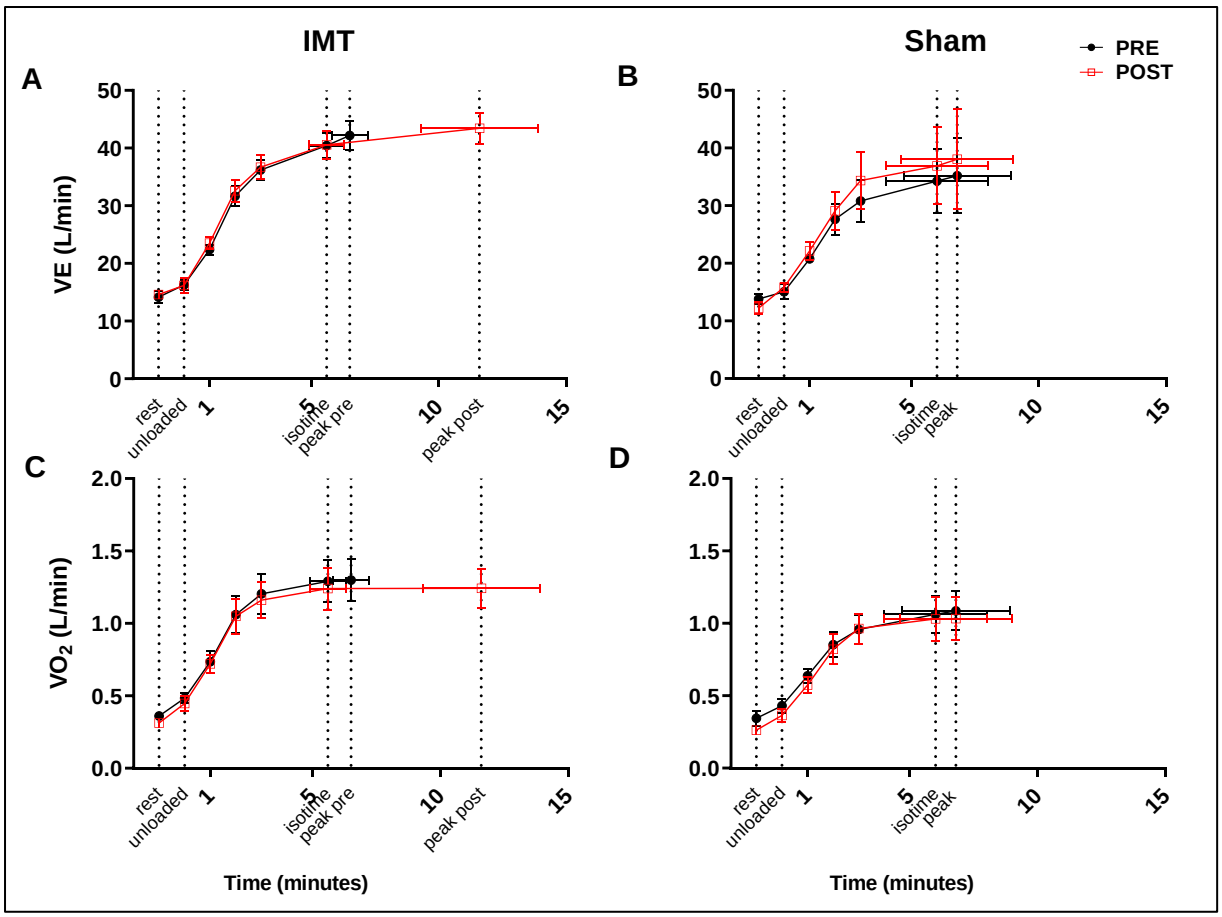

Figure 5.3 Minute ventilation (VE) and oxygen consumption (VO_2) during rest and every minute of CWR cycling test at pre- and post- measurement of the IMT group (A and C) and sham group (B and D).

Symptoms response

There was a trend for an improvement in the pre-post intervention change of Borg scores of dyspnea intensity in the IMT group after the training compared to the sham group (p=0.0660, Table 5.4 Figure 5.4 A, B), (Cohen's d effect size 0.60). There was no significant improvement in the pre-post

intervention change scores of Borg scores of leg discomfort, and unpleasantness of breathing in the IMT group compared to the sham group (Table 5.4 Figure 5.4 C-F). (Cohen's d effect size 0.46 and 0.29 respectively). Borg scores of dyspnea intensity, leg discomfort, and unpleasantness of breathing were slightly increased within-group in the sham-IMT group and slightly decreased within-group in the IMT group at post-measurement compared to pre-measurement. However, the changes did not reach statistical significance (Table 5.4 Figure 5.4 A-F).

All the patients in the sham group reported dyspnea and combination of dyspnea and leg discomfort both at pre and at post-measurement as the reasons for stopping the cycling test. Eight patients in the IMT group reported dyspnea and combination of dyspnea and leg discomfort as the main reason to stop exercise at pre-measurement and post-measurement. Three patients in the IMT group reported leg discomfort as the main reason for stopping exercise at pre-measurement vs. two at the post, and one patient reported back pain as the reason (Table 5.3).

Table 5.3 reason for stopping CWR cycling exercise

Reason for stopping cycling	Pre-measurement (number of patients)		Post-measurement (number of patients)	
	sham IMT	IMT	sham IMT	IMT
Breathing discomfort	3	2	4	4
Leg discomfort	-	3	-	2
Combination (breathing + leg discomfort)	2	6	1	4
others	-	-	-	1 (back pain)
Total	5	11	5	11

Table 5.4 Responses to IMT during CWR cycle ergometer test at iso-time

Measurements during symptom-limited CWR cycle ergometer exercise at iso-time	Sham IMT			IMT			Mean difference of change Sham IMT -IMT (95% CI) or differences between medians	P values Between group
	Pre	Post	P values within group	Pre	Post	P values within group		
Work rate, W (% Max)	53 ± 21 (79 ± 6)		-	65 ± 21 (74 ± 5)		-	-	-
Exercise time at Isotime, min	6.0 ± 4.5		-	5.6 ± 2.2		-	-	-
Dyspnea, Borg Units§	4.2 ± 1.9	5.0 ± 1.9	0.2302	6.5± 2.4	5.7 ± 2.6	0.1027	2.2 (-0.2 to 4.5)	0.0660
Leg Discomfort, Borg Units	4.0 ± 2.1	4.6 ± 2.3	0.4263	6.9 ± 1.9	5.9 ± 2.7	0.1449	1.6 (-0.7 to 3.8)	0.1503
Unpleasantness, Borg Units	4.0 ± 2.1	4.2 ± 1.9	0.6213	6.7 ± 2.7	5.8 ± 3.0	0.4785	1.1 (-1.4 to 3.6)	0.3586
Change from rest of Dyspnea, Borg Units	3.5 ± 1.1	4.1 ± 1.4	0.5012	5.5 ± 2.2	4.8 ± 2.0	0.3409	1.3 (-1.29 to 3.9)	0.2942
Change from rest of Leg Discomfort, Borg Units§	3.0 ± 1.0	3.4 ± 2.1	>0.9999	5.9 ± 1.8	5.0 ± 2.4	0.2368	1.3 (-1.3 to 3.9)	0.2914
Change from rest of Unpleasantness, Borg Units	3.8 ± 2.0	3.4 ± 1.1	0.6213	5.6 ± 2.4	4.8 ± 2.5	0.2962	0.5 (-2.3 to 3.2)	0.7224
VO_2, L/min	1.15 ± 0.27 (36 ± 13)	1.10 ± 0.30 (35 ± 14)	0.2393	1.36 ± 0.41 (48 ± 16)	1.26 ± 0.36 (44 ± 13)	***0.0251****	0.05 (-0.08 to 0.19)	0.4109
HR, beats/min	122 ± 34 (81 ± 19)	123 ± 37 (81 ± 21)	0.8083	124 ± 11 (79 ± 7)	119 ± 11 (77 ± 7)	0.1243	5 (-4 to 14)	0.2607
SpO_2, %	88.0 ± 5.8	88.6 ± 4.5	0.4946	89.5 ± 3.6	89.5 ± 4.5	0.9756	0.6 (-2.0 to 3.1)	0.6403
Breathing pattern								
Ventilation, L/min	33.9 ± 11.1	37.0 ± 13.4	0.1057	41.6 ± 7.3	40.9 ± 6.8	0.7334	3.8 (-2.8 to 10.4)	0.2339
Ventilation, %MVV	76 ± 11	83 ± 10	0.1250	79 ± 12	79 ± 14	0.8532	7 (-3 to 17)	0.1442
Vt, L	1.09 ± 0.27	1.15 ± 0.33	0.0917	1.46 ± 0.38	1.43 ± 0.32	0.6875	0.14 (-0.03 to 0.31)	0.0887
BF, breaths/ min	32.9 ± 4.5	32.3 ± 5.9	0.7005	28.9 ± 5.5	29.5 ± 5.8	0.4960	-1.2 (-4.6 to 2.2)	0.4595
IC, L§	1.28 ± 0.32	1.28 ± 0.31	0.8750	1.89 ± 0.38	1.88 ± 0.51	0.8588	0.02 (-0.29 to 0.32)	0.9072
IRV, L	1.04 ± 0.28	1.15 ± 0.33	0.0562	1.32 ± 0.44	1.30 ± 0.37	0.6765	0.13 (-0.03 to 0.28)	0.1078
Ti/Ttot, %	37 ± 6	36 ± 5	0.2000	41 ± 3	41 ± 5	0.9063	-1 (-6 to 4)	0.7341
Muscle activation								
EMGdi/EMGdi,max, %	80 ± 8	82 ± 3	0.8829	78 ± 10	57 ± 21	***0.0462****	22 (-13 to 57)	0.1690
EMGscm/EMGscmmax, %§	48 ± 10	44 ± 8	0.6250	29 ± 15	33 ± 21	0.4651	8 (-10 to 25)	0.3717
EMGsca/EMGscamax, %§	57 ± 7	64 ± 14	0.4157	33 ± 21	37 ± 23	0.6266	-4 (-27 to 20)	0.7379
EMGabd/EMGabdmax, %	32 ± 18	36 ± 22	0.4536	23 ± 17	23 ± 17	0.8268	-4 (-12 to 5)	0.3268
Pressures and effort of breathing								
Pes,tidal, cmH_2O	24 ± 5	26 ± 8	0.4867	32 ± 10	33 ± 10	0.8438	-1 (-13 to 10)	0.7627
Inspiratory Pes, cmH_2O	-22 ± 2	-21 ± 2	0.2048	-18 ± 4	-18 ± 6	0.9671	1 (-8 to 9)	0.8345
Inspiratory Pes/Pes,sniff, %	39 ± 13	35 ± 13	***0.0073****	29 ± 7	27 ± 12	0.3737	-2 (-12 to 8)	0.6152
Inspiratory Pdi, cmH_2O	30 ± 2	30 ± 0	0.9145	26 ± 8	29 ± 12	0.3711	-4 (-22 to 14)	0.5892
Inspiratory Pdi/Pdi,sniff, %	41 ± 3	40 ± 10	0.8938	27 ± 6	28 ± 11	0.7364	-2 (-21 to 16)	0.7764

Values are means ± SD. *p<0.05 within-group difference pre vs post intervention by paired t-test (or Wilcoxon match-pairs signed rank test). ¹P < 0.05 by unpaired t-test (or Mann-Whitney test compares ranks) comparing treatment differences for IMT vs. sham IMT. §P < 0.05 by unpaired t-test (or Mann-Whitney test compares ranks) comparing treatment differences for baseline values IMT vs. sham IMT. *Abbreviations*: VO_2 = oxygen consumption; HR = heart rate; SpO_2 = oxygen saturation by pulse oximetry; MVV = maximal voluntary ventilation; Vt = tidal volume; BF= breathing frequency; IC = inspiratory capacity; IRV = inspiratory reserve volume; Ti/Ttot = inspiratory duty cycle; EMGdi = electromyogram of the diaphragm measured during tidal inspiration; EMGdimax = the largest value of the diaphragm during a maximum inspiratory maneuver; EMGscm= electromyogram of the sternocleidomastoid measured during tidal inspiration; EMGscmmax= the largest value of the sternocleidomastoid during a maximum inspiratory maneuver; EMGsca= electromyogram of the scalene measured during tidal inspiration; EMGscamax=the largest value of the scalene during a maximum inspiratory maneuver; EMGabd= electromyogram of the abdominal (rectus abdominis) muscles measured during tidal expiration; EMGabd,max= the largest value of the abdominal (rectus abdominis) muscles during a maximum force vital capacity maneuver; Pestidal = the tidal swing of Pes; inspiratory Pes = the most negative Pes during a tidal inspiration; inspiratory Pdi = the most positive Pdi during a tidal inspiration

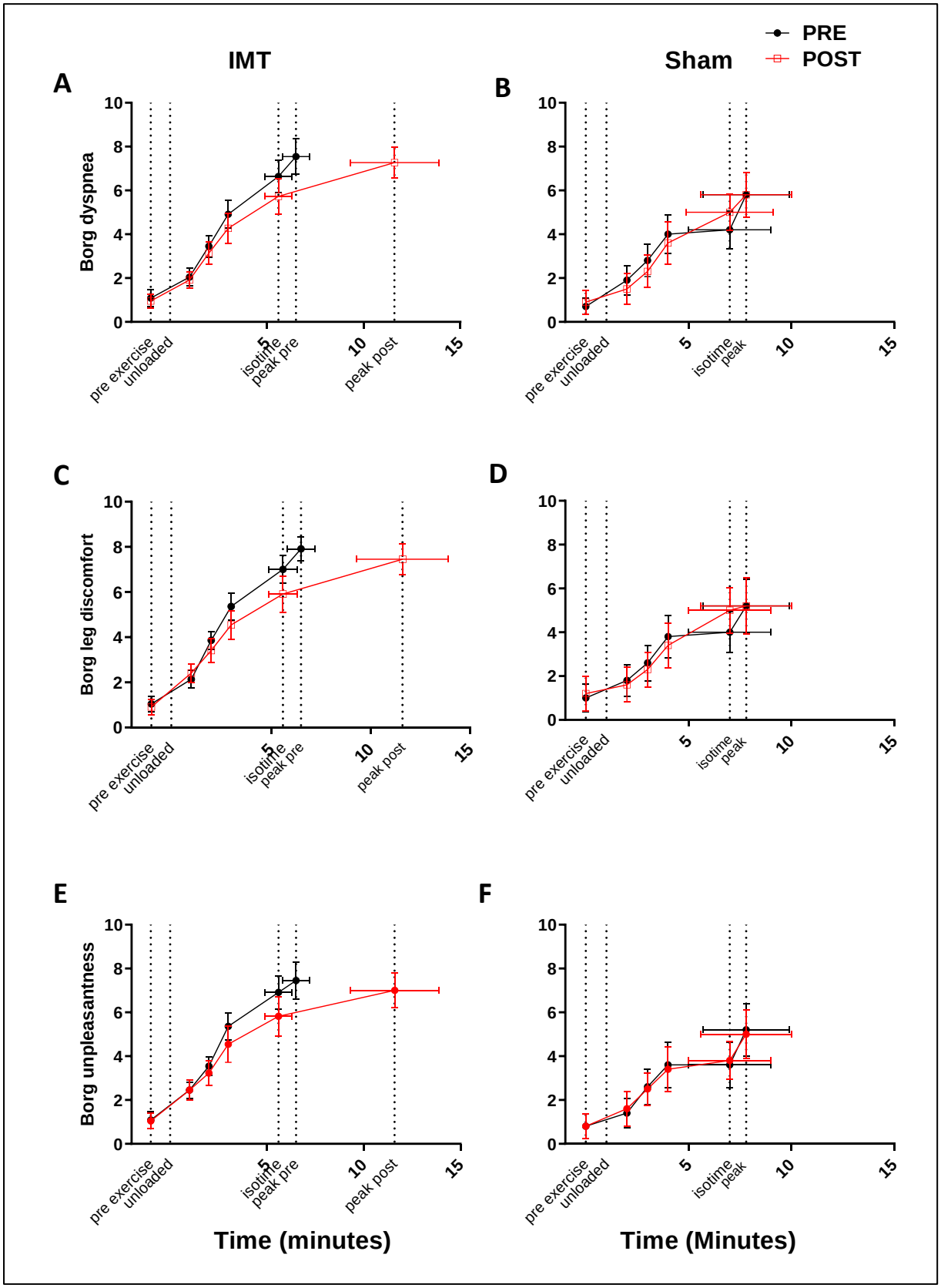

Figure 5.4 Borg dyspnea, leg discomfort and unpleasantness scores during rest and every minute of CWR cycling test at pre- and post- measurement of the IMT group (A, C and E) and sham group (B, D and F).

Respiratory pressure and effort of breathing responses

There was no significant improvement in the pre-post intervention changes of esophageal pressures (Pes), and transdiaphragmatic pressures (Pdi) at iso-time in the IMT group compared to the sham group (Table 5.4). Pes and Pdi were not changed within-group at iso-time during the constant work rate exercise after intervention either in the IMT or the sham group compared to pre-intervention (Table 5.4).

The pre-post intervention change scores of global inspiratory muscle effort (Inspiratory Pes/Pes,sniff) and diaphragmatic effort (Pdi/Pdi,sniff) were not changed after the intervention at iso-time during the CWR cycling exercise in the IMT group compared to the sham group (Table 5.4). Pes/Pes,sniff was significantly reduced within-group after the intervention at iso-time during the CWR cycling exercise in the sham group. Pdi/Pdi,sniff was not changed within-group after the intervention at iso-time during the CWR cycling exercise in both groups (Table 5.4).

Muscle activation response

At pre-measurement, the activation of the diaphragm was similar in both groups. The activation of sternocleidomastoid and scalene were significantly higher in the sham IMT group ($p=0.0417$ and 0.0238, respectively) (Table 5.4 and Figure 5.5 C, D, E, and F). Patients in the sham IMT group activated slightly more of rectus abdominis muscle compared to the IMT group, but the values did not reach statistical significance (Table 5.4 and Figure 5.6 A and B).

There was no statistical difference of pre-post intervention change scored of diaphragm activation (EMGdi/EMGdiMax%) after the intervention at iso-time during the CWR cycling exercise in the IMT group compared to the sham group (Cohen's d effect size of 0.64). At iso-time, EMGdi/EMGdiMax% decreased within-group significantly at post-measurement for the IMT group compared to the pre-measurement. There was no within-group change in EMGdi/EMGdiMax% in the sham IMT group at iso-time at post-measurement compared to pre-measurement. However, patients in the sham IMT group activated the diaphragm slightly more towards the end of the CWR cycling test compared to the IMT group, while during resting breathing the activation of the diaphragm in the sham IMT group was slightly lower. (Table 5.4 and Figure 5.5. A and B).

The pre-post intervention changes of the extra-diaphragmatic respiratory muscles activations (EMG/EMGMax% of sternocleidomastoid, scalene and rectus abdominis) at iso-time were not changed at post-intervention in the IMT group compared to the sham group (Table 5.4, Figure 5.5 C-F, and Figure 5.6 A and B), (Cohen's d effect size 0.31, 0.11 and 0.34, respectively). The extra-diaphragmatic

respiratory muscles activations were not changed at iso-time during the CWR cycling exercise at post-measurement compared to pre-measurement in both groups (Table 5.4, Figure 5.5 C-F, and Figure 5.6 A and B).

Percent change from baseline (pre-measurement) of the tidal and maximal (during IC maneuver) activation of the diaphragm, sternocleidomastoid (SCM), scalene and rectus abdominis at iso-time during CWR cycling of the IMT group are presented in table 5.5. EMGdi/EMGdiMax% reduced from 78% at pre-measurement to 57% at post-measurement, this change was contributed by the reduction of tidal EMGdi together with the increase in EMGdiMax. For the extra-diaphragm respiratory muscles, the activation ratio (tidalEMG/EMGMax%) were similar between pre/post measurement. The tidal EMG and EMGMax of scalene and rectus abdominis were similar between pre/post measurement resulted in no change in activation ratios. However, for SCM, the unchanged activation ratio was due to an increase in both tidal and Max SCM EMG.

Table 5.5 Percent change from baseline (pre-measurement) of the tidal and maximal activation (during IC maneuver) of the diaphragm, sternocleidomastoid (SCM), scalene and rectus abdominis of the IMT group at iso-time during CRW cycling test.

	Diaphragm	SCM	Scalene	Rectus abdominis
change from baseline Tidal EMG (%)	-22	21	-0.9	-6
change from baseline EMG Max (%)	8	16	1	1
TidalEMG/EMGmax PRE (%)	78	29	33	23
TidalEMG/EMGmax POST (%)	57	33	37	23

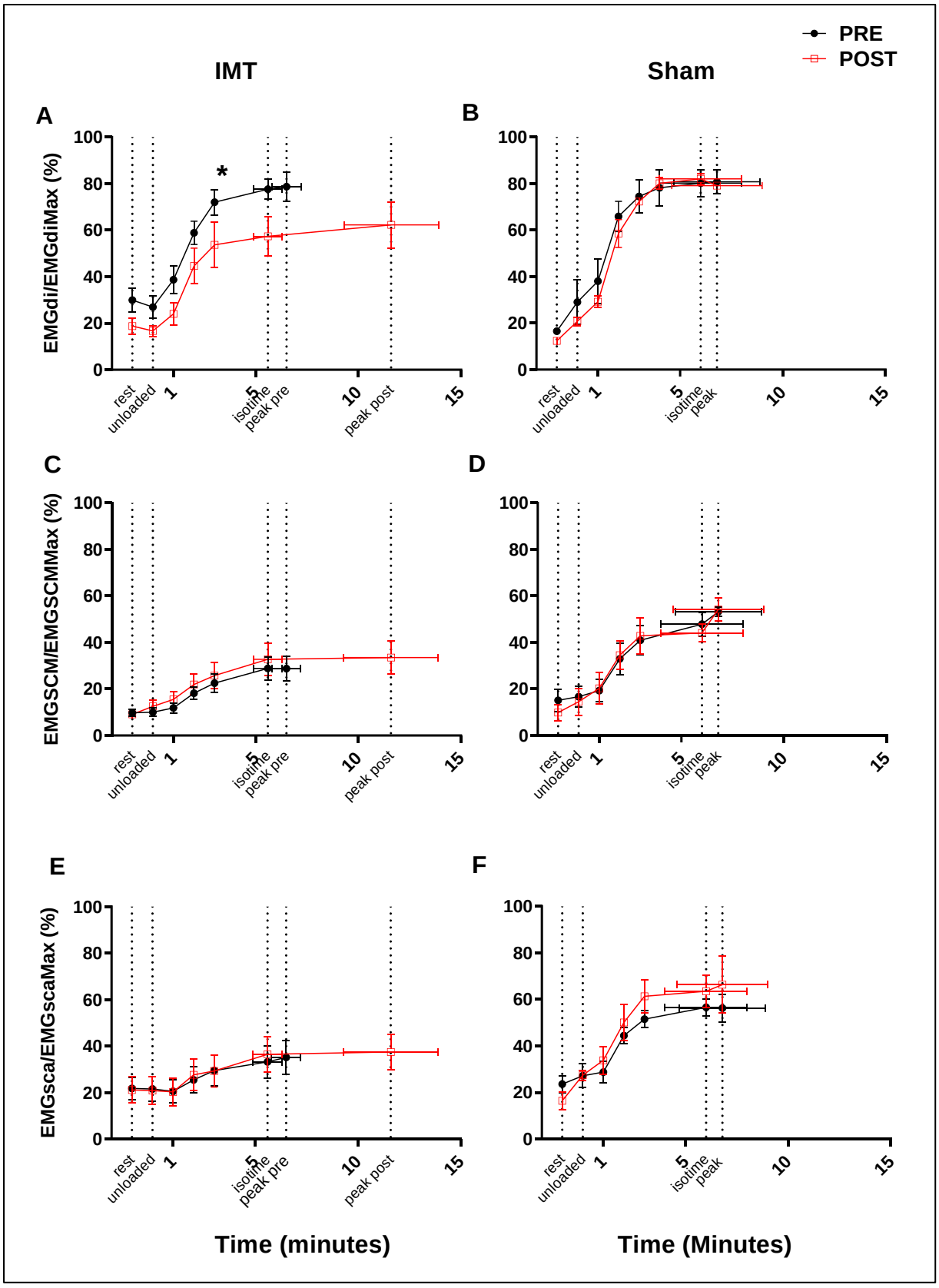

Figure 5.5 Muscle activation (EMG/EMGMax%) during rest and every minute of CWR cycling test at pre- and post-measurement of the IMT group of diaphragm (A), sternocleidomastoid (C), and scalene (E), and sham group of diaphragm (B), sternocleidomastoid (D), and scalene (F).

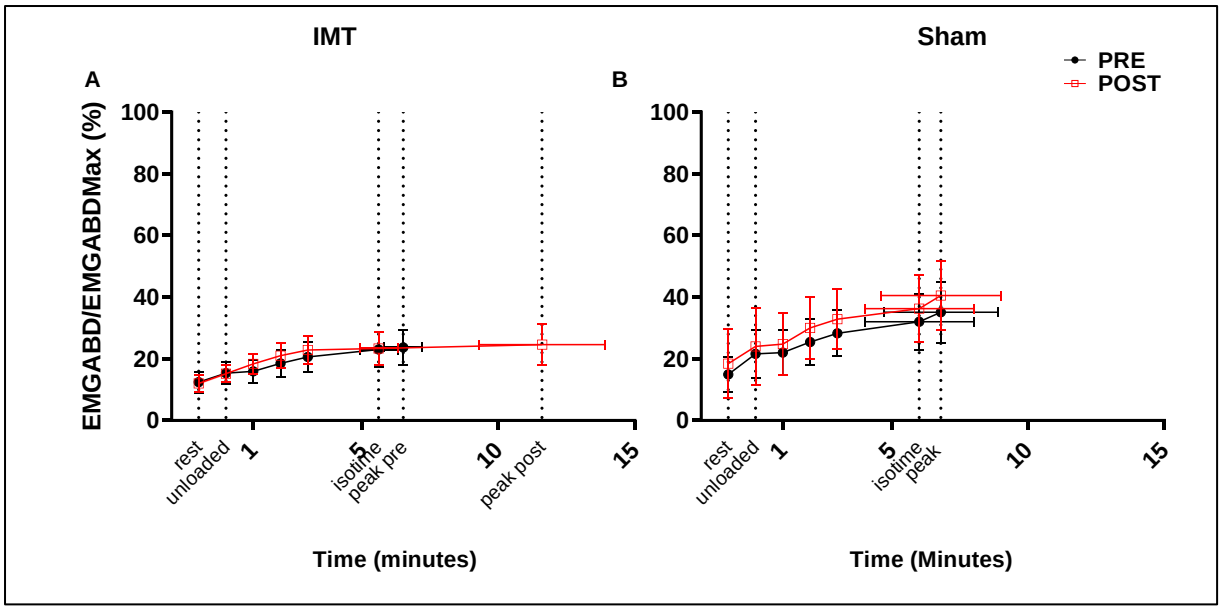

Figure 5.6 Abdominal (rectus abdominis) muscle activation (EMGABD/EMGABDMax%) during expiration at rest and every minute of CWR cycling test at pre- and post- measurement of the IMT group (A) and the sham IMT group (B).

Training responses at peak exercise during CWR cycling test

Exercise physiological response

Training responses to IMT at peak exercise during CWR cycling test are presented in Table 5.6. At the peak of the CWR cycling test, there was no significant difference in the pre-post intervention changes of oxygen consumption (VO_2), ventilation (VE), breathing pattern (Vt, BF), and oxygen saturation (SpO_2), in the IMT group after the intervention compared to the sham group (Table 5.6 and Figure 5.3 A-D). There were no changes in ventilation (VE), breathing pattern (VT, Fb), and oxygen saturation (SpO_2), in both groups after either intervention compared to pre-intervention (Table 5.6 and Figure 5.3 A-D). However, oxygen consumption (VO_2) was significantly lower at the post-measurement compared to pre-measurement in the sham IMT group. There was no significant difference in the pre-post intervention changes of tidal operating lung volumes (IRV, IC) in the IMT group compared to the sham group. Tidal operating lung volumes (IRV, IC) were not changed between pre/post measurements in both groups at the end of the constant work rate exercise (Table 5.6).

Symptom responses

There was no significant improvement in the pre-post intervention change scores of Borg scores of dyspnea, leg discomfort, and unpleasantness of breathing in the IMT group compared to the sham group (Table 5.6, Figure 5.4 A-F). There were no significant within-group differences of dyspnea, leg discomfort, and unpleasantness scores at the end of the cycling test after the intervention compared

to pre-intervention in both groups, even though the IMT group was cycling longer at the post-measurement (Table 5.6, Figure 5.4 A-F).

Respiratory pressure and effort of breathing responses

There were no significant differences in the pre-post intervention changes of esophageal pressures (Pes), and transdiaphragmatic pressures (Pdi) at the end of the CWR cycling test in the IMT group compared to the sham group (Table 5.6). Pes and Pdi were not changed at the end of the constant work rate exercise after the intervention in both groups compared to before the intervention (Table 5.6). The pre-post intervention change scores of global inspiratory muscle effort (Inspiratory Pes/Pes,sniff) and diaphragmatic effort (Pdi/Pdi,sniff) were not changed after the intervention at the end of the CWR cycling exercise in the IMT group compared to the sham group (Table 5.6). Pes/Pes,sniff and Pdi/Pdi,sniff were not changed at the end of the constant work rate exercise at post-measurement compared to pre-measurement in both groups (Table 5.6).

Muscle activation response

Diaphragm activation increased steeply at the beginning of the cycling test to the iso-time, afterwards, there was a small increase of the activation until the end of the exercise in the IMT group (Table 5.6 and Figure 5.5 A). However, there were no further increases in diaphragm activation in the sham IMT group, the activation steeply increased at the beginning of the cycling then reaching the plateau. The activation of the extra-diaphragm respiratory muscles were also increased from the beginning of the cycling until reaching the plateau and there were no further increases after the reaching plateau towards the end of the exercise in both groups even with the longer cycling time in the IMT group (Table 5.6 and Figure 5.5 C-F, Figure 5.6 A and B).

There was no statistical difference of pre-post intervention changes of all respiratory muscle activations (EMG/EMGMax% of diaphragm, sternocleidomastoid, scalene and rectus abdominis) after the intervention at the end of the CWR cycling exercise in the IMT group compared to the sham group (Table 5.6, Figure 5.5 A-F and 5.6 A and B). All respiratory muscles activations were not changed at the end of the CWR cycling exercise at post-measurement compared to pre-measurement in both groups (Table 5.6, Figure 5.5 A-F, and Figure 5.6 A and B).

Table 5.6 Responses to IMT at the end of the CWR cycle ergometer test

	Sham IMT		P values within group	IMT		P values within group	Mean difference of change Sham IMT -IMT (95% CI) or differences between medians	P values Between group
	Pre	Post		Pre	Post			
Dyspnea, Borg Units	5.8 ± 2.3	5.8 ± 2.3	>0.9999	7.5 ± 2.7	7.3 ± 2.4	0.9922	0.2 (-2.1 to 2.4)	0.8657
Leg Discomfort, Borg Units	5.2 ± 2.7	5.2 ± 2.9	>0.9999	7.9 ± 1.8	7.5 ± 2.3	0.3409	0.4 (-1.4 to 2.3)	0.6110
Unpleasantness, Borg Units	5.2 ± 2.7	5.0 ± 2.4	0.7040	7.5 ± 2.8	7.0 ± 2.6	0.6719	0.3 (-2.2 to 2.7)	0.8279
VO_2, L/min	1.16 ± 0.28 (43 ± 19)	1.10 ± 0.29 (41 ± 17)	***0.0476****	1.36 ± 0.40(48 ±16)	1.31 ± 0.39 (46 ± 15)	0.2946	-0.01 (-0.16 to 0.14)	0.8430
HR, beats/min	122 ± 34 (81 ± 19)	123 ± 37 (81 ± 21)	0.6864	124 ± 11 (79 ± 7)	126 ±16 (81 ± 10)	0.3940	-1 (-9 to 7)	0.7798
Breathing pattern								
BF, breaths/ min	34.0 ± 7.7	33.9 ± 8.0	0.9277	30.7 ± 6.8	32.0 ± 7.0	0.5994	-1.4 (-9.9 to 7.1)	0.7158
Vt, L	1.01 ± 0.28	1.11 ± 0.34	0.6312	1.43 ± 0.38	1.40 ± 0.35	0.0836	0.13 (-0.08 to 0.34)	0.2019
Ti/Ttot, %	38 ± 6	36 ± 5	0.2560	40 ± 4	40 ± 3	0.8805	-1 (-4 to 2)	0.3866
Ventilation, L/min	35.2 ± 13.1	38.0 ± 17.4	0.2735	42.2 ± 7.8	43.5 ± 8.0	0.5907	1.9 (-6.7 to 10.5)	0.6368
Ventilation, %MVV	79 ± 12	82 ± 8	0.4055	80 ± 11	83 ± 15	0.4679	1 (-13 to 15)	0.8786
Muscle activation								
EMGdi/EMGdi,max, %	81 ± 7	79 ± 2	0.6909	79 ± 15	62 ± 24	0.1055	14 (-23 to 52)	0.3728
EMGscm/EMGscmmax, %	53 ± 4	54 ± 10	0.8302	29 ± 16	34 ± 21	0.3737	-4 (-22 to 14)	0.6581
EMGsca/EMGscamax, %	56 ± 12	66 ± 25	0.5420	35 ± 22	38 ± 23	0.7118	8 (-21 to 37)	0.5613
EMGabd/EMGabdmax, %	35 ± 20	41 ± 23	0.3323	24 ± 17	25 ± 20	0.8038	5 (-9 to 18)	0.4617
Effort of breathing								
Inspiratory Pes/Pes,sniff, %	39 ± 15	33 ± 11	0.2602	31 ± 5	27 ± 9	0.0990	-2 (-11 to 7)	0.5606
Inspiratory Pdi/Pdi,sniff, %	41 ± 2	40 ± 6	0.7809	30 ± 8	26 ± 10	0.1981	1.7 (-7 to 10)	0.6486

Values are means ± SD. *p<0.05 within-group difference pre vs post intervention by paired t-test (or Wilcoxon match-pairs signed rank test). [ł]P < 0.05 by unpaired t-test (or Mann-Whitney test compares ranks) comparing treatment differences for IMT vs. sham. *Abbreviations*: VO_2 = oxygen consumption; HR = heart rate; SpO_2 = oxygen saturation by pulse oximetry; MVV = maximal voluntary ventilation; Vt = tidal volume; BF = breathing frequency; IC = inspiratory capacity; IRV = inspiratory reserve volume; Ti/Ttot = inspiratory duty cycle; EMGdi = electromyogram of the diaphragm measured during tidal inspiration; EMGdimax = the largest value of the diaphragm during a maximum inspiratory maneuver; EMGscm= electromyogram of the sternocleidomastoid measured during tidal inspiration; EMGscmmax= the largest value of the sternocleidomastoid during a maximum inspiratory maneuver; EMGsca= electromyogram of the scalene measured during tidal inspiration; EMGscamax=the largest value of the scalene during a maximum inspiratory maneuver; EMGabd= electromyogram of the abdominal (rectus abdominis) muscles measured during tidal expiration; EMGabd,max= the largest value of the abdominal (rectus abdominis) muscles during a maximum force vital capacity maneuver; Pestidal = the tidal swing of Pes; inspiratory Pes = the most negative Pes during a tidal inspiration; inspiratory Pdi = the most positive Pdi during a tidal inspiration

Responses during an IMT session of patients in the IMT group

Breathing pattern, respiratory symptoms, respiratory muscle activation, respiratory pressures and effort of breathing during an IMT session (breathing against 50% of patient's MIP) of the patients in the IMT group are reported in table 5.7.

Breathing pattern response

At the end of the IMT session (last minute) compared to resting breathing, tidal volume, and inspiratory flow were increased while the breathing frequency was decreased. The inspiratory time was increased, resulting in decreases in the duty cycle (Table 5.7).

Symptoms responses

The patients perceived higher Borg dyspnea score at the end of the IMT session compared to resting breathing (Table 5.7).

Respiratory pressure and effort of breathing responses

Esophageal pressures (Pes), and transdiaphragmatic pressures (Pdi), global inspiratory muscle effort (Inspiratory Pes/Pes,sniff) and diaphragmatic effort (Pdi/Pdi,sniff) were increased at the end of the IMT session compared to resting breathing (Table 5.7).

Muscle activation response

Inspiratory muscles activations (i.e., diaphragm, sternocleidomastoid, and scalene muscle) were increased during loaded breathing compared to resting breathing (Table 5.7, Figure 5.7). The activation of expiratory (rectus abdominis) muscles during loaded breathing was slightly increased from resting breathing but to a lesser extent compared to the inspiratory muscles (Table 5.7, Figure 5.7).

Table 5.7 Breathing pattern, muscle activation, respiratory pressures and effort of breathing during one IMT session (30 breaths) of the patients in the IMT group. The values are the average for 1 minute at rest and the end of the session.

	IMT	
	Rest	End
Pressure, cmH_2O (% MIP)	41 ± 11 (53 ± 7)	
Dyspnea, Borg Units	0.7± 1.2	4.9 ± 2.7
breathing effort, Borg Units	0.8 ± 1.1	5.3 ± 2.0
Unpleasantness, Borg Units	1.0 ± 1.4	6.0 ± 2.3
Breathing pattern		
Ventilation, L/min	12.6 ± 2.3	12.2 ± 3.9
Vt, L	0.78 ± 0.27	2.20 ± 0.92
BF, breaths/min	19.1 ± 6.9	6.1 ± 2.3
Inspiratory peak flow, L/s	0.89 ± 0.21	2.32 ± 1.00
Ti, s	1.38 ± 0.48	2.48 ± 0.97
Ti/Ttot, %	38 ± 6	24 ± 8
Muscle activation		
EMGdi/EMGdi,max, %	29 ± 19	66 ± 30
EMGscm/EMGscmmax, %	6 ± 4	63 ± 44
EMGsca/EMGscamax, %§	14 ± 14	67 ± 48
EMGabd/EMGabdmax, %	2 ± 3	6 ± 5
Pressure and effort of breathing		
Pes,tidal, cmH_2O	12 ± 4	38 ± 17
Inspiratory Pes, cmH_2O	-12 ± 5	-62 ± 26
Inspiratory Pes/Pes,sniff, %	19 ± 6	98 ± 16
Inspiratory Pdi, cmH_2O	22 ± 4	73 ± 17
Inspiratory Pdi/Pdi,sniff, %	23 ± 4	78 ± 13

Values are means ± SD. *Abbreviations*: Vt = tidal volume; Fb= breathing frequency; Ti' inspiratory time; Ti/Ttot = inspiratory duty cycle; EMGdi = electromyogram of the diaphragm measured during tidal inspiration; EMGdimax = the largest value of the diaphragm during a maximum inspiratory maneuver; EMGscm= electromyogram of the sternocleidomastoid measured during tidal inspiration; EMGscmmax= the largest value of the sternocleidomastoid during a maximum inspiratory maneuver; EMGsca= electromyogram of the scalene measured during tidal inspiration; EMGscamax=the largest value of the scalene during a maximum inspiratory maneuver; EMGabd= electromyogram of the abdominal (rectus abdominis) muscles measured during tidal expiration; EMGabd,max= the largest value of the abdominal (rectus abdominis) muscles during a maximum force vital capacity maneuver; Pestidal = the tidal swing of Pes; inspiratory Pes = the most negative Pes during a tidal inspiration; inspiratory Pdi = the most positive Pdi during a tidal inspiration

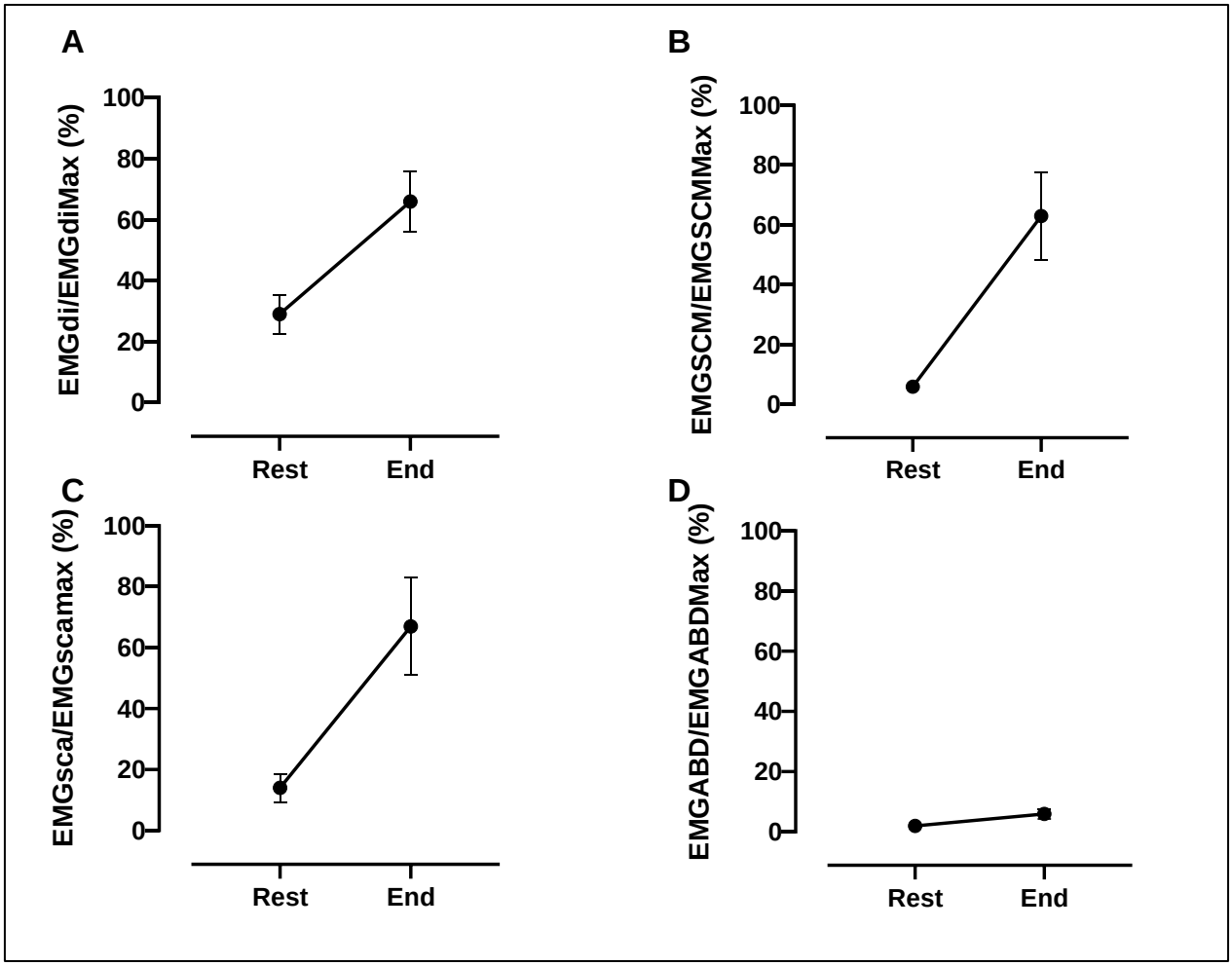

Figure 5.7 Muscle activation (EMG) during rest and the last minute of the IMT session of the IMT group; diaphragm (A), sternocleidomastoid (B), scalene (C), and abdominal (rectus abdominis) (D).

5.5 Discussion

Main findings

These preliminary results show that 8 weeks of IMT but not the sham IMT can improve inspiratory muscle strength measured from MIP, while a trend of dyspnea improvement during potential longer exercise duration was observed. These improvements after IMT during the exercise were accompanied by a potentially better function of the diaphragm and more reliance on the sternocleidomastoid muscle during exercise breathing. The dynamic breathing parameters and muscle activation during IMT session were different from those during exercise. Patients in the IMT group achieved greater tidal volume, SCM and scalene activation, pressures of breathing and breathing effort, while dyspnea rating, breathing frequency

and rectus abdominis activation were decreased at the end of IMT session compared to at the end of cycling test.

Baseline characteristics

Patients included in this study presented with significant activity-related dyspnea and exercise intolerance. Maximal incremental cardiopulmonary exercise test (CPET) confirmed low peak power output (61±18% predicted work rate), seven patients defined severe dyspnea (Borg 6) as the dominant exercise-limiting symptom (Table 5.1). Ventilatory limitation and critical inspiratory constraint were also contributed to exercise intolerance in all patients. At peak incremental exercise, the peak ventilation reached 92±15% of MVV in both groups. The inspiratory constrain was also presented in both group, at peak exercise, IRV declined to an average of 0.37±0.35 L (Table 5.1). The two groups were well matched for baseline activity-related dyspnea measured by baseline dyspnea index questionnaire (BDI).

Patients in the sham IMT group presented with more severe airflow obstruction (FEV_1 43±33% pred), greater static hyperinflation (FRC 168±14% pred, RV/TLC 63±5%) and lower diffusion capacity (47±7% pred) compared to the IMT group (Table 5.1). Of note, the sniff Pdi which indicates diaphragm function, was much better preserved in the IMT group (94±18 cmH_2O) compared to the sham IMT (72±10 cmH_2O), consequentially from the less lung hyperinflation in the IMT group (Table 5.2). The more severe pulmonary function in the sham group also contributed to greater activation of extra-diaphragmatic respiratory muscle during exercise in the sham IMT compared to the IMT group (Table 5.4, Figure 5.5 C-F, Figure 5.6 A and B). This is in line with the previous study that found that with an increase in airflow obstruction, the ventilatory pressure generation was shifted from generated by a diaphragm to inspiratory ribcage muscle with a significant contribution by the expiratory muscle (22).

Patients in the IMT group presented with higher body mass index and achieved higher VO_2 during peak incremental cycling test (VO_2 77±26%pred) (Table 5.1). A study found that increased in BMI in patients with COPD was shown to be potentially advantageous, resulting in lack of increase in dyspnea and exercise intolerance due to the alteration in elastic properties of the lung, raised intra-abdominal pressures, reduce lung hyperinflation and preserved inspiratory capacity (51). This emphasizes the potential bias of the results from the difference in baseline characteristic between patients groups.

Patients in the IMT group presented with slightly more reduction in functional capacity (6MWD = 69±16% predicted; Table 1), but slightly higher in quadriceps force compared to the sham group. The physical activity level of the patients in both groups was comparable the patients reaching more than 5000 steps

per day which above the step-defined sedentary lifestyle index (52) and enough steps to avoid severe physical inactivity in the patients with COPD (53).

The differences in baseline characteristic between the two groups presented above could lead to a discussion about the statistical analysis used in this study. The analysis of this study compared the mean of the change scores of the two groups using the unpaired t-test/Mann-Whitney test to estimate the treatment effect. However, once the average baseline scores are not the same in each group, this unpaired t-test/ Mann-Whitney test approach might not be the best suitable for the data because the test does not control for baseline imbalance. The analysis of covariance (ANCOVA) might be the better choice for the analysis. ANCOVA adjusts each patients' follow up scores by their own baseline score using the regression method, therefore, eliminate baseline imbalance (54). However, in this study, the use of ANCOVA is not appropriated at this moment due to the small sample size in these preliminary results. The assumptions needed in order to perform ANCOVA are not met, for example, the normal distribution of the data or the homogeneity of variance. Therefore, we hope that once the number of inclusion is increased, we will be able to use a more sophisticated test for the data analysis.

Effect of IMT on inspiratory muscle function at rest

After 8 weeks of training, patients in the IMT group were able to significantly improve their MIP compared to the sham group (mean difference -16 [95%CI -29, -3] cmH_2O) (Table 5.2) with the large effect size of 0.86. In the study of Langer and colleagues, applying the same regime of IMT program, a similar magnitude of improvement (mean difference -15 [95%CI -26, -3] cmH_2O) was found with the Cohen's d effect size of 1.04 (24). In the previous meta-analyses, the significant improvements in MIP of -12 to -13 cmH_2O were found in the IMT group compared to sham control (20, 21). A small increase in MIP,-4 cmH_2O, (Table 5.2) in the sham IMT group was also observed, in line with the previous study that found an improvement of -6 cmH_2O with the same sham intervention program (24). This suggests that the increase in MIP in the IMT group was not explained by the placebo effect or by the improved technique of test performance.

Even though the inspiratory muscle strength measured by Pes,sniff and Pdi,sniff was not significantly improved in the patient in the IMT group compared to the sham group, however, the values changed in the expected direction (mean between-group difference in the pre-post intervention changes of -7 [95%CI -28, 13] cmH_2O and 16 [95%CI -8, 39] cmH_2O respectively). Interestingly, the magnitude of the increase in Pdi,sniff was greater than Pes,sniff, and this accompanied with larger effect size (0.64 and 0.34 respectively), suggesting improvement in the diaphragm function in response to IMT. The improvement in

Pdi,sniff was slightly lower than what has been shown in the previous study (mean between-group difference in the pre-post intervention changes of 18 cmH_2O) (24).

There was no significant between-group difference in the pre-post intervention change scores of endurance breathing duration, power per breath, and total external work found in the IMT group compared to the control group (Table 5.2), with the small effect size. However, the endurance breathing duration (mean difference 2.2 [95%CI -3.4, 7.8] minutes), inspiratory power/breath (mean difference 1.3 [95%CI -0.9, 3.4] W), and total external inspiratory work (mean difference 52 [95%CI -234, 338] J) in the IMT group changed in the expected direction (increase) compared to the sham group (Table 5.2). This indicated that the patients in the IMT group were able to generate higher inspiratory volume and faster inspiratory flow in response to IMT. However, the mean difference of 2.2 minutes in the endurance breathing duration in the IMT compared to sham group was lower than that found in the previous study, ~6.0 minute (24). In the meta-analysis, the increased mean difference of ~4 minutes in the endurance breathing duration in the IMT compared to sham group was found (20).

The smaller improvement in between-group difference in the pre-post intervention change scores of endurance breathing duration compared to the previous study might be explained by the increase in endurance breathing duration in the sham IMT group at post-measurement compared to pre-measurement found in this study (Table 5.2; within-group difference of ~5 minutes in the IMT group and ~3 minutes in the sham IMT). The improvement of breathing duration in the sham IMT could have been, in part, the result of a learning effect. Another explanation could have been that the standardized initial resistance chosen for the endurance-breathing test was not challenging enough for the patients in the sham IMT group. From the previous protocol, the expected breathing duration at baseline during this endurance-breathing test should be in between 3-7 minutes (34). In our study, the baseline breathing time of 7 minutes in the sham IMT group was observed. Indeed this is a methodological shortcoming in the study that we could not follow the initial protocol. This emphasized the importance of choosing suitable breathing resistance for the test to avoid the ceiling effect. In contrast with the IMT group, the increase in endurance time in the sham IMT group (~ 3 minutes) was not associated with an increased power or work of breathing (Table 5.2), indicating no improvement in the quality of breathing in the sham IMT group.

Effect of IMT on Dyspnea and Exercise Performance

There was no significant between groups difference in the activity-related dyspnea measured by TDI. However, the improvement in TDI was greater in the IMT group compared to the sham group (mean

difference 1.6 unit), which exceed the minimal clinically important differences of 1 unit (55). However, in the previous study, the improvement in TDI in the IMT group was greater compared to the sham IMT (mean difference 3.1 [95%CI 0.5, 5.7] unit) (24). This lower improvement between groups of TDI in our study could be explained by the improvement in TDI in the sham group. For the within-group differences, the TDI score improved by approximately 4 units in the IMT group and 2 units in the sham IMT group. The degree of improvement in the IMT group was similar to what has been found in previous studies (24, 35, 56). However, it is difficult to explain why the improvement also occurred in the sham IMT group. This contrasts with the finding of the previous study, which found small improvement in the sham IMT group with an increased score of 1.2 unit (24). This could have been the effect of control breathing exercise during the sham IMT that, in part, could improve the activity related dyspnea.

The mMRC was used to evaluate functional impairment due to dyspnea in clinical practice. There was no significant between groups difference in the pre-post intervention change scores of mMRC scores observed. Baseline scores of mMRC were slightly higher in the sham IMT group (2.0 unit) compared to the IMT group (1.1 unit). At post-measurement, no significant within-group changes were observed in either group. The mMRC scores of both groups at baseline indicates a low symptom level, which is in contrast to the BDI score at baseline, (6.5 and 7), which indicated more severe dyspnea. It has been found that the mMRC had a moderate to poor correlation to the BDI (0.61 to 0.37), however, the magnitude of the discrepancies between these tools was not reported (57). The mMRC and BDI cannot be used interchangeably; the BDI has the potential advantage of covering the sensory component of dyspnea, which is not the case of the mMRC. Therefore, it is not uncommon that the patients who rate low symptoms in mMRC could rate high symptom in BDI (57). With the now underpowered trial, the assumption of the lack of significant improvement between groups cannot be confirmed.

There were no significant between-group differences found in the pre-post intervention change scores of cycling duration. However, the cycling duration in the IMT group compared to the sham group changed in the expected direction (increase) (mean difference 5 minutes), with medium effect size 0.50 (Table 5.2). Minute ventilation and breathing pattern (BF, Vt, and Ti/Ttot) were comparable between groups at iso-time (Table 5.4). These results are in line with the previous study that found an increase of 4 minutes cycling duration in the IMT group after the same IMT program with comparable VE, and breathing pattern (24). For the within-group differences, minute ventilation and breathing pattern (BF, Vt, and Ti/Ttot) were comparable between pre and post measurement in the IMT group. The patients in the sham IMT group breathed deeper and with the same breathing frequency compared to pre-measurement resulting in

higher ventilation at post-measurement. However, no significant difference between pre and post-measurement was found.

Interestingly, regarding the potential improvement in exercise capacity in response to IMT, the physical activity (step/day) were not improved. However, similar to the reasons why physical activity does not improve following a conventional exercise training that exercise training is not designed to change the behavior of these patients (58). It has been suggested that in patients with sufficient exercise tolerance, specific interventions that aim to change the inactive behavior is needed to improve their physical activity (59).

At iso-time, the patients in the sham IMT group reached lower minute ventilation and VO_2 during the cycling test compared to the IMT group (Table 5.4 and Figure 5.4 A, B, C, and D). This is in line with more severe airflow obstruction, greater static hyperinflation, and lower diffusion capacity in the sham IMT compared to the IMT group (Table 5.1). While the ventilatory limitation (VE/MVV%) in both groups were comparable during CWR cycling at iso-time, however, the sham IMT group presented with greater inspiratory constraint (Table 5.4). As a direct consequence, the extra-diaphragmatic respiratory muscles were recruited more in the sham group (Table 5.4, Figure 5.5 C-F, Figure 5.6 A and B).

At iso-time, there was a significant lower VO_2 at post-measurement compared to pre-measurement in the IMT group (Table 5.4). This finding is in contrast with the previous study (24) that did not find any changes in VO_2 at iso-time during the CWR cycling test. The reduction of VO_2 together with the reduction in diaphragm activation (EMGdi/EMGdiMax%) at iso-time in response to IMT might indicate a better diaphragm function to generate the same work of breathing (generated the same ventilation with the same respiratory pressures; Table 5.4).

Dyspnea, Neural Respiratory Drive, and Respiratory Muscle Activation during Exercise

There was a trend of between-group difference in the pre-post intervention change scores of dyspnea Borg score (mean difference -2.2 [95%CI -4.5, 0.2], p=0.07; Table 5.4, figure 5.4 A, B) with a medium effect size of 0.60, while the between-group difference in the pre-post intervention change scores of the diaphragm activation (EMGdi/EmGdimax%) changed in the expected direction (reduce) (mean difference -22 [95%CI -57, 13], p=0.17; Table 5.4, figure 5.4A, B), with a medium effect size of 0.64 at iso-time, despite a very small sample size of EMGdi in the sham group (n=2). With the comparable minute ventilation, breathing pattern, operating lung volume and pressure of breathing generated, the pre-post intervention changes of diaphragm activation during cycling was significantly reduced (from 78±10% to 57±21%,

p=0.046) in the IMT group compared to the unchanged activation in the sham IMT group (Table 5.4 and Figure 5.5 A, B). The pre-post intervention change scores of the diaphragm activation in the IMT group compared to the sham group was also found in the previous studies (24) with a similar degree of reduction (mean difference -24 [95%CI -39, -9] %). This reduction of percent activation of the diaphragm could have been due to the increased in EMGdiMax (inspiratory muscle strength) assumable coming from the potential neuronal adaptation leading to more recruitment of the motor units during the maximal voluntary activation (60, 61).

Of note, the potential reduction in the pre-post intervention change scores of diaphragm activation was not presented with the reduction in the pre-post intervention change scores of diaphragmatic effort (Inspiratory Pdi/Pdi,sniff%) (Table 5.4). In contrast, in the previous study, mean difference of -8 [95%CI -16, 0] % of the pre-post intervention change scores of diaphragmatic effort was found (24). There was no significant between-group difference in the pre-post intervention change scores of global inspiratory effort (Inspiratory Pes/Pes,sniff%), in contrast to the previous study that found the reduction in the pre-post intervention change scores of Pes/Pes,sniff (mean difference -9 [95%CI -16, 2]%) (24). The significant pre-post within-group reduction of -4% of global inspiratory effort was found in the sham IMT group (Table 5.4). However, at this point, it is difficult to explain what could cause this reduction (Table 5.4), possibly because of a very small sample size in this group. The pressures of breathing were analyzed from only 2 patients in the sham IMT group.

The potential reduction in EMGdi/EMGdiMax% reflects reduction of the neural respiratory drive (NRD) which closely related to dyspnea in patients with COPD (62, 63). Dyspnea occurs when the level NRD or neuromuscular effort of breathing is inappropriately high relative to pressure generating capacity that can be achieved from the mechanical/muscular response (16, 64). It was unclear whether the extra-diaphragmatic respiratory muscles were contributing to this improved in mechanical response during breathing. At iso-time during the exercise test, the pre-post intervention change scores of the extra-diaphragmatic respiratory muscles activations reported as ratios of maximal activation (EMG/EMGMax%) were not found in the IMT compared to the sham group. However, these unchanged ratios might come from the different proportion between the “tidal” EMG and the EMGMax. At post-measurement, these muscles could become stronger, therefore, the EMGmaxes were increased. To remain the same ratio of the EMG/EMGmax as in the pre-measurement, the “tidal” activation of these muscles actually needed to be higher. The higher tidal EMG indicated more motor unit recruited at post-measurement of these muscles. Therefore, the reduction in the activation of the diaphragm might also come from the

"unloading" of the diaphragm by these extra-diaphragmatic respiratory muscles. From the reason mentioned above, we looked into the data from the IMT group of our study and calculated the percent changes of tidal EMG and EMGMax from baseline of the diaphragm, sternocleidomastoid (SCM), scalene and rectus abdominis (Table 5.5).

The data in table 5.4 show that for diaphragm at post-measurement compare to pre, and there was an 8% increase of EMGdimax, however, the tidal EMGdi reduce in the much more degree. For SCM there was a 16% increase in SCMmax, however, the tidal SCM also increased in the more or less the same degree (21%) to remain the "unchanged" ratio. This indicates an increased reliance on SCM after the intervention. The changes in both EMGmax and EMG tidal for scalene and rectus abdominis were small and indicated no changes. Therefore, we concluded that the improvement in inspiratory muscle strength and potential improve in exercise capacity after IMT were a combination of the better function of the diaphragm and more reliance on the sternocleidomastoid muscle (even though EMG/EMGmax ratio remained constant).

In these preliminary results, the reduction in the pre-post intervention change scores of dyspnea sensation on Borg during CWR cycling test at iso-time in the IMT compared to the sham group did not reach the significant difference (mean difference -2.2 [95%CI -4.5, 0.2], p=0.07), with a medium effect size of 0.60. However, the magnitude of the improvement reached the minimal clinically important difference of dyspnea Borg score of 1 unit (65). Together with other pieces of evidence of the improvement in inspiratory muscle strength, quality of breathing during endurance breathing test, some degree of improvement in exercise capacity is suggested. We would assume that once the inclusions are completed with the expected sample size, the effect of IMT on dyspnea during exercise would be more evident and reach statistical significance.

The patients in the sham IMT group, in general, activated more of all respiratory muscles (diaphragm, sternocleidomastoid, scalene, and rectus abdominis muscles) compare to IMT (Figure 5.5 and 5.6). They also generated slightly higher respiratory pressure (Pes, Pdi) and effort of breathing (Pes/Pes,sniff, Pdi/Pdisniff) compared to the IMT group during both pre and post-measurement (Table 5.3). It has been proposed that the measurement of diaphragm activation can be used as an objective physiological measurement of disease severity in COPD (62). In line with this assumption, the patients in the sham IMT group presented with more severe airway disease, indicated by the lower FEV_1 %pred (Table 5.1 and 5.2). Higher dynamic hyperinflation (significantly lower in IC and lower IRV) (Table 5.2) also supports the higher activation of the diaphragm in the sham IMT group (66). The higher activation of extra-diaphragmatic muscles could also be explained by the higher level of the hyperinflation of the patients in the sham IMT

group. This was also found in a previous study that the activation of the inspiratory muscle of the rib cage and expiratory muscle increased their contribution to generating pressure when the diaphragm function is altered (22).

Dyspnea, Neural Respiratory Drive, and Respiratory Muscle Activation during an IMT session

During the IMT session compared to rest, the patients in the IMT group breathe deeper with higher inspiratory flow and lower breathing frequency (Table 5.7). Inspiratory pressures (Pes and Pdi) during the loaded breathing were also increased compared to rest (Table 5.7). The patients generated higher inspiratory flow, which enhanced inspiratory muscle shortening and resulted in higher power output, in line with previous studies (34, 67).

Tidal volume during IMT (2.20 L) was higher than that at the end of pre-measurement CWR cycling test (1.43 L), (Table 5.6 and 5.7). With the lower breathing frequency during IMT (6.1 bpm) compared to at the end of the pre-measurement CWR cycling test (30.7 bpm), the minute ventilation was also lower during IMT (Table 5.6 and 5.7). The pressures of breathing were much higher during IMT (Pes = -62 and Pdi = 73 cmH_2O) compared to at the end of the pre-measurement CWR cycling test (Pes = -18 and Pdi = 26 cmH_2O) (Table 5.4 and 5.7). The higher volume and pressure of breathing during IMT compared to during CWR cycling test contributed to higher work of breathing or energy generated by the patients (35).

During an IMT session, diaphragm activation (Table 5.7, Figure 5.7 A; EMGdi/EMGdiMax 66±44%) was lower compared to the end of cycling (Table 5.6, Figure 5.5 A; EMGdi/EMGdiMax 79±15%). The extra-diaphragmatic inspiratory muscles were activated more during the IMT session (Table 5.7, Figure 5.7 B, and C) compared to at the end of the cycling (Table 5.6, Figure 5.5 C, and E). The activation of rectus abdominis muscles during the expiration phase of the IMT session was slightly increased (4%) compared to resting breathing and the activation was much less (6±5%) compared to the activation at the peak of CWR cycling (24±17%) (Table 5.6 and 5.7, Figure 5.6 A and 5.7 D). This finding suggests different muscle activation patterns between spontaneous exercise breathing during the cycling test and breathing during the IMT session. It is important to point out that the IMT session is the replication of one session of 8 weeks IMT training program, even though the patients in the IMT group were trained with this high activation of extra-diaphragmatic inspiratory muscles for 8 weeks, however, the muscle activation pattern during CWR cycling was not changed.

The effort of breathing (Pes/Pes,sniff and Pdi/Pdi,sniff) was higher during the IMT (Table 5.7; 98±16% and 78±13% respectively) compared to the effort of breathing at peak CWR cycling (Table 5.6; 31±8% and

30±8% respectively). This confirmed that a loaded (resistive) breathing task at 50%MIP is considered as a high-intensity load IMT, a high effort of breathing from the patients is required to complete the IMT session. However, the perceived dyspnea and effort of breathing after 30 breaths of IMT (Table 5.7; Borg dyspnea 4.9±2.7 and Borg breathing effort 5.3±2.0) showed a moderate score, which reflexes the feasibility to perform the training. The maximal effort of breathing is stimulated during the IMT session, while only moderate perceived Borg score was presented.

Strengths and limitations

This study is the first that offers a more comprehensive measurement of NRD by incorporated the measurement of extra-diaphragmatic respiratory muscles (sternocleidomastoid, scalene, and rectus abdominis muscle) activation via surface EMG. This allows us to evaluate the specificity of IMT; recruitment and activation patterns of different respiratory muscles during exercise in response to the training. We also offer a comprehensive measurement of physiological variables (breathing parameters, muscle activation, detailed metabolic, pressures of breathing) that could contribute to changes in response to IMT. These variables were continuously synchronized with sophisticated data acquisition software, which allows all data to be considered by the breath-by-breath analysis.

This study is also the first study that presented the activation of the diaphragm and extra-diaphragmatic respiratory muscles during the IMT session. This information could enhance the understanding of what stimulus was given during the IMT training itself. It can also help with the elaboration on the effect of IMT during the exercise.

The main limitation of our study now is indeed the relatively small sample size. Once more data being analyzed the finding of the study can potentially change. During the inclusion period of 2 years and 6 months, there were only 16 patients participated in the study from, considering, a big pool of patients in the University hospital. Seven of the patients either refused to swallow the esophageal electrode catheter or could not tolerate the insertion of the catheter despite the used of local anesthesia due to high irritation and strong gag reflex. This reflects the difficulty of the inclusion due to the invasiveness of the method and the intense of the protocol that required a lot of effort and time from the patients to participate. We hope that once the number of inclusions is completed, more precise insight of the outcome measured can be shown.

The methodological shortcoming was also presented in this study for the endurance breathing test protocol. The baseline endurance breathing during was quite longer than initially anticipated. The

explanation could be that the resistance chosen for the test was not challenging enough for the testing. This reflects the ceiling effect when the sham control group could also improve their endurance breathing time at post measurement.

5.6 Conclusions

In patients with moderate to severe COPD, an intensive home-based and partially supervised IMT program of 8 weeks tended to improve inspiratory muscle strength with the large effect size and possibly improved endurance exercise performance and symptoms of dyspnea during exercise with the medium effect sizes. These improvements presumably due to a combination of improved functioning of the diaphragm (improvement in Pdi,sniff, and reduction of diaphragm activation during exercise with medium effect sizes) and more reliance on the sternocleidomastoid (SCM) muscle. The more reliance on the SCM potentially was contributed by higher SCM stimulation during IMT.

References

1. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380(9859):2095-128.
2. Vestbo J, Hurd SS, Agusti AG, Jones PW, Vogelmeier C, Anzueto A, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med. 2013;187(4):347-65.
3. Mannino DM, Buist AS. Global burden of COPD: risk factors, prevalence, and future trends. Lancet. 2007;370(9589):765-73.
4. Sundh J, Ekstrom M. Persistent disabling breathlessness in chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis. 2016;11:2805-12.
5. O'Donnell DE, Laveneziana P. Dyspnea and activity limitation in COPD: mechanical factors. Copd. 2007;4(3):225-36.
6. Rennard S, Decramer M, Calverley PMA, Pride NB, Soriano JB, Vermeire PA, et al. Impact of COPD in North America and Europe in 2000: subjects' perspective of Confronting COPD International Survey. Eur Respir J. 2002;20(4):799-805.
7. O'Donnell DE, Banzett RB, Carrieri-Kohlman V, Casaburi R, Davenport PW, Gandevia SC, et al. Pathophysiology of dyspnea in chronic obstructive pulmonary disease: a roundtable. Proc Am Thorac Soc. 2007;4(2):145-68.
8. Parshall MB, Schwartzstein RM, Adams L, Banzett RB, Manning HL, Bourbeau J, et al. An official American Thoracic Society statement: update on the mechanisms, assessment, and management of dyspnea. Am J Respir Crit Care Med. 2012;185(4):435-52.
9. Decramer M. Respiratory muscles in COPD: regulation of trophical status. Verh K Acad Geneeskd Belg. 2001;63(6):577-602; discussion -4.
10. Hamilton AL, Killian KJ, Summers E, Jones NL. Muscle strength, symptom intensity, and exercise capacity in patients with cardiorespiratory disorders. Am J Respir Crit Care Med. 1995;152(6):2021-31.
11. Killian KJ, Jones NL. Respiratory muscles and dyspnea. Clin Chest Med. 1988;9(2):237-48.
12. Decramer M. Effects of hyperinflation on the respiratory muscles. Eur Respir J. 1989;2(4):299-302.
13. Leblanc P, Bowie DM, Summers E, Jones NL, Killian KJ. Breathlessness and exercise in patients with cardiorespiratory disease. Am Rev Respir Dis. 1986;133(1):21-5.
14. De Troyer A, Wilson TA. Effect of acute inflation on the mechanics of the inspiratory muscles. J Appl Physiol. 2009;107(1):315-23.
15. Polkey MI, Hamnegård C-H, Hughes PD, Rafferty GF, Green M, Moxham J. Influence of acute lung volume change on contractile properties of human diaphragm. J Appl Physiol. 1998;85(4):1322-8.
16. O'Donnell DE, Ora J, Webb KA, Laveneziana P, Jensen D. Mechanisms of activity-related dyspnea in pulmonary diseases. Respir Physiol Neurobiol. 2009;167(1):116-32.
17. Moxham J, Jolley C. Breathlessness, fatigue and the respiratory muscles. Clin Med. 2009;9(5):448-52.
18. McCarthy B, Casey D, Devane D, Murphy K, Murphy E, Lacasse Y. Pulmonary rehabilitation for chronic obstructive pulmonary disease. Cochrane Database Syst Rev. 2015(2):Cd003793.
19. Spruit MA, Singh SJ, Garvey C, ZuWallack R, Nici L, Rochester C, et al. An official American Thoracic Society/European Respiratory Society statement: key concepts and advances in pulmonary rehabilitation. Am J Respir Crit Care Med. 2013;188(8):e13-64.
20. Gosselink R, De Vos J, van den Heuvel SP, Segers J, Decramer M, Kwakkel G. Impact of inspiratory muscle training in patients with COPD: what is the evidence? Eur Respir J. 2011;37(2):416-25.
21. Beaumont M, Forget P, Couturaud F, Reychler G. Effects of inspiratory muscle training in COPD patients: A systematic review and meta-analysis. Clin Respir J. 2018;12(7):2178-88.

22. Martinez FJ, Couser JI, Celli BR. Factors influencing ventilatory muscle recruitment in patients with chronic airflow obstruction. Am Rev Respir Dis. 1990;142(2):276-82.
23. Aliverti A, Cala SJ, Duranti R, Ferrigno G, Kenyon CM, Pedotti A, et al. Human respiratory muscle actions and control during exercise. J Appl Physiol. 1997;83(4):1256-69.
24. Langer D, Ciavaglia C, Faisal A, Webb KA, Neder JA, Gosselink R, et al. Inspiratory muscle training reduces diaphragm activation and dyspnea during exercise in COPD. J Appl Physiol (1985). 2018;125(2):381-92.
25. Doig GS, Simpson F. Randomization and allocation concealment: a practical guide for researchers. J Crit Care. 2005;20(2):187-91; discussion 91-3.
26. Mahler DA, Weinberg DH, Wells CK, Feinstein AR. The measurement of dyspnea. Contents, interobserver agreement, and physiologic correlates of two new clinical indexes. Chest. 1984;85(6):751-8.
27. Bestall JC, Paul EA, Garrod R, Garnham R, Jones PW, Wedzicha JA. Usefulness of the Medical Research Council (MRC) dyspnoea scale as a measure of disability in patients with chronic obstructive pulmonary disease. Thorax. 1999;54(7):581-6.
28. Hsu KY, Lin JR, Lin MS, Chen W, Chen YJ, Yan YH. The modified Medical Research Council dyspnoea scale is a good indicator of health-related quality of life in patients with chronic obstructive pulmonary disease. Singapore Med J. 2013;54(6):321-7.
29. Wanger J, Clausen JL, Coates A, Pedersen OF, Brusasco V, Burgos F, et al. Standardisation of the measurement of lung volumes. Eur Respir J. 2005;26(3):511-22.
30. Graham BL, Brusasco V, Burgos F, Cooper BG, Jensen R, Kendrick A, et al. 2017 ERS/ATS standards for single-breath carbon monoxide uptake in the lung. Eur Respir J. 2017;49(1).
31. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J. 2005;26(2):319-38.
32. ATS/ERS Statement on respiratory muscle testing. Am J Respir Crit Care Med. 2002;166(4):518-624.
33. Laveneziana P, Albuquerque A, Aliverti A, Babb T, Barreiro E, Dres M, et al. ERS Statement on Respiratory Muscle Testing at Rest and during Exercise. Eur Respir J. 2019:1801214.
34. Langer D, Charususin N, Jacome C, Hoffman M, McConnell A, Decramer M, et al. Efficacy of a Novel Method for Inspiratory Muscle Training in People With Chronic Obstructive Pulmonary Disease. Phys Ther. 2015;95(9):1264-73.
35. Langer D, Jacome C, Charususin N, Scheers H, McConnell A, Decramer M, et al. Measurement validity of an electronic inspiratory loading device during a loaded breathing task in patients with COPD. Respir Med. 2013;107(4):633-5.
36. Borg GA. Psychophysical bases of perceived exertion. Med Sci Sports Exerc. 1982;14(5):377-81.
37. Eakin EG, Sassi-Dambron DE, Ries AL, Kaplan RM. Reliability and validity of dyspnea measures in patients with obstructive lung disease. Int J Behav Med. 1995;2(2):118-34.
38. Witek TJ, Jr., Mahler DA. Minimal important difference of the transition dyspnoea index in a multinational clinical trial. Eur Respir J. 2003;21(2):267-72.
39. Mahler DA, Wells CK. Evaluation of clinical methods for rating dyspnea. Chest. 1988;93(3):580-6.
40. Hajiro T, Nishimura K, Tsukino M, Ikeda A, Koyama H, Izumi T. Analysis of clinical methods used to evaluate dyspnea in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 1998;158(4):1185-9.
41. Luo YM, Moxham J. Measurement of neural respiratory drive in patients with COPD. Respir Physiol Neurobiol. 2005;146(2-3):165-74.
42. Luo YM, Li RF, Jolley C, Wu HD, Steier J, Moxham J, et al. Neural Respiratory Drive in Patients with COPD during Exercise Tests. Respiration. 2011;81(4):294-301.
43. Luo YM, Hart N, Mustfa N, Lyall RA, Polkey MI, Moxham J. Effect of diaphragm fatigue on neural respiratory drive. J Appl Physiol (1985). 2001;90(5):1691-9.

44. Sinderby C, Beck J, Spahija J, Weinberg J, Grassino A. Voluntary activation of the human diaphragm in health and disease. J Appl Physiol (1985). 1998;85(6):2146-58.
45. ATS/ACCP Statement on Cardiopulmonary Exercise Testing. Am J Respir Crit Care Med. 2003;167(2):211-77.
46. Louvaris Z, Spetsioti S, Andrianopoulos V, Chynkiamis N, Habazettl H, Wagner H, et al. Cardiac output measurement during exercise in COPD: A comparison of dye dilution and impedance cardiography. Clin Respir J. 2019.
47. Guenette JA, Chin RC, Cory JM, Webb KA, O'Donnell DE. Inspiratory Capacity during Exercise: Measurement, Analysis, and Interpretation. Pulm Med. 2013;2013:13.
48. Neder JA, Andreoni S, Lerario MC, Nery LE. Reference values for lung function tests. II. Maximal respiratory pressures and voluntary ventilation. Braz J Med Biol Res. 1999;32(6):719-27.
49. Blackie SP, Fairbarn MS, McElvaney GN, Morrison NJ, Wilcox PG, Pardy RL. Prediction of maximal oxygen uptake and power during cycle ergometry in subjects older than 55 years of age. Am Rev Respir Dis. 1989;139(6):1424-9.
50. Troosters T, Gosselink R, Decramer M. Six minute walking distance in healthy elderly subjects. Eur Respir J. 1999;14(2):270-4.
51. Ora J, Laveneziana P, Wadell K, Preston M, Webb KA, O'Donnell DE. Effect of obesity on respiratory mechanics during rest and exercise in COPD. J Appl Physiol (1985). 2011;111(1):10-9.
52. Tudor-Locke C, Craig CL, Thyfault JP, Spence JC. A step-defined sedentary lifestyle index: <5000 steps/day. Appl Physiol Nutr Metab. 2013;38(2):100-14.
53. Depew ZS, Novotny PJ, Benzo RP. How many steps are enough to avoid severe physical inactivity in patients with chronic obstructive pulmonary disease? Respirology. 2012;17(6):1026-7.
54. Vickers AJ, Altman DG. Analysing controlled trials with baseline and follow up measurements. BMJ. 2001;323(7321):1123-4.
55. Mahler DA, Witek TJ, Jr. The MCID of the transition dyspnea index is a total score of one unit. Copd. 2005;2(1):99-103.
56. Lisboa C, Villafranca C, Leiva A, Cruz E, Pertuze J, Borzone G. Inspiratory muscle training in chronic airflow limitation: effect on exercise performance. Eur Respir J. 1997;10(3):537-42.
57. Perez T, Burgel PR, Paillasseur JL, Caillaud D, Deslee G, Chanez P, et al. Modified Medical Research Council scale vs Baseline Dyspnea Index to evaluate dyspnea in chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis. 2015;10:1663-72.
58. Blondeel A, Demeyer H, Janssens W, Troosters T. The role of physical activity in the context of pulmonary rehabilitation. Copd. 2018;15(6):632-9.
59. Troosters T, Blondeel A, Rodrigues FM, Janssens W, Demeyer H. Strategies to Increase Physical Activity in Chronic Respiratory Diseases. Clin Chest Med. 2019;40(2):397-404.
60. Hakkinen K, Newton RU, Gordon SE, McCormick M, Volek JS, Nindl BC, et al. Changes in muscle morphology, electromyographic activity, and force production characteristics during progressive strength training in young and older men. J Gerontol A Biol Sci Med Sci. 1998;53(6):B415-23.
61. Moritani T. Neuromuscular adaptations during the acquisition of muscle strength, power and motor tasks. J Biomech. 1993;26 Suppl 1:95-107.
62. Jolley CJ, Luo YM, Steier J, Reilly C, Seymour J, Lunt A, et al. Neural respiratory drive in healthy subjects and in COPD. Eur Respir J. 2009;33(2):289-97.
63. Jolley CJ, Luo YM, Steier J, Rafferty GF, Polkey MI, Moxham J. Neural respiratory drive and breathlessness in COPD. Eur Respir J. 2015;45(2):355-64.
64. Jolley CJ, Moxham J. Dyspnea Intensity: A Patient-reported Measure of Respiratory Drive and Disease Severity. Am J Respir Crit Care Med. 2016;193(3):236-8.
65. Ries AL. Minimally clinically important difference for the UCSD Shortness of Breath Questionnaire, Borg Scale, and Visual Analog Scale. Copd. 2005;2(1):105-10.

66. Sinderby C, Spahija J, Beck J, Kaminski D, Yan S, Comtois N, et al. Diaphragm activation during exercise in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2001;163(7):1637-41.
67. Villafranca C, Borzone G, Leiva A, Lisboa C. Effect of inspiratory muscle training with an intermediate load on inspiratory power output in COPD. Eur Respir J. 1998;11(1):28-33.

Chapter 6

Impact of Inspiratory Muscle Training on Respiratory and Locomotor Muscle Perfusion, Oxygenation and Locomotor Muscle Fatigue during Exercise in Patients with COPD

Sauwaluk Dacha, Zafeiris Louvaris, Lotte Janssens,

Rik Gosselink, & Daniel Langer

Preliminary results

6.1 Abstract

Background: It has been postulated that metabolites accumulating in both respiratory and locomotor muscles during high-intensity contractions can cause an increase in group III and IV afferent activity leading to a sympathetically mediated vasoconstriction of both sets of muscle vasculature (the so-called muscle metaboreflex). This sympathetic vasoconstrictor activity might contribute to limiting muscle blood flow and oxygen availability, accelerating the development of muscle fatigue and consequently limiting exercise performance. In healthy subject, it has been demonstrated that strengthening the respiratory muscles following an inspiratory muscle training (IMT) program can contribute to a delayed onset of the respiratory muscle metaboreflex by attenuating the onset of reflex vasoconstriction of limb muscles during whole-body exercise. Whether improvements in respiratory muscle function following IMT can attenuate the respiratory muscle metaboreflex at a given level of exercise in patients with COPD is unclear.

Aim and hypothesis: To evaluate the effects of 8 weeks IMT or sham training on changes in respiratory and locomotor muscle blood flow and locomotor muscle fatigue during cycling exercise in patients with COPD. The hypothesis was that 8 weeks IMT would decrease the relative load on the inspiratory muscles (by increasing maximal pressure-generating capacity) and therefore attenuate the respiratory muscle metaboreflex. This was hypothesized to result in improvements in respiratory and locomotor muscle blood flow, and reduced locomotor muscle fatigue during cycling exercise at iso-time after the intervention period in comparison to the sham training group.

Methods: Blood flow index (BFI) and muscle oxygenation ($StiO_2$) of respiratory muscles (scalene, 7^{th} intercostal, and rectus abdominis) and locomotor muscle (vastus lateralis) were assessed at rest, iso-time and at the end of exercise (task failure) during a constant work rate (CWR) cycling test (performed at 75% of peak workrate). Respiratory and locomotor muscle BFI was assessed by near-infrared spectroscopy (NIRS) in conjunction with injections of the light-absorbing tracer indocyanine green (ICG) dye. $StiO_2$ was also assessed continuously by NIRS. Central hemodynamic responses (i.e. cardiac output and systemic oxygen delivery) were also assessed. Lower limb muscle fatigue following CWR cycling was evaluated by assessing potentiated quadriceps twitch force pre and post-intervention. In addition, breathing pattern, respiratory pressure generation (Pes and Pdi), andEMG responses of respiratory muscle (diaphragm, scalene, sternocleidomastoid, and rectus abdominis) were continuously assessed during exercise.

Results: There was a potentially prolonged endurance cycling time post-intervention (mean difference in the pre-post intervention change scores 5.0 [95%CI -1.9, 11.9] min, p=0.14) in the IMT group compared to

the sham group with the Cohen's d effect size of 0.50. The pre-post intervention difference of Borg scores of dyspnea intensity and leg discomfort in the IMT group after the training compared to the sham group were changed in the expected direction (decrease) (mean difference -2.2 [95%CI -4.5, 0.2], p=0.07, Cohen's d effect size of 0.60 and -1.6 [95%CI -3.8, 0.7], p=0.15, Cohen's d effect size 0.46, respectively). There was no between-group difference in potentiated quadriceps twitch force observed at iso-time. At iso-time, absolute values of cardiac output and changes from rest in BFI and $StiO_2$ of scalene and 7^{th} intercostal were comparable at pre and post intervention between IMT and sham group at iso-time. At iso-time, there was a significant increase in the pre-post intervention difference of changes from rest of BFI of rectus abdominis muscle in the IMT group compared to sham. At post-intervention, patients in the IMT group demonstrated a further increase of the perfusion of all respiratory and locomotor muscles (scalene +1.24 ± 2.97nM/s p=0.25 [+30%], intercostal +0.85 ± 2.75nM/s p=0.36 [+24%] rectus abdominis +0.75±1.49nM/s p=0.14 [+20%], vastus lateralis +1.27± 2.09nM/s p=0.10 [+32%]), with preserved $StiO_2$ during the additional 5 minutes from iso-time towards the end of cycling exercise despite the plateaus in cardiac output and systemic oxygen delivery.

Conclusions: These results suggest that the potential improvement in endurance cycling time following IMT can probably not be attributed to improvements in respiratory or locomotor muscle perfusion and fatigue secondary to the attenuation of the muscle metaboreflex. The effects are more likely to result from a potential reduction of dyspnea sensation. Since we observed plateaus in cardiac output and systemic oxygen delivery from iso-time on to the new time to task failure of CWR cycling at post-measurement in the IMT group, the increases in perfusion of both respiratory and locomotor muscles are more likely to result from reallocation of available blood flow from other tissues to the working muscles following IMT.

Keywords: Inspiratory Muscle Training (IMT), muscle blood flow, near infrared spectroscopy, muscle perfusion, muscle fatigue, COPD

6.2 Introduction

Dyspnea and locomotor muscle fatigue are primary causes that contribute to impaired exercise capacity in patients with COPD (1-3). In addition to ventilatory, gas exchange, and circulatory impairment, respiratory and locomotor muscle strength is frequently reduced in patients with COPD (4). Respiratory muscle dysfunction is an important feature of patients with COPD. The mechanical disadvantages imposed by pulmonary hyperinflation, systemic inflammation, and oxidative stress are considered the main causes of respiratory muscle dysfunction. In addition, the adoption of a sedentary lifestyle by the patients together with other factors, such as exacerbations, and malnutrition, contribute to muscle dysfunction (5). Furthermore, in patients with COPD, a restriction in oxygen delivery to the respiratory and locomotor muscles during exercise might indicate the inability of the circulatory system to satisfy their energy demands (6). All these factors by their interplay could accelerate the development of both respiratory and locomotor muscle fatigue, contributing to exercise limitation (7-9).

During high-intensity exercise, a so-called muscle metaboreflex which is mediated by chemically sensitive nerves located in the muscle parenchyma can be activated by the accumulation of metabolites in the exercising muscles (10-12). The efferent response to metaboreflex activation can induce sympathetically mediated vasoconstriction in respiratory and locomotor muscles leading to premature termination of exercise secondary to restriction in blood flow and oxygen delivery to the working muscles, and then resulting in development of both respiratory and locomotor muscle fatigue (7). Indeed, recent notions suggest that sympathetic vasoconstrictor activity is a 2-way street which can be stimulated by both limb and respiratory muscle metaboreceptors during exercise (13) by increasing group III and IV muscle afferent activity during fatiguing contraction patterns in both muscle groups (13).

In healthy subjects, it has been demonstrated that inspiratory muscle training (IMT) can attenuate the respiratory muscle metaboreflex resulting in improvements in limb muscle blood flow, oxygenation, and delayed onset of limb muscle fatigue (14). In addition, it has been shown in healthy subjects that following six weeks of IMT, the oxygen requirements of respiratory muscle were significantly lower during isocapnic hyperpnea exercise thus providing an insight into the mechanism(s) explaining the reported improvements in whole-body endurance performance following IMT (15). A study also showed that in patients with chronic heart failure (CHF), IMT could improve blood flow to resting and exercising limb muscle along with the improvements in exercise capacity (16).

In patients with COPD, improvements in respiratory muscle function after IMT can be translated into improvements in exercise capacity (17-20), however, the exact mechanisms underlying these improvements are not entirely clear. A study demonstrated a reduction in leg muscle fatigue after respiratory muscle unloading (proportional assist ventilation, heliox, and supplemental O_2) in patients with COPD (21). The effects of IMT (i.e. respiratory muscle strengthening) on respiratory and locomotor blood flow and leg muscle fatigue have not yet been studied in this patient population. Along these lines, whether high-intensity IMT can attenuate the respiratory and locomotor muscle metaboreflex resulting in improvements in respiratory and locomotor muscle blood flow and decrease in locomotor muscle fatigue at a given level of exercise has not been investigated before in patients with COPD.

Accordingly, the aims of the study were to evaluate the effect of eight weeks of high-intensity IMT in comparison with sham training on changes in respiratory and locomotor muscle blood flow, oxygenation and locomotor muscle fatigue during cycling exercise. We hypothesized that eight weeks of IMT would decrease the relative load on the inspiratory muscles (by increased maximal pressure-generating capacity) and therefore attenuate the respiratory muscle metaboreflex that would be manifested improvements in respiratory and locomotor muscle blood flow, and reduced locomotor muscle fatigue during cycling exercise at iso-time as compared to the sham training.

6.3 Materials and methods

This study is a part of the larger clinical trial (NCT03240640; S58513). The subjects included in this study were the same patients who participated in the same clinical trial in chapter 5. The inclusion/exclusion criteria and the initial screening for eligibility for the study were explained in chapter 5. The measurement of cardiopulmonary exercise testing (CPET), pulmonary function and respiratory muscle, EMG measurement and analysis, and CWR cycling test were performed according to the explanation in chapter 5.

6.3.1 Study design

The patients performed a cardiopulmonary exercise test (CPET), and a constant work rate (CWR) cycling test. The protocol of CWR cycling test was described in chapter 5. In short, the patient was cycling at 75% of their maximal work rate during CPET until symptom limitation. The CWR cycling in chapter 5 was performed until symptom limitation pre and post measurement in order to allow subjects to reach a new time to task failure. To evaluate locomotor muscle fatigue, an additional CWR cycling test was performed with the measurement of locomotor muscle fatigue before and after the cycling. At post-measurement,

this additional CWR test was stopped at the time limit of the pre-intervention cycling test in order to evaluate changes in leg fatigue at iso-time.

6.3.2 Muscle perfusion and oxygenation

Respiratory and locomotor muscle blood flow was assessed by near-infrared spectroscopy (NIRS) in conjunction with the light-absorbing tracer indocyanine green (ICG) dye. Muscle oxygenation ($StiO_2$) was continuously assessed by NIRS (22, 23).

6.3.2.1 Near-infrared spectroscopy (NIRS)

NIRS is a noninvasive measurement of oxygen-related indexes in the microcirculation. The principle of NIRS measurement is based on the distinctly different absorbance of infrared light (wavelength 700-900 nm) by oxygenated (HbO_2) and deoxygenated (Hb) hemoglobin. A NIRS probe consists of the emission and receiving optodes of the NIRS light, which incorporated the specific wavelengths of infrared (24). These two optodes (i.e., emission and receiving) were attached in the area of interest with a maximum separation distance of 40 mm that allows a penetration depth of the NIRS light into the body of 20 mm. Muscle oxygen saturation index ($StiO_2$) expressed in percentage (%), is the ratio of oxygenated to total tissue hemoglobin concentration expressed as *[(oxyhemoglobin/(oxyhemoglobin+deoxyhemoglobin)] × 100%* and commonly adopted as an absolute index of oxygen availability that depict the dynamic balance between local tissue oxygen supply (delivery) and utilization (extraction)(25, 26). In this study, two commercial Near-Infrared Spectroscopy (NIRS; NIRO-200 and a NIRO-200NX; HAMAMATSU Photonics KK) devices were used that both incorporate the Spatially Resolved Spectroscopy method (SRS) and the Modified Beer-Lambert method (MBL) in a near-infrared spectrum of 735, 810, and 850 nm. NIRS optodes were placed at the right posterior triangle of the neck, left 7^{th} intercostal space, abdominal (rectus abdominis) and left anterolateral upper leg to measure scalene, intercostal, rectus abdominis, and vastus lateralis muscles perfusion respectively. Arterial blood gas analysis was performed at baseline to acquire hemoglobin values for $StiO_2$ calculation.

6.3.2.2 Blood flow index (BFI) by NIRS and Indocyanine green (ICG) injection (NIRS-ICG)

NIRS-ICG is a reliable, robust, and essentially noninvasive tool for assessing relative changes in respiratory and locomotor muscle perfusion in healthy and patients with COPD (23, 27). To obtain BFI, intravenous injection of ICG dye was performed via a venous catheter placed at the antecubital forearm in line as previously validated for patients with COPD (23). ICG injections were performed at rest and at the last 30

seconds of CWR cycling. The concentration curve of ICG was recorded on the NIRS device. BFI was calculated by dividing the ICG peak concentration (nM) by the rise time (second) from 10-90% of peak (23). At post-measurement, the ICG was injected at rest and at the same time limit of the pre-test as well as at the end of exercise if prolonged (i.e., post-peak).

6.3.3 Central hemodynamics

Cardiac output, heart rate, and stroke volume were continuously measured by impedance cardiography device (PhysioFlowPF50; Manatec Biomedical, Macheren, France) previously validated for COPD patients (28). Estimated systemic oxygen delivery was calculated by the product of cardiac output and arterial oxygen content; the latter was calculated as the product of 1.39 x hemoglobin concentration [Hb] and $\%SpO_2$ (29). Hemoglobin concentration [Hb] values were obtained from arterial blood sample from all patients.

6.3.4 Assessment of quadriceps strength and fatigue

Locomotor exercise-induced quadriceps contractile fatigue was measured by supra-maximal stimulation of the femoral nerve with a double twitch stimulation technique. Maximum voluntary contraction (MVC) and potentiated quadriceps twitch force (Ptw) were assessed at baseline and after 8 weeks IMT using the previously established protocol (30, 31). The patient was sitting in a recumbent chair with 120° hip extension and 90° knee flexion, both arms crossed on the chest, with a waist strap to the chair. The right leg of the patient was attached at the ankle level to a fixed strain gauge dynamometer connected with signal analog force transducer (546QD; CDS Milan, Italy) and amplifier (Biopac MP150; Biopac Systems, Goleta, California, USA). The strain gauge signals of quadriceps force (kg) were stored on the computer via AcqKnowledge data acquisition software (AcqKnowledge 4.0, BIOPAC Systems, Inc., Santa Barbara, CA, USA). The femoral nerve was stimulated through a 45-mm, figure-of-eight coil powered by a double Magstim stimulator (Magstim Co Ltd, Whitland, UK). Twitch forces were measured at 30, 50, 70, 80, 90, 95 and 100% of the maximum stimulator output. Maximality of the non-volitional contraction was ensured by that the twitch force did not further increase between 90% and 100% of the power output (supramaximal stimulation). The patient performed five isometric maximal voluntary contractions (MVC) for 3 seconds. Superimposed twitches were obtained during the preceding MVC to ensure maximal potentiation. TWqpot was systematically measured 3 seconds after the end of each MVC maneuver. The highest TWqpot was taken for the calculation.

The twitch measurements were performed at rest, 10 and 35 minutes after CWR cycling test to symptom limitation. At post-measurement, after 8 weeks of training, the patients were asked to stop cycling at the same end time of the pre-measurement (iso-time). A fall of TWqpot more than 15% at 10 minutes after

CWR cycling test was defined as significant contractile fatigue (31). Ptw at 35 minutes after the cycling test was measured to investigate the recovery of fatigue after exercise (21).

6.3.5 Statistical analysis

Responses were compared at an iso-time point of the exercise (i.e., the end time of the shortest cycling test), between pre and post IMT intervention thus ensuring that the leg muscles performed the same amount of work. The detail of the statistical analysis is described in chapter 5. In short, paired t-tests were used to compare responses at the comparable exercise time point (i.e., rest; iso-time) between baselines and post eight weeks intervention. In case the normality was not met, or the sample size was too small to test for normality, the nonparametric tests were performed. The level of significance for all analyses was set at $P < 0.05$.

6.4 Results

Preliminary results of intermediate analyses are reported in this chapter. Baseline characteristics at study enrolment are presented in chapter 5. Additional baseline variables regarding central hemodynamic parameters are presented in Table 6.1. Hemodynamic parameters at rest were comparable between the two groups at baseline. Hemoglobin values were in the normal range in all the patients.

Table 6.1 Baseline characteristics (note: other baseline values are presented in Table 5.1, chapter 5)

	All subjects (n=16)	Sham IMT (n=5)	IMT (n=11)
Resting central hemodynamic			
Oxygen uptake (VO_2), l /min	0.32 ± 0.09	0.31 ± 0.12	0.33 ± 0.08
Heart rate, bpm	80 ± 12	77 ± 8	82 ± 14
SpO_2, %	94.8 ± 1.8	95.4 ± 1.1	94.5 ± 2.1
Cardiac output, l/min	5.51 ± 1.35	5.33 ± 1.60	5.59 ± 1.31
Stroke Volume ml/beat	68.7 ± 13.0	67.9 ± 14.5	69.1 ± 13.0
Arterial O_2 content (CaO_2), mlO_2/dl	18.93 ± 1.76	18.38 ± 0.77	19.18 ± 2.04
Systemic O_2 delivery, lO_2/min	1.03 ± 0.24	0.98 ± 0.30	1.06 ± 0.22
Hemoglobin, g/dl Men 14.0-18.0 Women 12.0-16.0	 14.8 ± 1.6 13.9 ± 0.4	 13.8 ± 0.6 13.9 ± 0.6	 15.0 ± 1.7 13.8 ± 0.2
Symptom-limited incremental cycle ergometer exercise test			
Peak Cardiac output, l/min	12.02 ± 2.16	11.58 ± 2.33	12.23 ± 2.17
Nadir SpO_2, %	89.3 ± 4.0	90.6 ± 4.8	88.7 ± 3.7

Central hemodynamic and metabolic responses (VO_2) during CWR cycling exercise

Central hemodynamic parameters during cycling for both groups are presented in Table 6.2. No significant within-group difference were found in hemodynamic parameters between pre versus post measurements

(Table 6.2) in either group. Change from rest to iso-time of arterial oxygen saturation (SpO_2), and systemic oxygen delivery (Figure 6.1 A and B) during CWR cycling were also comparable between pre versus post measurements within each group. The values at the new time to task failure during cycling exercise (Post Peak) of the IMT group are also presented in Figures 6.1 A and 6.1 B, and in Table 6.2. For the IMT group, all central hemodynamic values at Post Peak were not significantly different from those at iso-time (Figures 6.1 A and 1B, and Table 6.2).

Table 6.2 Central hemodynamic responses during CWR exercise before (PRE) and after the intervention period (POST) both at iso-time and at the new time to task failure (Post Peak).

ISO-time	Sham IMT (n=5)			IMT (n=11)			
	PRE	POST (ISO-time)	differences	PRE	POST (ISO-time)	differences	POST (Peak)
Cardiac output, l/min	11.55 ± 3.68	11.77 ± 3.38	0.22 ± 1.27	11.50 ± 2.38	11.93 ± 2.80	0.41 ± 1.60	12.03 ± 2.97
Heart rate, bpm	122 ± 34	123 ± 33	1 ± 5	124 ± 11	119 ± 38	-4 ± 10	126 ± 16
Stroke Volume ml/beat	94.3 ± 12.9	86.4 ± 13.6	-7.3 ± 8.7	94.4 ± 16.1	99.4 ± 18.3	6.0 ± 16.0	94.8 ± 16.3
Mean arterial blood pressure, mmHg	113 ± 14	116 ± 11	2 ± 7	114 ± 11	113 ± 10	-1 ± 11	117 ± 11
SpO_2, %	88.0 ± 5.8	88.6 ± 4.5	1.1 ± 1.4	89.5 ± 3.6	89.5 ± 4.5	-0.9 ± 4.2	88.6 ± 4.1
Arterial O_2 content (CaO_2), mlO_2/dl	16.85 ± 0.62	17.07 ± 0.44	0.22 ± 0.28	17.95 ± 2.14	18.17 ± 2.32	0.23 ± 0.84	18.01 ± 2.20
Systemic O_2 delivery, lO2/min	1.95 ± 0.63	2.00 ± 0.61	-0.10 ± 0.18	2.06 ± 0.36	2.13 ± 0.47	0.08 ± 0.35	2.13 ± 0.52

Values are means ± SD. *p<0.05 within-group difference of pre vs post measurement paired t-test (or Wilcoxon match-pairs signed rank test).
Abbreviations: SpO_2 = oxygen saturation by pulse oximetry.

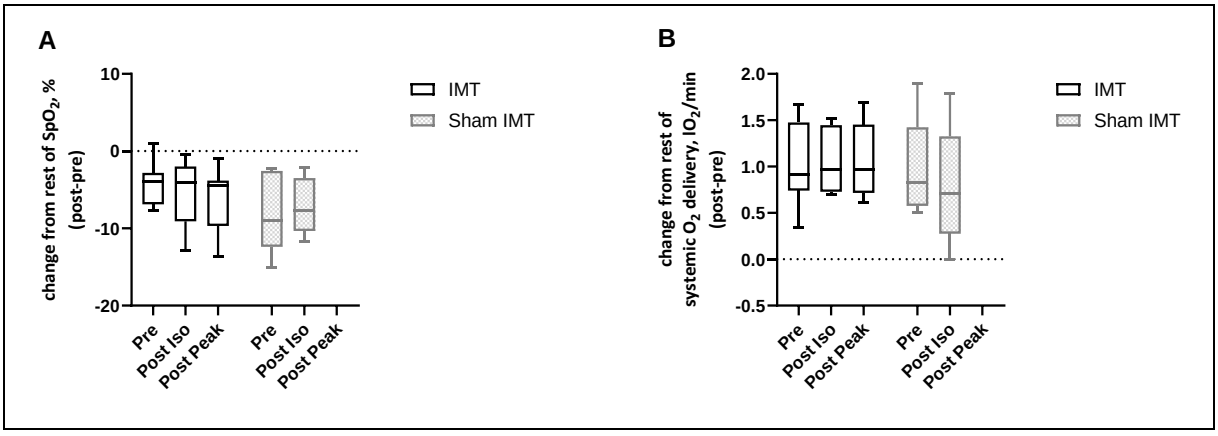

Figure 6.1 Values are presented as median, min and max. Change from rest to iso-time and peak exercise of arterial oxygen saturation pre and post 8 weeks training measured during CWR cycling A; Change from rest to iso-time and peak exercise of systemic oxygen delivery pre and post 8 weeks training measured during CWR cycling B.

Blood flow index (BFI) and oxygenation ($StiO_2$) of respiratory and locomotor muscles during CWR cycling exercise

Changes from rest of the respiratory and locomotor muscles blood flow index (BFI, nM/s) at iso-time pre and post-intervention during CWR cycling test for IMT and sham group are presented in Figure 6.2. The

changes from rest to iso-time in BFI of scalene, 7th intercostal, and vastus lateralis muscles at pre and post-intervention were comparable between the IMT and sham group. There was a significant between-group difference in BFI of rectus abdominis muscles (Figure 6.2 C).

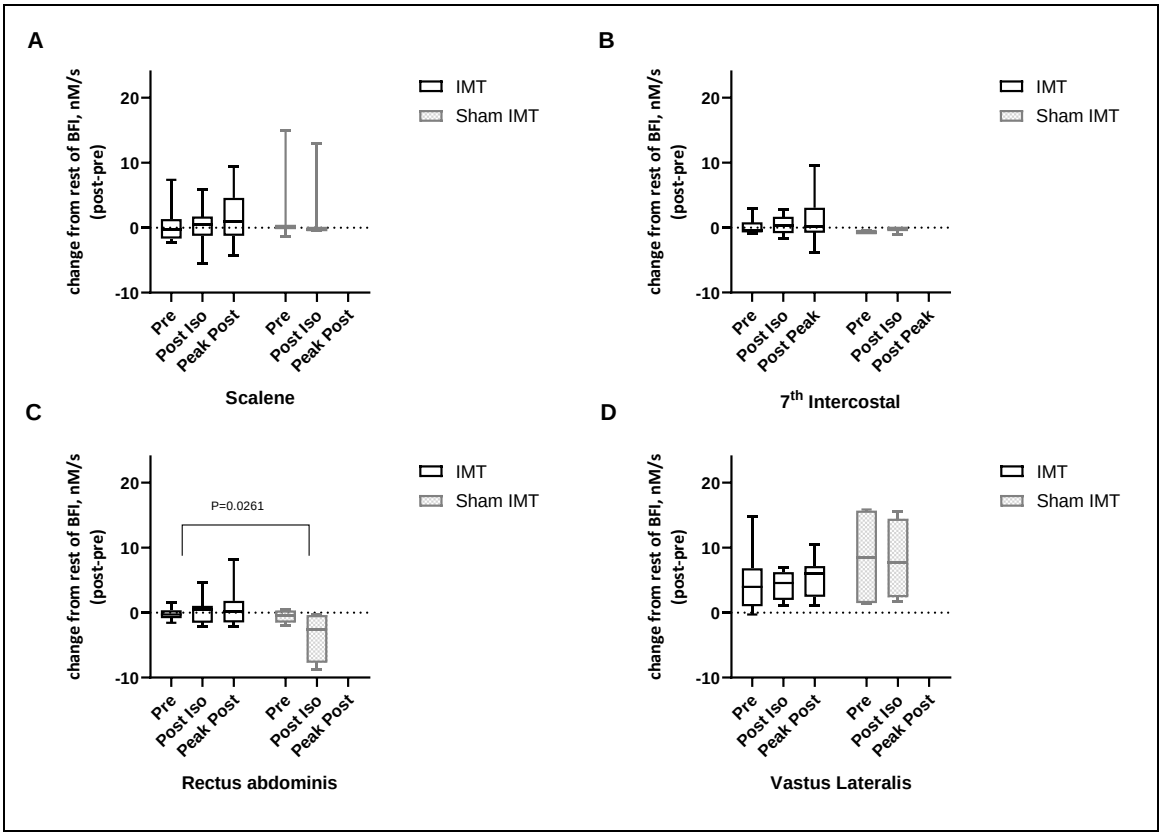

Figure 6.2 Values are presented as median, min and max. Change from rest to iso-time of blood flow index (BFI, nM/s) pre and post 8-week training during cycling of Scalene A; 7th intercostal B; rectus abdominis muscle C; and quadriceps D.

Changes from rest in muscle oxygenation ($StiO_2$) of respiratory and locomotor muscles pre and post 8-week intervention during CWR cycling test are presented in Figure 6.3. At iso-time following IMT, inspiratory, expiratory, and leg muscle oxygenation remained comparable to that observed before IMT (Figure 6.3).

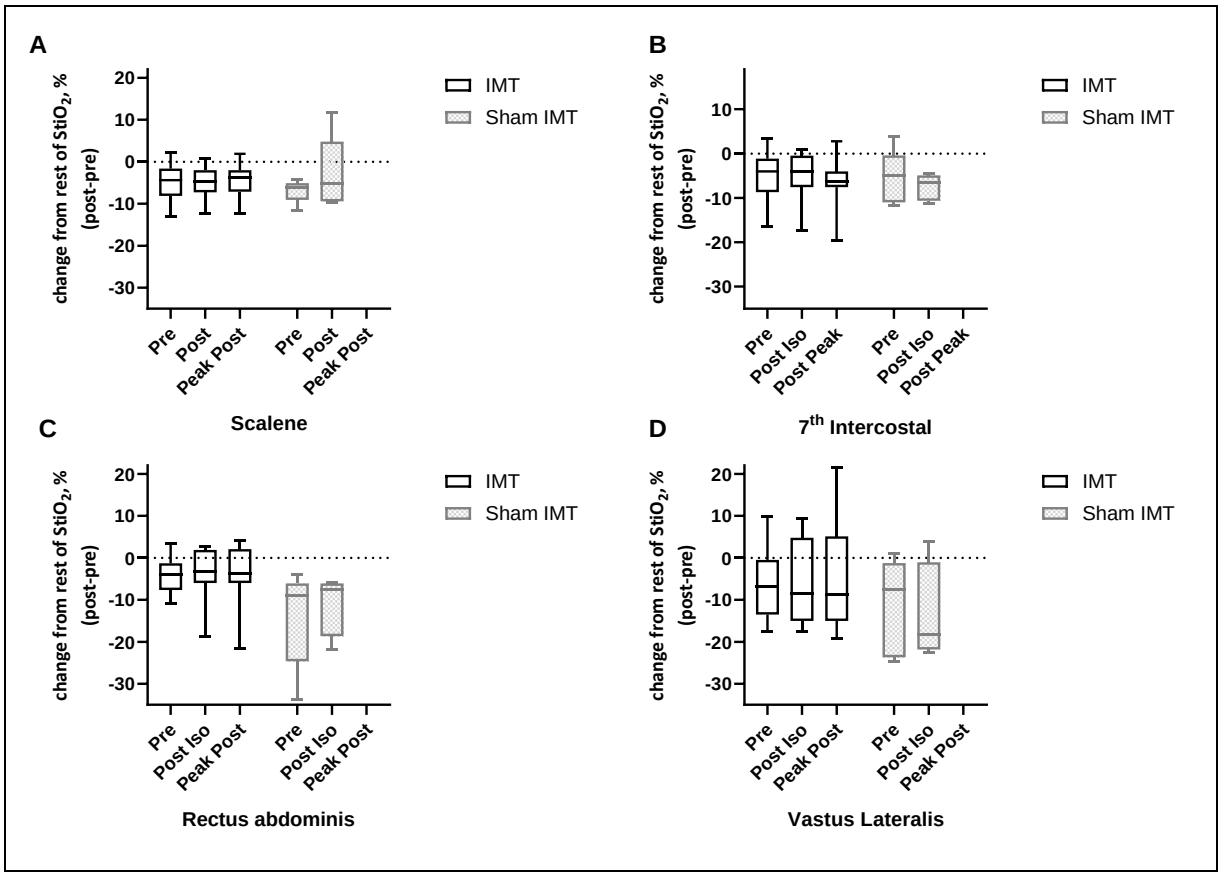

Figure 6.3 Values are presented as median, min and max. Changes from rest to iso-time of muscle oxygen saturation ($StiO_2$, %) pre and post 8-week training during cycling of Scalene A; 7th intercostal B; rectus andominis muscle C; and quadriceps D.

Central and local hemodynamic and oxygenation responses during CWR cycling for the IMT group at the end of (peak) exercise

Figure 6.4 presents the changes from rest in CO, BFI, and $StiO_2$ during CWR cycling exercise in the IMT group at rest, iso-time pre and post-intervention, and the end of (peak) exercise post-intervention. At iso-time CO was increased ~6 L at pre and post measurements, with no further increase at the end of exercise (Figure 6.4 A) during post measurements in the IMT group. At the end of cycling exercise post-intervention, patients in the IMT group demonstrated a further increase of the perfusion of all respiratory and locomotor muscle as compared to that at iso-time (Figure 6.4 B, D, F and H) (scalene +1.24±2.97nM/s p=0.25; [+30%], intercostal +0.85±2.7nM/s p=0.36; [+24%] rectus abdominis +0.75±1.49nM/s p=0.14; [+20%] (vastus lateralis +1.27±2.09 nM/s p=0.10; [+32%]) while $StiO_2$ values for scalene, 7th intercostal, rectus abdominis and vastus lateralis muscles remained preserved and comparable to the values recorded at iso-time (Figure 6.4 C, E, G, and I).

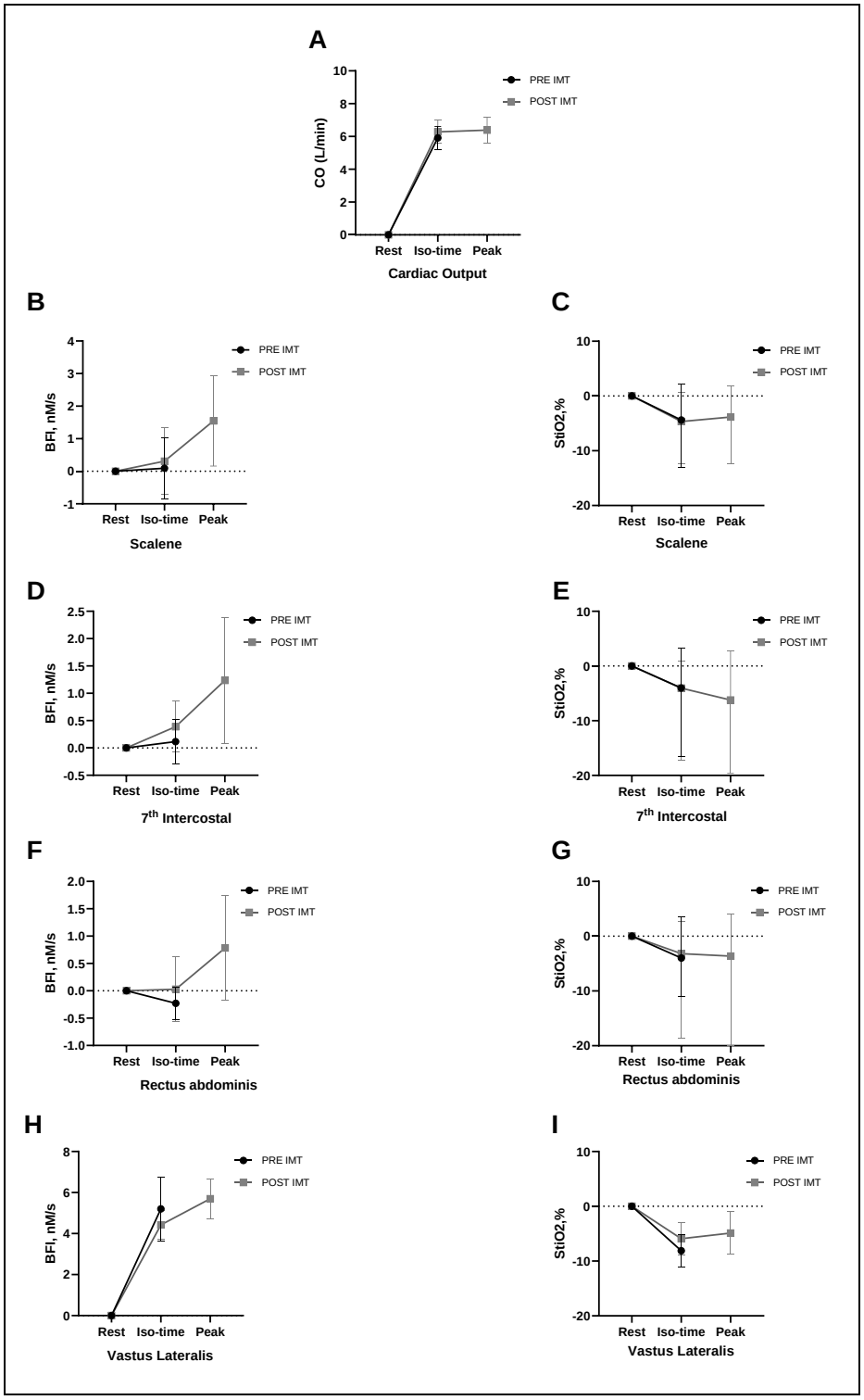

Figure 6.4 Change from rest of cardiac output at rest, iso-time and end exercise during CWR cycling test at pre and post measurement of the IMT group A; change from rest of BFI at rest, iso-time and end exercise during CWR cycling test at pre and post measurement of scalene B; 7th intercostal D; Abdominal (rectus abdominis) F; and Vastus Lateralis H; change from rest of $StiO_2$ at rest, iso-time and end exercise during CWR cycling test at pre and post measurement of scalene C; 7th intercostal E; Abdominal (rectus abdominia) G; and Vastus Lateralis I.

Lower limb muscle fatigue following CWR cycling

Potentiated quadriceps twitch forces (PtW) expressed as “percent change from pre-exercise baseline” at 10 minutes after CWR cycling test are presented in Figure 6.5. There was no difference in the percentage change of PtW between pre-post interventions among the two groups.

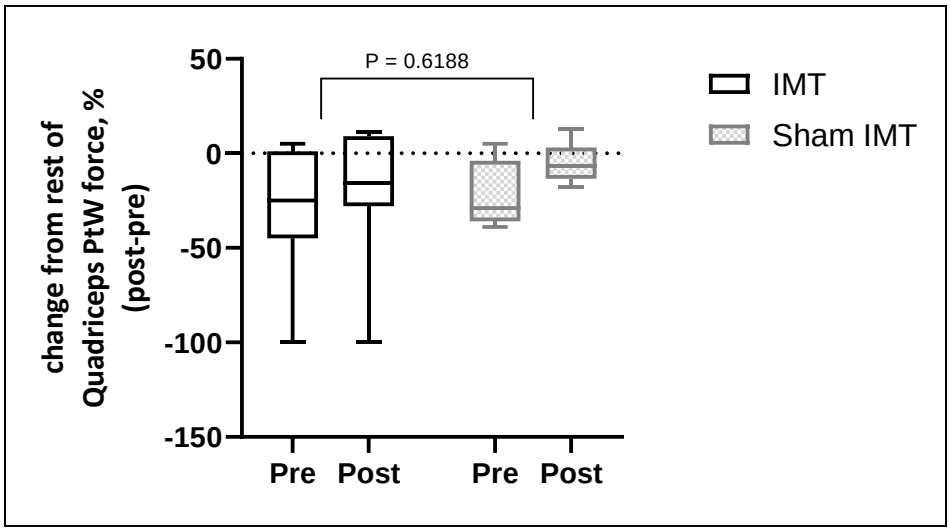

Figure 6.5 Values are presented as median, min, and max. Potentiated quadriceps twitch force (PtW) expressed as differences from pre-exercise baseline to 10 minutes after CWR cycling test of both groups pre and post-intervention.

6.5 Discussion

Main findings

The main findings of this study is that in response to IMT, respiratory and locomotor muscle blood flow, muscle oxygenation, and limb muscle fatigue were not changed in the IMT group compared to the sham group at iso-time during post-intervention cycling (Figures 6.2, 6.3 and 6.5). However, there was a potential improvement in endurance cycling duration accompanied by a trend of reduction in dyspnea sensation and potential reduction in leg discomfort (results reported in chapter 5 table 5.4) at post-measurement in the IMT group compared to the sham group. Despite plateaus in cardiac output and systemic oxygen delivery from iso-time to the new time to task failure of CWR cycling test in the IMT group after the intervention, there was a further increase in the perfusion of all respiratory and locomotor muscles (Figure 6.4).

Patient characteristics, functional capacity, physical activity, and locomotor muscle strength

Central hemodynamic variables were comparable between the two groups at rest. The hemoglobin level of the patients in both groups was in the normal range, indicating that there was sufficient hemoglobin to carry oxygen to individual cells in the body. At post-measurement, there was no significant difference in functional capacity, locomotor muscle strength, and physical activity in both groups (results reported in chapter 5, Table 5.1). The patients in both groups reduced their daily step count slightly at post-measurements (results reported in chapter 5), excluding a major role for changes in physical activity levels to contribute to changes is physical fitness.

Central hemodynamic response during CWR cycling

At iso-time, there was no significant change in central hemodynamic parameters between pre/post-intervention in each group (Table 6.2, Figure 6.1 A, and B). At (new) peak cycling test at post-measurement of the IMT group, these hemodynamic values were also comparable with those at the iso-time (Table 6.2, Figure 6.1 A, and B). This suggests no effect of IMT or sham IMT on central hemodynamic values after eight weeks of training. These findings are in line with previous studies that investigated the effects of inspiratory muscle training in patients with heart failure with preserved ejection fraction on echocardiogram parameters. Specifically, a meta-analysis (32) that included three randomized controlled trials, one non-randomized controlled trial, and one pre-post study (total of 228 individuals) indicated no significant improvements in any of echocardiographic parameters such as diastolic function, ventricular volumes and ejection fraction following IMT. Two more recent randomized studies in patients with CHF supported the previous findings by showing that despite a significant increase in exercise capacity of the

patients randomized to IMT, this increase was not associated with improvements in the diastolic function of the heart (33, 34).

Respiratory and locomotor muscles perfusion (BFI) and oxygenation ($StiO_2$), and locomotor muscle fatigue during CWR cycling exercise at iso-time

At iso-time during cycling exercise, there were no differences in changes from rest to iso-time of BFI and $StiO_2$ observed in any of the muscles in the IMT group compared to the sham group (Figure 6.2 and 6.3), except for a significant increase in change from rest to iso-time of BFI to rectus abdominis muscle in the IMT group compared to the sham group (p=0.026, Figure 6.2 C). This between-group difference in change from rest to iso-time of BFI to rectus abdominis muscle was mainly influenced by a reduction in BFI to rectus abdominis muscle at post-measurement in the sham group, which was not expected and might partly be explained by the small sample size. The differences in changes from rest to iso-time of $StiO_2$ to rectus abdominis muscle between the IMT and the sham group was also comparable. Despite no changes in cardiac output and systemic oxygen delivery found at iso-time in both group (Table 6.2), this finding seems to indicate that at iso-time perfusion and oxygenation of all muscles was not influenced by the IMT intervention. There was no significant between-group difference in the pre-post intervention change of percent change from pre-exercise baseline of potentiated quadriceps twitch force (PtW) at 10 minutes after iso-time CWR cycling test (P=0.6188; Figure 6.5). This indicates no effect of IMT or sham IMT on locomotor muscle fatigue at iso-time of CWR cycling test.

Several studies showed that unloading respiratory muscles (reduce work of breathing) using heliox, oxygen or proportional assisted ventilation can improve respiratory and locomotor muscle blood flow and oxygenation during exercise, reduce dyspnea and increase endurance exercise duration in patients with COPD (8, 29, 35-37). In addition, by supplementing oxygen, heliox, or using proportional assisted ventilation, the reductions in locomotor muscle fatigue was observed (21). However, in our study the potential improvement in endurance cycling duration, trend of improvement in dyspnea sensation and potential reduction in perceived leg discomfort during exercise (results reported in chapter 5 Table 5.2 and 5.3) were presented in the absence of changes in leg muscle perfusion, oxygenation, and objectively measured leg muscle fatigue (quadriceps twitch force).

Several factors could be speculated to explain these unexpected findings. First, the impact of better respiratory muscles capacity (significant improvement in MIP, chapter 5; Table 5.2) on exercise limitation is not as great as the impact of respiratory muscles unloading. The stronger respiratory muscles after IMT could perhaps delay the fatiguing respiratory muscle contraction pattern during exercise to some degree,

as indicated by potential prolonged endurance exercise and reduction in symptoms. However, as the cycling exercise prolongs, the continuously increasing load on the respiratory muscle (due to dynamic hyperinflation) could potentially stimulate a fatiguing respiratory muscles contraction pattern and contribute to the early development of leg muscle fatigue via respiratory muscle metaboreflex (38).

Secondly, in our patients, the symptoms of dyspnea might be the most dominant contributor to exercise limitation rather than leg fatigue. From the results presented in chapter 5, 75% of the patients in the IMT group report dyspnea or combination of dyspnea and leg discomfort as the main reason to stop cycling. They also rated the dyspnea intensity at the new peak time to task failure at post-measurement the same as in the rating at the peak of pre-measurement, indicating that dyspnea is the main factor of limiting exercise. The example can be made by comparing the results of our study to the study of Amann et al. (21). The patients in our study presented with more reduction in respiratory muscle strength and more static hyperinflation. During the baseline cycling test with the comparable workrate the patients in the study of Amann could cycle for 3 minutes longer. Even though the main symptom of stopping exercise in both studies was dyspnea, however, in our study, the dyspnea symptom was more pronounced and occurred earlier compared to the study of Amann. Therefore, it could have been that at the moment of stopping exercise in our study, the muscle metaboreflex was not yet stimulated by fatigue of the respiratory muscle.

The findings from our study so far did not support the hypothesis regarding the impact of IMT on respiratory muscle metaboreflex, in contrast to the previous studies that found the improvements in limb muscle blood flow, oxygenation, delayed onset of limb muscle fatigue in healthy subjects (14) and patients with chronic heart failure (16) following IMT. However, it is important to point out that there are several differences in the study protocol of those trials compared to our study. First, in those previous studies, respiratory muscle fatigue was induced by inspiratory loading, while in our study there was no external load on the respiratory muscles. Second, the exercise-induced locomotor muscle fatigue was not a whole-body exercise in these studies; one study used a duration of a control performance for calf plantar flexion exercise as a measurement of locomotor muscle fatigue, while the other used time to task failure while the individuals were performing the handgrip exercise protocol. Therefore, it could have been that in our study, the spontaneous breathing during CWR cycling exercise could not induce respiratory muscle fatigue to the point that respiratory muscle metaboreflex was triggered. Another explanation might be that due to the excessive blood flow demand to both respiratory and locomotor muscles during the whole-body exercise in our study, the improvement in the respiratory muscle capacity was probably not enough

to attenuate the muscle metaboreflex. Especially, when the sympathetic vasoconstrictor activity is a 2-way street which can be stimulated by both limb and respiratory muscle metaboreceptors during exercise (13).

Respiratory and locomotor muscles perfusion (BFI) and oxygenation ($StiO_2$), from iso-time to the new time to task failure during CWR cycling exercise in the patients in the IMT group

At post-measurements, the patients in the IMT group could prolong the exercise time by 5.0 [95%CI -1.9, 11.9 minutes (75% increased) compared to the sham group. Interestingly, the patients in the IMT group demonstrated a capacity to further increase the perfusion of all respiratory muscles and locomotor muscle by 24-32% during this final part of the test compared to iso-time (Figure 6.4). Despite the plateaus in cardiac output and systemic oxygen delivery (Table 6.1 and Figure 6.2), this increase in respiratory and locomotor muscle perfusion was adequate to preserve the oxygenation ($StiO_2$) in both respiratory and locomotor muscles until the end of the exercise (Figure 6.3 and 6.4).

These results suggest reallocation of available blood flow from other sources to the working muscles following IMT. Potential contributions of blood flow could have come from other muscles body organs that are activated less during exercise. Another possibility is that blood flow reallocation from diaphragm, according to the results found in our study (a significant within-group reduction in activation of the diaphragm (EMGdi/EMGdiMax%) in the IMT group at post-measurement compared to pre-measurement (from 78±10% to 57±21%, p=0.046, chapter 5 table 5.4, Figure 5.5 A). A reduce activation of the diaphragm might provide more blood flow availability to be distributed to the more active muscles during exercise. However, the measurement of diaphragm blood flow is not possible, therefore, the speculation has to be considered with caution.

Strengths and limitations

This is the first study that aimed to explore mechanisms, in addition to changes in dyspnea perception, underlying the IMT-induced improvements in exercise endurance capacity in COPD. It is the first study evaluating changes in central and local muscle hemodynamic variables and locomotor muscle fatigue in response to IMT in these patients. Another novelty of this study is that simultaneous measurements of local muscle hemodynamics in both the inspiratory and vastus lateralis muscles were performed while controlling for changes in cardiac output and breathing parameters. This enabled us to investigate the potential of IMT to alter the distribution of blood flow to these muscle groups.

The study also has several important limitations. The major problem is that with the limited sample size, the study is still underpowered. Therefore to confidently explain the results without the prove of

statistically significant results is difficult. We also did not objectively measure diaphragm fatigue and measuring blood flow in the diaphragm is still not possible non-invasively by NIRS. Therefore, the evidence of change in blood flow to the diaphragm, which is the most important respiratory muscle and requires a large proportion of respiratory muscle blood supply, could not be monitored.

6.6 Conclusions

The findings in this study indicate that in our patient population the potential improvements in endurance time following IMT can probably not be attributed to improvements in respiratory and/or locomotor muscle perfusion, oxygenation and limb muscle fatigue secondary to the attenuation of the muscle metaboreflex. The effects are more likely to result from a potential reduction in dyspnea sensation. The further increases in perfusion of both respiratory and locomotor muscles from iso-time on to the new time to task failure in the intervention group despite the plateaus in cardiac output and systemic oxygen delivery might suggest a reallocation of available blood flow from other tissues to the working muscles following IMT.

References

1. Killian KJ, Leblanc P, Martin DH, Summers E, Jones NL, Campbell EJ. Exercise capacity and ventilatory, circulatory, and symptom limitation in patients with chronic airflow limitation. Am Rev Respir Dis. 1992;146(4):935-40.
2. Spruit MA, Singh SJ, Garvey C, ZuWallack R, Nici L, Rochester C, et al. An official American Thoracic Society/European Respiratory Society statement: key concepts and advances in pulmonary rehabilitation. Am J Respir Crit Care Med. 2013;188(8):e13-64.
3. Saey D, Debigaré R, LeBlanc P, Mador MJ, Côté CH, Jobin J, et al. Contractile Leg Fatigue after Cycle Exercise. Am J Respir Crit Care Med. 2003;168(4):425-30.
4. Hamilton AL, Killian KJ, Summers E, Jones NL. Muscle strength, symptom intensity, and exercise capacity in patients with cardiorespiratory disorders. Am J Respir Crit Care Med. 1995;152(6):2021-31.
5. Gea J, Pascual S, Casadevall C, Orozco-Levi M, Barreiro E. Muscle dysfunction in chronic obstructive pulmonary disease: update on causes and biological findings. J Thorac Dis. 2015;7(10):E418-E38.
6. Vogiatzis I, Athanasopoulos D, Habazettl H, Aliverti A, Louvaris Z, Cherouveim E, et al. Intercostal muscle blood flow limitation during exercise in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2010;182(9):1105-13.
7. Dempsey JA, Romer L, Rodman J, Miller J, Smith C. Consequences of exercise-induced respiratory muscle work. Respir Physiol Neurobiol. 2006;151(2-3):242-50.
8. Dempsey JA, Sheel AW, St Croix CM, Morgan BJ. Respiratory influences on sympathetic vasomotor outflow in humans. Respir Physiol Neurobiol. 2002;130(1):3-20.
9. Gagnon P, Bussieres JS, Ribeiro F, Gagnon SL, Saey D, Gagne N, et al. Influences of spinal anesthesia on exercise tolerance in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2012;186(7):606-15.
10. Hopkinson NS, Dayer MJ, Moxham J, Polkey MI. Abdominal muscle fatigue following exercise in chronic obstructive pulmonary disease. Respir Res. 2010;11:15.
11. Mador MJ, Kufel TJ, Pineda LA, Sharma GK. Diaphragmatic fatigue and high-intensity exercise in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2000;161(1):118-23.
12. Boushel R. Muscle metaboreflex control of the circulation during exercise. Acta Physiol (Oxf). 2010;199(4):367-83.
13. Sheel AW, Boushel R, Dempsey JA. Competition for blood flow distribution between respiratory and locomotor muscles: implications for muscle fatigue. J Appl Physiol. 2018;125(3):820-31.
14. McConnell AK, Lomax M. The influence of inspiratory muscle work history and specific inspiratory muscle training upon human limb muscle fatigue. J Physiol. 2006;577(Pt 1):445-57.
15. Turner LA, Tecklenburg-Lund SL, Chapman RF, Stager JM, Wilhite DP, Mickleborough TD. Inspiratory muscle training lowers the oxygen cost of voluntary hyperpnea. J Appl Physiol (1985). 2012;112(1):127-34.
16. Chiappa GR, Roseguini BT, Vieira PJ, Alves CN, Tavares A, Winkelmann ER, et al. Inspiratory muscle training improves blood flow to resting and exercising limbs in patients with chronic heart failure. J Am Coll Cardiol. 2008;51(17):1663-71.
17. Charususin N, Gosselink R, Decramer M, Demeyer H, McConnell A, Saey D, et al. Randomised controlled trial of adjunctive inspiratory muscle training for patients with COPD. Thorax. 2018;73(10):942-50.
18. Langer D, Ciavaglia C, Faisal A, Webb KA, Neder JA, Gosselink R, et al. Inspiratory muscle training reduces diaphragm activation and dyspnea during exercise in COPD. J Appl Physiol (1985). 2018;125(2):381-92.
19. Petrovic M, Reiter M, Zipko H, Pohl W, Wanke T. Effects of inspiratory muscle training on dynamic hyperinflation in patients with COPD. Int J Chron Obstruct Pulmon Dis. 2012;7:797-805.

20. Gosselink R, De Vos J, van den Heuvel SP, Segers J, Decramer M, Kwakkel G. Impact of inspiratory muscle training in patients with COPD: what is the evidence? Eur Respir J. 2011;37(2):416-25.
21. Amann M, Regan MS, Kobitary M, Eldridge MW, Boutellier U, Pegelow DF, et al. Impact of pulmonary system limitations on locomotor muscle fatigue in patients with COPD. Am J Physiol Regul Integr Comp Physiol. 2010;299(1):R314-24.
22. Louvaris Z, Habazettl H, Asimakos A, Wagner H, Zakynthinos S, Wagner PD, et al. Heterogeneity of blood flow and metabolism during exercise in patients with chronic obstructive pulmonary disease. Respir Physiol Neurobiol. 2017;237:42-50.
23. Louvaris Z, Habazettl H, Wagner H, Zakynthinos SG, Wagner PD, Vogiatzis I. Near-infrared spectroscopy using indocyanine green dye for minimally invasive measurement of respiratory and leg muscle blood flow in patients with COPD. J Appl Physiol (1985). 2018.
24. Boushel R, Langberg H, Olesen J, Gonzales-Alonzo J, Bulow J, Kjaer M. Monitoring tissue oxygen availability with near infrared spectroscopy (NIRS) in health and disease. Scand J Med Sci Sports. 2001;11(4):213-22.
25. Panagiotou M, Vogiatzis I, Louvaris Z, Jayasekera G, MacKenzie A, McGlinchey N, et al. Near infrared spectroscopy for the assessment of peripheral tissue oxygenation in pulmonary arterial hypertension. Eur Respir J. 2016;48(4):1224-7.
26. Boushel R, Langberg H, Olesen J, Nowak M, Simonsen L, Bulow J, et al. Regional blood flow during exercise in humans measured by near-infrared spectroscopy and indocyanine green. J Appl Physiol (1985). 2000;89(5):1868-78.
27. Habazettl H, Athanasopoulos D, Kuebler WM, Wagner H, Roussos C, Wagner PD, et al. Near-infrared spectroscopy and indocyanine green derived blood flow index for noninvasive measurement of muscle perfusion during exercise. J Appl Physiol (1985). 2010;108(4):962-7.
28. Louvaris Z, Spetsioti S, Andrianopoulos V, Chynkiamis N, Habazettl H, Wagner H, et al. Cardiac output measurement during exercise in COPD: A comparison of dye dilution and impedance cardiography. Clin Respir J. 2019.
29. Borghi-Silva A, Oliveira CC, Carrascosa C, Maia J, Berton DC, Queiroga F, Jr., et al. Respiratory muscle unloading improves leg muscle oxygenation during exercise in patients with COPD. Thorax. 2008;63(10):910-5.
30. Machado Rodrigues F, Demeyer H, Hornikx M, Camillo CA, Calik-Kutukcu E, Burtin C, et al. Validity and reliability of strain gauge measurement of volitional quadriceps force in patients with COPD. Chron Respir Dis. 2017;14(3):289-97.
31. Burtin C, Saey D, Saglam M, Langer D, Gosselink R, Janssens W, et al. Effectiveness of exercise training in patients with COPD: the role of muscle fatigue. Eur Respir J. 2012;40(2):338-44.
32. Taylor RS, Davies EJ, Dalal HM, Davis R, Doherty P, Cooper C, et al. Effects of exercise training for heart failure with preserved ejection fraction: a systematic review and meta-analysis of comparative studies. Int J Cardiol. 2012;162(1):6-13.
33. Palau P, Dominguez E, Lopez L, Ramon JM, Heredia R, Gonzalez J, et al. Inspiratory Muscle Training and Functional Electrical Stimulation for Treatment of Heart Failure With Preserved Ejection Fraction: The TRAINING-HF Trial. Rev Esp Cardiol (Engl Ed). 2019;72(4):288-97.
34. Palau P, Dominguez E, Nunez E, Schmid JP, Vergara P, Ramon JM, et al. Effects of inspiratory muscle training in patients with heart failure with preserved ejection fraction. Eur J Prev Cardiol. 2014;21(12):1465-73.
35. Chiappa GR, Queiroga F, Jr., Meda E, Ferreira LF, Diefenthaeler F, Nunes M, et al. Heliox improves oxygen delivery and utilization during dynamic exercise in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2009;179(11):1004-10.

36. Louvaris Z, Zakynthinos S, Aliverti A, Habazettl H, Vasilopoulou M, Andrianopoulos V, et al. Heliox increases quadriceps muscle oxygen delivery during exercise in COPD patients with and without dynamic hyperinflation. J Appl Physiol (1985). 2012;113(7):1012-23.
37. Louvaris Z, Vogiatzis I, Aliverti A, Habazettl H, Wagner H, Wagner P, et al. Blood flow does not redistribute from respiratory to leg muscles during exercise breathing heliox or oxygen in COPD. J Appl Physiol (1985). 2014;117(3):267-76.
38. Moxham J, Jolley C. Breathlessness, fatigue and the respiratory muscles. Clin Med. 2009;9(5):448-52.

Chapter 7

Impact of Inspiratory Muscle Training on The Perception and Neural Processing of Respiratory Sensations in COPD

Sauwaluk Dacha, Thomas Reijnders, Ysys Denutte,

Andreas von Leupoldt, Rik Gosselink, & Daniel Langer

Preliminary results

7.1 Abstract

Background: Dyspnea is the sensation of breathing discomfort. It is regarded as a multidimensional symptom which can be subdivided into a sensory-perceptual domain (intensity and quality of dyspnea) and an affective distress domain (unpleasantness). Dyspnea is an important symptom in patients with COPD, contributing to limitations in daily activities and impaired quality of life. Inspiratory muscle training (IMT) has been shown to improve inspiratory muscle capacity (strength and endurance), reduce sensory intensity of exertional dyspnea perception, and improve exercise capacity in patients with COPD. However, whether IMT has an impact on the perception of affective distress (unpleasantness) of dyspnea, dyspnea-related fear and the underlying neural processing of symptom perception in COPD is unknown.

Aim: 1) To investigate the effects of eight weeks of IMT in comparison with sham training on changes in the perception of affective unpleasantness of dyspnea and underlying neural processing of respiratory sensations in patients with COPD. We specifically aimed to focus on the higher order cognitive-affective processing reflected in the later components of respiratory-related evoked potentials (RREPs) recorded in the electroencephalogram (EEG) in response to short inspiratory occlusions during unloaded breathing. 2) To assess the impact of IMT on general anxiety symptoms and dyspnea-specific fears.

Methods: RREPs in the EEG elicited by short inspiratory occlusions during unloaded breathing were assessed before and after the intervention period. Mean amplitudes of later peak components of the RREPs (P2 and P3) were recorded and changes after the intervention period were compared between groups. In addition, intensity and unpleasantness ratings in response to occlusions during unloaded breathing, general anxiety levels, and disease-specific fear ratings were assessed before and after the intervention period and compared between groups.

Results: Intensity and unpleasantness ratings in the IMT group after the training compared to the sham group changed in the expected direction (i.e. reduction) (Mean between-group differences in the pre-post intervention change scores of -0.7 [95%CI -2.4, 0.9], p=0.34, Cohen's d effect size 0.30 for intensity and -0.9 [95%CI -2.6, 0.7], p=0.25, Cohen's d effect size 0.36 for unpleasantness were observed on a modified Borg-scale). Further, there was a trend for a reduction in the mean amplitude of the P2 component of the RREP in the IMT group after IMT compared to the sham group (mean between-group difference in the pre-post intervention change scores of -2.48 [95%CI -5.73, 0.77] microvolts, p=0.12, Cohen's d effect size 0.50). Ratings on the dyspnea-related fear subscale of the Breathlessness Beliefs Questionnaire (BBQ) were also changed in the expected direction (i.e. reduction) in the IMT group compared to the sham group after IMT

(mean between-group differences in the pre-post intervention change scores of -1.8 [95%CI -6.3, 4.9] units, p=0.42, Cohen's d effect size 0.25).

Conclusions: Based on these preliminary data collected during unloaded breathing we conclude that potential reductions in the affective unpleasantness and intensity of respiratory sensations in the IMT group (with small effect sizes) might be paralleled by changes in the neural processing of these respiratory sensations (with a medium effect size) as well as by improvements in dyspnea-related fears (small effect size). It could be speculated that the repeated experience of dyspnea during IMT might contribute to modify the affective unpleasantness of respiratory sensations (next to the sensory intensity) and dyspnea-related fears in patients with COPD as a result of habituation in the neural processing. Enlarging the sample size and additional assessments under conditions of loaded breathing and exercise-induced dyspnea would be required to confirm these initial trends.

Keywords: Inspiratory Muscle Training (IMT), dyspnea perception, EEG, respiratory-related evoked potential, COPD

7.2 Introduction

Dyspnea is the sensation of breathing discomfort. It is oftentimes operationalized as a multidimensional construct that consists of qualitatively distinct sensations varying in intensity, unpleasantness, and emotional and behavioral significance (1). The sensory-perceptual domains of dyspnea (intensity and quality) and several biological variables associated with these sensations have been studied intensively in patients with COPD. However, the perception of the respiratory distress component of dyspnea (affective unpleasantness perceived for a given intensity) and its association with several psychological variables has received considerably less attention (2, 3). A recent study showed that improving inspiratory muscle function in COPD patients with inspiratory muscle weakness by inspiratory muscle training (IMT) can reduce respiratory neural drive as measured with diaphragmatic electromyogram (EMGdi) and the sensory intensity of dyspnea during exercise (4). Another study compared the sensory intensity and affective unpleasantness of dyspnea between patients with asthma and healthy subjects. Both groups showed similar ratings of the sensory intensity of dyspnea; however, in patients with asthma, this was associated with ratings of less affective unpleasantness of dyspnea (5). The authors suggested that altered neural processing in patients with asthma (possibly related to gradual habituation to dyspnea stimuli by frequent exposure) might explain this reduction in the affective unpleasantness component of dyspnea at a similar intensity of the sensation (5).

It has been suggested that neuromechanical uncoupling between mechanical/muscular responses of the respiratory system to elevated levels of respiratory motor drive could increase activation of central limbic structures related to dyspnea processing, resulting in increased affective respiratory distress (2). De-activation of the limbic system might, therefore, play a central role in desensitization processes of this affective unpleasantness component of dyspnea (2). The reduction of the affective unpleasantness of dyspnea in patients with COPD in response to rehabilitation was often explained by the mechanism of desensitization to dyspnea (6, 7). However, only a few studies have systematically evaluated this potential mechanism (8, 9), especially in patients with COPD (10-13). Therefore, it remains unclear whether the affective unpleasantness component of dyspnea and the associated central neural processing can be modified by interventions such as respiratory muscle training in COPD patients. However, it has been suggested that respiratory muscle training might be able to alter the perception of dyspnea despite the absence of changes in ventilatory capacity (14). This implies that changes in the perception of dyspnea could occur separately from other physiological changes, but the involvement of respective neural processes and specific psychological factors herein remains widely unknown. An important psychological

factor impacting on the perception of dyspnea is anxiety and fear (15-19). Both, increased levels of general (non-specific) anxiety and dyspnea-specific fear have been shown to be related to increased dyspnea reports in patients with COPD (16-18), but their associations with the neural processing of dyspnea and effects of IMT are not well understood.

The neural processing of respiratory sensations such as dyspnea can be investigated by using the non-invasive technique of measuring respiratory-related evoked potentials (RREPs) recorded from the electroencephalogram (EEG). The RREP is a measurement of cerebral cortical activity, which is elicited by the activation of lung and muscle mechanoreceptors due to short inspiratory occlusions or resistive loads (20). The early peaks of the RREP (Nf, P1, and partly N1) reflect the initial arrival and first-order processing of afferent respiratory signals in sensorimotor regions (21). The later peaks (P2 and P3) characterize subsequent higher-order cognitive processing and are vulnerable to cognitive and affective processes (21). N1, however, can also be affected by attention and affect related processes (22).

In healthy subjects, a significant increase in the magnitude of N1, P2, and P3 peaks was found when breathing became more difficult and unpleasant. This indicates an increase in the neural processing of respiratory sensations reflective of higher order cognitive-affective processing (23). Moreover, a previous study investigated the effects of inspiratory muscle training on the RREP in healthy individuals and hypothesized that once the inspiratory muscles become stronger, the inspiratory motor drive would be decreased. Although respective decreases in the motor drive were indeed achieved after training, this study found no changes in early sensory-related RREP components (24). However, the effect of inspiratory muscle training on later cognitive-affective components of the RREP has not yet been studied.

This study aimed at 1) Investigating the effects of eight weeks inspiratory muscle training on changes in the affective unpleasantness component of dyspnea in relation to the neural processing of respiratory sensations, specifically in the higher order cognitive-affective processing reflected by the later components of the RREP (P2 and P3) in response to short inspiratory occlusions during unloaded breathing. 2) Assessing the impact of IMT on general anxiety and dyspnea-specific fear. In addition, the correlations between the changes in these later peak components of the RREP in response to IMT with changes in the perceived intensity and affective unpleasantness of respiratory sensations during unloaded breathing, dyspnea-specific fear and general anxiety were investigated. The hypotheses were that 1) after eight weeks of IMT, the affective unpleasantness of respiratory sensation would be reduced, which would be associated with the reduction in the amplitudes of the later RREP components (P2 and P3). 2) IMT could

reduce general anxiety levels and dyspnea-specific fears and that these reductions might be associated with the changes in the later peak component of the RREP.

7.3 Materials and methods

7.3.1 Design

The neural processing of respiratory sensations (dyspnea) was investigated using the non-invasive technique of measuring respiratory-related evoked potentials (RREPs) recorded from the electroencephalogram (EEG) (20-22). Patients in this study were the same subjects who participated in experiments summarized in chapters 3 and 4. The measurements were performed at baseline (before starting IMT or sham IMT) and again after 8 weeks of intervention. The patients were asked to complete the following questionnaires: Hospital anxiety and depression scale (HADS) and the Breathlessness Beliefs Questionnaire (BBQ) regarding general anxiety and dyspnea-specific fear, respectively. The investigators who performed the tests in this study were blinded for the patient's group allocation.

7.3.2 Electroencephalogram (EEG) measurement

The measurement of RREP using EEG was performed similarly to the protocol established in previous studies (25, 26).

7.3.2.1 The setup

The patient was sitting in a test room separated from the investigator facing a computer screen which presented the standardized instructions. A copy of the display of the patient's computer screen was also visible for the investigator. The patient breathed via a mouthpiece through a breathing circuit with a nose clip attached. The breathing circuit consisted of a two-way non-rebreathing valve connected in series to a pneumotachograph, a loading manifold, and a pressure-activated occlusion valve. The mouthpiece and the valve system were held with a suspensor to eliminate the need for the patient to hold or bite the mouthpiece. The investigator could observe the patient via an intercom system.

The patient then was acquainted with the setting by using the mouse of the computer and breathing through the mouthpiece while wearing a nose clip. Thereafter, patients underwent a brief resistive loaded breathing task, which will be reported elsewhere. After a rest period, the RREP task followed as described below.

7.3.2.2 The Respiratory-Related Evoked Potentials (RREP) during unloaded breathing

The EEG sensor net [129-channel system, Electrical Geodesics Inc., Eugene, USA] was positioned on the patient scalp. The first block of the task consisted of 3 minutes breathing through the breathing circuit without external load ("unloaded breathing"). During breathing, the computer screen displayed a central fixation cross on a gray background. The investigator interrupted the patient's inspiration briefly for 600

milliseconds at the onset of inspiration every two to six breaths by manually activating the occlusion valve with pressurized air through a trigger box. These occlusions induced the RREP during the unloaded breathing. The patient then rated the perceived intensity and unpleasantness of the occlusions by mouse click on the modified Borg Scale (27) that appeared on the computer screen. Then the patient rested for two minutes. The second block of the task was comparable to the first block, but in addition, an inspiratory resistive load was added to the breathing circuit ("loaded breathing"). Thereafter, both first (unloaded) and second (loaded) block were repeated in a similar way.

The main outcome measurement analyzed during this task were the ratings of intensity and unpleasantness of occlusions and the RREP components P2 and P3 during unloaded breathing. Responses during loaded breathing will be analyzed separately. An illustration of typically P2 and P3 peak components of the RREP and related scalp topographies provided in figure 7.1.

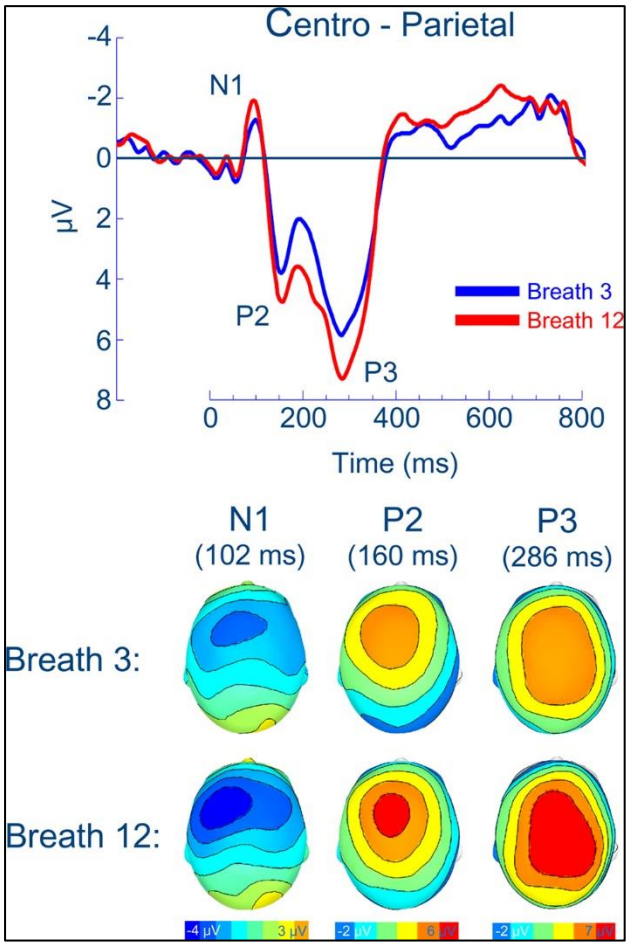

Figure 7.1 Illustration of typically observed group means for the respiratory-related evoked potential and related scalp topographies (at their peak latencies) at centro-parietal regions.

Adapted from: Von Leupoldt A, Bradley MM, Lang PJ, Davenport PW. Neural processing of respiratory sensations when breathing becomes more difficult and unpleasant. Front Physiol. 2010;1:144.(23)

7.3.3 Modified 10-point Borg scale

The modified 10-point Borg scale was developed to measure overall exertion during physical activity. It was modified from the original perceived exertion scale described by Borg comprised a scale ranging from 6 to 20. The modified Borg scale consists of a scale ranging from 0 (nothing at all) to 10 (Maximal), including written indicators of severity to anchor specific numbers of the scale (27). The scale has been used widely to quantify various "perceived symptom" for example breathlessness and muscle fatigue during exercise. The validity and reliability of Borg ratings of breathlessness during exercise have been evaluated in several studies (28, 29). The minimal clinically important difference of the dyspnea Borg score is 1 unit with moderate effect size (30).

7.3.4 General anxiety measurement

The Hospital Anxiety and Depression Scale (HADS) (31) was used to measure general anxiety levels at baseline and 8 weeks after IMT or sham IMT. The HADS consists of 7 items of the anxiety subscale and 7 items of the depression subscale. In this study, only the 7 items of the anxiety subscale were used. The HADS was validated and showed good reliability in somatic, psychiatric, and primary care patients and the general population (32). Items are scored on a scale from 0 to 3, with total scores ranging between 0 and 21. Higher scores indicate higher anxiety levels (31).

7.3.5 Dyspnea-specific fear measurement

The Breathlessness Beliefs Questionnaire (BBQ) (33) was used to measure dyspnea specific fear. The BBQ is a self-administered questionnaire and, consists of two subscales divided into 6 items of the "Activity Avoidance (BBQ-AA)" subscale and 5 items of the "Somatic Focus or dyspnea-related fear (BBQ-SF)" subscales (33). High scores on the BBQ-AA reflect the belief that physical activity might make the patients' disease and dyspnea worse (physical activity-related fear) and should be avoided. An example of BBQ-AA is "It's really not safe for a person with a condition like mine to be physically active". High scores on the BBQ-SF-scale reflect the belief that breathlessness is a signal of potential bodily harm (dyspnea-related fear). An example of BBQ-SF is "Whenever I feel short of breath my body is telling me I have something seriously wrong." Each item is rated on a five-point Likert-type scale, ranging from 1 (totally disagree) to 5 (totally agree) with a total score of 30 on BBQ-AA and 25 on BBQ-SF subscale. The BBQ shows good reliability and has been validated in patients with chronic respiratory disease (33).

7.3.6 Statistical analysis

All EEG data were processed offline using the software package BESA Research 6.0 (BESA, Gräfelfing, Germany). After filtering of data (high-pass filter: 0.1Hz, low-pass filter: 30Hz, notch filter: 50Hz), and the correction and removal of artifacts (for example eye blinks and movement artifacts), data were re-referenced to the original average reference. The remaining epochs of interest were extracted 200ms

before, and 1200ms after the onset of the occlusions and thereafter averaged across all occlusions of the two unloaded blocks. Based on earlier studies (34, 35), the RREP components were identified as P2 (=positive peak – in the central scalp region, latency: 160–230ms) and P3 (=positive peak – in the centro-parietal scalp region, latency: 250–350ms). The mean amplitudes were calculated around each peak in a latency window of +/-20ms for both P2 and P3, and entered into the subsequent statistical analysis.

The ratings of intensity and unpleasantness of occlusions (Borg) and the mean amplitudes of the RREP components(μV) were averaged across the two unloaded breathing blocks. Then the comparisons between pre/post measurement within-group, and the pre/post differences between groups were performed. Statistical significance was considered at $p<0.05$. Data are presented as means ± SD. Tests of normality were performed using Kolmogorov-Smirnov and/or Shapiro-Wilk tests. Paired t-tests were used to test the within-group differences between pre and post-intervention measurements. In case the normality was not met, or the sample size was too small to test for normality, nonparametric tests were performed. Wilcoxon signed rank test, and Mann-Whitney test were used instead of paired and unpaired t-test, respectively. As a measure of effect size for these tests, Cohen's d was calculated.

Correlations between the amplitude changes in the later peak components of the RREP in response to IMT with changes in the ratings for unpleasantness and intensity of occlusions, disease-specific fear, and general anxiety were performed using Pearson's correlation coefficient (r) or Spearman's correlation (rho), when the normal distribution of the data was not met. Statistical analyses were performed using GraphPad Prism version 8 for Windows (GraphPad Software, La Jolla, California, USA)

7.4 Results

Due to large delays in the recruitment of patients, only preliminary results are reported in this chapter. Baseline characteristics of patients at study enrolment are presented in chapter 5 (Table 5.1).

Perceived intensity and unpleasantness of the occlusions

Perceived intensity and unpleasantness ratings on the modified Borg scale in response to occlusions during unloaded breathing are presented in Table 7.1. The intensity and unpleasantness ratings in the IMT group after the training compared to the sham group were changed in the expected direction (reduce) (mean between-group differences in the pre-post intervention change scores of -0.7 [95%CI -2.4, 0.9], p=0.34, Cohen's d effect size of 0.30 and -0.9 [95%CI -2.6, 0.7], p=0.25, Cohen's d effect size 0.36, for the intensity and unpleasantness ratings, respectively (Table 7.1). There was a trend of within-group reduction in the

ratings for unpleasantness and intensity in the IMT group at post-measurement compared to pre-measurement (within-group differences -1.0±1.5, p=0.06 and -1.1±1.5 p=0.06, respectively).

Respiratory-Related Evoked Potential (RREP)

Amplitudes (microvolts) of the P2 and P3 components of the Respiratory-Related Evoked Potential (RREP) during unloaded breathing at baseline and after 8 weeks of sham IMT or IMT are presented in Table 7.1. A trend of improvement (reduction) in P2 amplitude was found in the IMT group compared to the sham group (mean between-group difference in the pre-post intervention change scores of -2.48 [95%CI -5.73, 0.77] μV, p=0.12), with the Cohen's d effect size of 0.50. There was no significant between-group difference in the pre-post intervention change scores in the amplitude of P3 observed (mean between-group difference in the pre-post intervention change scores of -0.76 [95%CI -4.44, 2.92] μV, p=0.66), with the Cohen's d effect size of 0.13. There was no significant within-group difference observed in both P2 and P3. However, the P2 amplitude in the IMT group changed in the expected direction (reduction) at post-measurement compared to pre-measurement (within-group differences -1.20±2.83, p=0.19) while the P2 amplitude was increased in the sham group (within-group differences 1.28±1.47, p=0.13) (Table 7.1).

General anxiety

The results regarding the HADS anxiety and depression scores of the patients in both groups at pre and post-measurement are presented in Table 7.1. There were no significant changes observed in the HADS anxiety and depression scores within and between groups in response to the intervention. The Cohen's d effect sizes were 0.09 and 0.10, respectively, for HADS anxiety and depression.

Dyspnea-specific fears

Dyspnea-specific fears measured with the BBQ are presented in Table 7.1. The BBQ scores are presented separately for activity avoidance (BBQ-AA) and dyspnea-related fear (BBQ-SF) subscales. There were no significant differences in the pre-post-intervention change scores of BBQ-AA and BBQ-SF between the IMT group and the sham group (Table 7.1). The Cohen's d effect sizes were 0.07 and 0.25, respectively, for BBQ-AA and BBQ-SF. However, there was a trend towards a significant reduction in the dyspnea-related fear score (BBQ-SF) in the IMT group at post-measurement compared to pre-measurement (within-group differences -2.0±3.8 p=0.12 Table 7.1).

Table 7.1 Ratings for occlusions and mean amplitudes (microvolts) of the later peak components of the Respiratory-Related Evoked Potential (RREP) during unloaded breathing, general anxiety levels and dyspnea-specific fear at baseline and after 8 weeks of sham IMT or IMT training

	Sham IMT (n=4)			IMT (n=11)			Mean difference of change Sham IMT-IMT (95%CI) or Differences between medians	P values between group
	PRE	POST	Differences (P values within group)	PRE	POST	Differences (P values within group)		
Perceived intensity and unpleasantness								
Occlusion Intensity (Borg)	1.8±1.0	1.6±1.2	-0.2±0.4 (0.3910)	2.9±1.2	2.0±0.8	-1.1±1.5 (0.0597)	-0.7 (-2.4 to 0.9)	0.3378
Occlusion Unpleasantness (Borg)	2.3±1.3	2.1±1.3	-0.1±0.6 (0.7177)	2.9±1.2	1.8±1.0	-1.0±1.5 (0.0547)	-0.9 (-2.6 to 0.7)	0.2531
RREP								
P2 (μV)	0.02±1.73	1.30±1.11	1.28±1.47 (0.1250)	1.76±2.54	0.56±2.33	-1.20±2.83 (0.1885)	-2.48 (-5.73 to 0.77)	0.1234
P3 (μV)	1.90±1.77	2.87±3.09	0.98±4.46 (0.6913)	1.92±1.78	2.14±1.95	0.22±2.27 (0.7566)	-0.76 (-4.44 to 2.92)	0.6645
Questionnaires								
HADS Anxiety (0-21)	6.0±1.4	6.5±0.6	0.5±1.3 (0.7500)	7.3±2.7	8.2±3.0	0.9±2.5 (0.2640)	0.4 (-2.5 to 3.3)	0.7673
HADS Depression (0-21)	2.3±1.5	2.8±2.1	0.5±1.0 (0.3910)	5.2±3.0	6.1±2.6	0.9±2.3 (0.2272)	0.4 (-2.3 to 3.1)	0.7452
BBQ Activity avoidance (0-30)	11.5±3.1	12.0±2.2	0.5±1.0 (0.3910)	15.4±4.4	15.4±5.1	0.0±4.2 (>0.9999)	-0.5 (-5.2 to 4.2)	0.7810
BBQ Dyspnea related fear (0-25)	13.3±6.3	13.0±3.6	-0.3±2.8 (0.8675)	16.9±3.2	14.9±3.2	-2.0±3.8 (0.1154)	-1.8 (-6.3 to 2.8)	0.4232

Values are mean ± SD; *P < 0.05, within-group difference, pre- vs. post-intervention by paired t-test (or Wilcoxon signed rank test); ǂP < 0.05 by unpaired t-test (or Mann-Whitney test) comparing differences of pre-post intervention change scores for IMT vs. sham IMT; (μV) = microvolts

Correlations between the changes in response to IMT between the mean amplitudes of the RREP, the intensity and, the affective unpleasantness ratings, dyspnea-specific fear, and anxiety scores

Across subjects from both groups (IMT and sham IMT), there was no significant correlation between the changes of the mean amplitude of later peak components (P2 and P3) and the changes in the intensity and the affective unpleasantness ratings for occlusions, changes in anxiety scores and changes in dyspnea-specific fear scores (Table 7.2). However, there was a trend for a positive linear relationship (r=0.361, p=0.18) between the reduction of P2 mean amplitudes and the reduction in the affective unpleasantness ratings for occlusions (Figure 7.2).

Table 7.2 Correlation (across subjects from both groups (IMT and sham IMT)) between changes in mean amplitudes of later RREP components (P2, and P3) and changes in intensity, unpleasantness, general anxiety and dyspnea-specific fear (BBQ) scores.

Changes in	Occlusion intensity (Borg)	Occlusion unpleasantness (Borg)	General Anxiety	BBQ Activity avoidance	BBQ dyspnea-related fear (fear/avoidance of dyspnea)
P2 (µV)	r = 0.281 p = 0.31	r = 0.361 p = 0.18	r = 0.026 p = 0.93	r = -0.308 p = 0.26	r = 0.035 p = 0.90
P3 (µV)	r = 0.278 p = 0.32	r = 0.156 p = 0.58	r = -0.480 p = 0.23	r = -0.325 p = 0.24	r = -0.334 p = 0.90

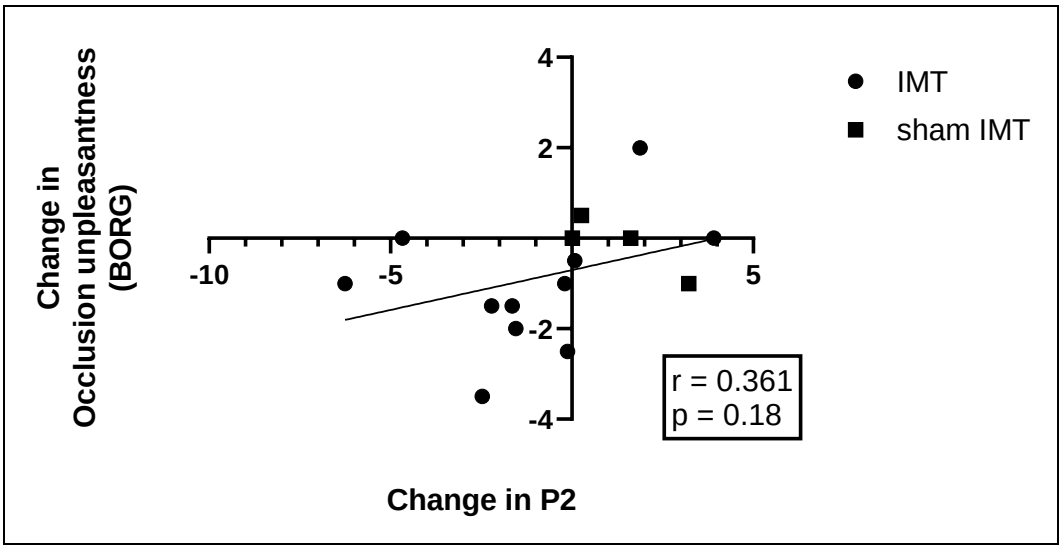

Figure 7.2 correlation across subjects from both groups (IMT and sham IMT) between the pre-post intervention reductions of P2 mean amplitude (µV) to the reduction of occlusion unpleasantness (Borg) ratings.

7.5 Discussion

Main findings

Based on preliminary findings and in the absence of any statistically significant between-group differences we found that the perceived unpleasantness and intensity of respiratory sensations were changed in the expected direction (reduction) during unloaded breathing in response to 8 weeks of IMT with small effect sizes. These observations are in line with changes in symptom perception that have been observed in chapter 5 during exercise breathing. In parallel, we observed a trend for differences in changes in the P2 component of the RREP between groups with a medium effect size. We did not observe any changes in general anxiety symptoms in response to IMT. However, we found a trend for a within-group reduction in dyspnea-specific fear in the intervention group with a small effect size (Table 7.1) and a trend for a positive linear relationship between the reductions of the mean amplitude of P2 and the reduction of affective unpleasantness scores (p=0.18)(Figure 7.2).

Changes in Respiratory-Related Evoked Potentials (RREPs) and, perceived intensity and unpleasantness of the occlusions in response to IMT

The RREP results show the trend for a reduction in the P2 component at post EEG measurement in the IMT group compared to the sham group with a medium effect size. This was paralleled with a potential reduction in the intensity and unpleasantness ratings in the IMT group after the training compared to the sham group (mean between-group differences in the pre-post intervention change scores of -0.7 [95%CI -2.4, 0.9], p=0.34 and -0.9 [95%CI -2.6, 0.7], p=0.25, for the intensity and unpleasantness ratings, respectively on a modified Borg-scale) with small effect sizes. Even though the reduction of perceived respiratory sensations was not statistically significant; however the reduction of about 1 unit Borg score (especially for the affective unpleasantness ratings) is equal to a minimally clinically important change of dyspnea scores (30). During cycling exercise (chapter 5, Table 5.4), a comparable reduction of dyspnea intensity and unpleasantness ratings was also found in the IMT group compared to the sham group (mean between-group difference in the pre-post intervention change scores of -2.2 [95%CI -4.5, 0.2]; p=0.07 Cohen's d effect size 0.60 and -1.1 [95%CI -3.6, 1.4]; p=0.36 Cohen's d effect size 0.29 for intensity and unpleasantness respectively on a modified Borg-scale). Noteworthy, the reductions of the dyspnea intensity and unpleasantness during exercise breathing were more pronounced compared to the ratings of occlusions during unloaded breathing. This could be explained by the higher baseline symptoms during exercise leading to more pronounced reduction. Therefore, it is important that the RREPs are also evaluated during the task that can stimulate higher dyspnea sensation, for example, during loaded breathing or exercise breathing in addition to resting breathing.

The later peak RREP components P2 and P3 are vulnerable to cognitive-affective processes not related to the respiration per se. Attentional distraction and affective processes have been shown to be related to the amplitudes of these later components (22, 34, 36-38). For example, von Leupoldt and colleagues showed that higher state, as well as trait levels of anxiety, were related to changes in the amplitudes of the P2 and P3 RREP components (34, 36). Moreover, previous studies suggested that repeated exposure to dyspnea could result in a decrease (habituation/desensitization) of dyspnea perception (11-13) both in the affective and sensory dimension (8, 11-13). A study found that repeated exposure to resistive load-induced dyspnea over several days could reduce the impairing effect of dyspnea on response inhibition (inhibition of automatic, prepotent, or inappropriate responses) (39). Another study also found a reduction in the perception of respiratory sensations together with the reduction of the P2 and P3 RREP component (in addition to the N1 peak) during the repeated presentation of inspiratory occlusions between early and late experimental periods in healthy subjects, suggesting the potential mechanism of habituation in the neural processing of respiratory sensations (9). In line with these results, the observed reductions in the intensity and unpleasantness ratings for the occlusions reported by IMT patients could potentially constitute the result of habituation in the neural processing of repeated experience of dyspnea and occlusion sensations during the IMT (9).

It has been suggested that habituation is a general adaptation process to save the neural resources for other more relevant tasks when repeated sensory information becomes redundant and loses significance (9). Therefore it could be implied that the repeated exposure to the dyspnea during the IMT training stimulated this habituation mechanism resulting in less respiratory sensation during unloaded breathing and exercise. Whereas in some populations, for example, patients with asthma, habituation of dyspnea might not be favorable because it could lead to delay in treatment and critical under-medication (5, 9, 40), in patients with COPD habituation of dyspnea might be able to prevent the patients from avoiding physical activity (8). Avoiding activity in patients with COPD contributes to further deconditioning of respiratory and limb muscles leading to worsened physical fitness and dyspnea severity (41). The observed reduction of activity-related dyspnea (TDI) in our study was present without any changes in physical activities of the patients (results presented and discussed in chapter 5 and 6), again supporting the notion of a potential habituation effect of the course of IMT.

Changes in levels of general anxiety and dyspnea-specific fears in response to IMT

Anxiety is a prevalent comorbidity in patients with COPD, related to a worse course of the disease (42-44) and affects the perception of respiratory symptoms (45, 46). In the present study, after 8 weeks of the training, there were no significant changes in the general anxiety scores within and between groups. Therefore, from the limited evidence that we have at the moment, we can only conclude that

IMT does not affect general anxiety levels in patients with COPD. This is in contrast with a previous study that found a significant reduction in general anxiety ($p<0.001$) after 6 months of pulmonary rehabilitation together with improvement in other outcomes measured (e.g., functional capacity, dyspnea, and quality of life) (17). These contrasting results might be related to the rather low baseline levels of general anxiety with the mean HADS-A scores of the patients in this study being 6 - 7.3, which might have prevented stronger reductions over the course of intervention in the sense of a floor effect. This hypothesis would be in line with findings from Harrison et al. (2012), showing greater reductions in anxiety after pulmonary rehabilitation in COPD with higher baseline anxiety levels, but only small reduction in those with low baseline anxiety levels (47).

Moreover, it has been suggested that dyspnea-specific fear is more closely related to the daily experiences of dyspneic patients and can capture the source of anxiety better in patients with COPD in comparison to general anxiety levels (18, 48). For example, a study showed that greater dyspnea-specific fears were associated with worse activity-related dyspnea, functional exercise capacity, health-related quality of life, health status, and depression at the start and end of pulmonary rehabilitation, even after controlling for baseline levels of general anxiety (49). In this study, dyspnea-related fear was measured using the Dutch version of the Breathlessness Beliefs Questionnaire (BBQ) (33).

In the present study, with no significant between-group differences observed, we found a trend for a reduction in the dyspnea-related fear subscale (BBQ-SF), but not in the activity avoidance subscale (BBQ-AA) at post-measurement in the IMT group, but not in the sham group (Table 7.1). Based on this finding, we could speculate that IMT might be able to improve the fear of dyspnea by the repeated exposure to the dyspnea during the daily IMT, but not further impact on the avoidance of activities associated with dyspnea. This interpretation converges with other findings in healthy subjects (39) showing that daily dyspnea exposure with resistive loads reduces dyspnea over time in the absence of physiological or behavioral changes, which is again suggestive of a habituation effect.

Future research direction

The data presented in this chapter provide a first indication that changes in disease-specific fears and higher order neural processing of respiratory sensations might be involved in modulating the unpleasantness of the dyspnea sensation in these patients in response to IMT. However, in order to confirm these initial observations, these findings would need to be replicated in a larger group of subjects. Moreover, it would be necessary to confirm these trends by evaluating changes in dyspnea unpleasantness also at similar levels of dyspnea intensity as before the intervention. This could be

achieved by performing RREP evaluations while breathing against resistive loads eliciting similar dyspnea intensities as prior to the intervention or by using exercise-induced dyspnea.

Strengths and limitations

This is the first study that investigated the neural processing of respiratory sensations in response to this intervention (IMT) in patients with COPD. In addition, this study also included the measurements of respiratory sensation (intensity and unpleasantness), general anxiety, and dyspnea-specific fear in response to IMT.

The limitation of this study was that the sample size at this moment is rather small. The results therefore need to be interpreted with great caution and need confirmation in a larger sample. The evaluation of the RREPs pre and post-measurement during a task that elicits higher dyspnea intensity (i.e., during loaded breathing or exercise) would also be valuable.

7.6 Conclusion

In conclusion, the results of this study show preliminary signs that 8 weeks of IMT might lead to reductions in respiratory sensations (both intensity and unpleasantness) with small effect sizes, corresponding with a trend of parallel reductions in the P2 component of the RREP with medium effect size. Even though there were no changes in general anxiety in response to IMT, however, we found a trend for a reduction in dyspnea-related fear with small effect size and a mild positive linear relationship between the reductions of P2 amplitudes pre-post-intervention with the potential reductions of the affective unpleasantness of respiratory sensations. Therefore, these findings suggest that repeated experience of dyspnea during IMT might not only be able to modify the intensity, but also the affective unpleasantness of respiratory sensations, dyspnea-related fears and the higher-order neural processing of respiratory sensations in patients with COPD, which might constitute a habituation process in neural processing.

Reference

1. Parshall MB, Schwartzstein RM, Adams L, Banzett RB, Manning HL, Bourbeau J, et al. An official American Thoracic Society statement: update on the mechanisms, assessment, and management of dyspnea. Am J Respir Crit Care Med. 2012;185(4):435-52.
2. O'Donnell DE, Ora J, Webb KA, Laveneziana P, Jensen D. Mechanisms of activity-related dyspnea in pulmonary diseases. Respir Physiol Neurobiol. 2009;167(1):116-32.
3. Lansing RW, Gracely RH, Banzett RB. The multiple dimensions of dyspnea: review and hypotheses. Respir Physiol Neurobiol. 2009;167(1):53-60.
4. Langer D, Ciavaglia C, Faisal A, Webb KA, Neder JA, Gosselink R, et al. Inspiratory muscle training reduces diaphragm activation and dyspnea during exercise in COPD. J Appl Physiol (1985). 2018;125(2):381-92.
5. von Leupoldt A, Sommer T, Kegat S, Eippert F, Baumann HJ, Klose H, et al. Down-regulation of insular cortex responses to dyspnea and pain in asthma. Am J Respir Crit Care Med. 2009;180(3):232-8.
6. Bianchi R, Gigliotti F, Romagnoli I, Lanini B, Castellani C, Binazzi B, et al. Impact of a rehabilitation program on dyspnea intensity and quality in patients with chronic obstructive pulmonary disease. Respiration. 2011;81(3):186-95.
7. Agle DP, Baum GL, Chester EH, Wendt M. Multidiscipline treatment of chronic pulmonary insufficiency. 1. Psychologic aspects of rehabilitation. Psychosom Med. 1973;35(1):41-9.
8. Stoeckel MC, Esser RW, Gamer M, Buchel C, von Leupoldt A. Brain mechanisms of short-term habituation and sensitization toward dyspnea. Front Psychol. 2015;6:748.
9. von Leupoldt A, Vovk A, Bradley MM, Lang PJ, Davenport PW. Habituation in neural processing and subjective perception of respiratory sensations. Psychophysiology. 2011;48(6):808-12.
10. Cooper CB. Desensitization to dyspnea in COPD with specificity for exercise training mode. Int J Chron Obstruct Pulmon Dis. 2009;4:33-43.
11. Donesky D, Nguyen HQ, Paul SM, Carrieri-Kohlman V. The affective dimension of dyspnea improves in a dyspnea self-management program with exercise training. J Pain Symptom Manage. 2014;47(4):757-71.
12. Wadell K, Webb KA, Preston ME, Amornputtisathaporn N, Samis L, Patelli J, et al. Impact of pulmonary rehabilitation on the major dimensions of dyspnea in COPD. Copd. 2013;10(4):425-35.
13. Herigstad M, Faull OK, Hayen A, Evans E, Hardinge FM, Wiech K, et al. Treating breathlessness via the brain: changes in brain activity over a course of pulmonary rehabilitation. Eur Respir J. 2017;50(3).
14. Levine S, Weiser P, Gillen J. Evaluation of a ventilatory muscle endurance training program in the rehabilitation of patients with chronic obstructive pulmonary disease. Am Rev Respir Dis. 1986;133(3):400-6.
15. Lou P, Zhu Y, Chen P, Zhang P, Yu J, Zhang N, et al. Prevalence and correlations with depression, anxiety, and other features in outpatients with chronic obstructive pulmonary disease in China: a cross-sectional case control study. BMC Pulm Med. 2012;12:53.
16. Oga T, Tsukino M, Hajiro T, Ikeda A, Nishimura K. Analysis of longitudinal changes in dyspnea of patients with chronic obstructive pulmonary disease: an observational study. Respir Res. 2012;13:85.
17. von Leupoldt A, Taube K, Lehmann K, Fritzsche A, Magnussen H. The impact of anxiety and depression on outcomes of pulmonary rehabilitation in patients with COPD. Chest. 2011;140(3):730-6.
18. Janssens T, De Peuter S, Stans L, Verleden G, Troosters T, Decramer M, et al. Dyspnea perception in COPD: association between anxiety, dyspnea-related fear, and dyspnea in a pulmonary rehabilitation program. Chest. 2011;140(3):618-25.
19. Keil DC, Stenzel NM, Kühl K, Vaske I, Mewes R, Rief W, et al. The impact of chronic obstructive pulmonary disease-related fears on disease-specific disability. Chron Respir Dis. 2014;11(1):31-40.

20. von Leupoldt A, Chan PY, Esser RW, Davenport PW. Emotions and neural processing of respiratory sensations investigated with respiratory-related evoked potentials. Psychosom Med. 2013;75(3):244-52.
21. Chan PY, Davenport PW. Respiratory related evoked potential measures of cerebral cortical respiratory information processing. Biol Psychol. 2010;84(1):4-12.
22. Davenport PW, Chan PY, Zhang W, Chou YL. Detection threshold for inspiratory resistive loads and respiratory-related evoked potentials. J Appl Physiol (1985). 2007;102(1):276-85.
23. von Leupoldt A, Bradley MM, Lang PJ, Davenport PW. Neural processing of respiratory sensations when breathing becomes more difficult and unpleasant. Front Physiol. 2010;1:144.
24. Huang CH, Martin AD, Davenport PW. Effect of inspiratory muscle strength training on inspiratory motor drive and RREP early peak components. J Appl Physiol (1985). 2003;94(2):462-8.
25. Herzog M, Sucec J, Vukovic M, Van Diest I, Van den Bergh O, von Leupoldt A. Experimental social rejection increases dyspnoea perception and neural processing of respiratory sensations in healthy subjects. Eur Respir J. 2018:1801409.
26. Herzog M, Sucec J, Van Diest I, Van den Bergh O, Sarah Chan P-Y, Davenport P, et al. Reduced neural gating of respiratory sensations is associated with increased dyspnoea perception. Eur Respir J. 2018:1800559.
27. Borg GA. Psychophysical bases of perceived exertion. Med Sci Sports Exerc. 1982;14(5):377-81.
28. Leblanc P, Bowie DM, Summers E, Jones NL, Killian KJ. Breathlessness and exercise in patients with cardiorespiratory disease. Am Rev Respir Dis. 1986;133(1):21-5.
29. Mador MJ, Rodis A, Magalang UJ. Reproducibility of Borg scale measurements of dyspnea during exercise in patients with COPD. Chest. 1995;107(6):1590-7.
30. Ries AL. Minimally clinically important difference for the UCSD Shortness of Breath Questionnaire, Borg Scale, and Visual Analog Scale. Copd. 2005;2(1):105-10.
31. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand. 1983;67(6):361-70.
32. Bjelland I, Dahl AA, Haug TT, Neckelmann D. The validity of the Hospital Anxiety and Depression Scale. An updated literature review. J Psychosom Res. 2002;52(2):69-77.
33. De Peuter S, Janssens T, Van Diest I, Stans L, Troosters T, Decramer M, et al. Dyspnea-related anxiety: The Dutch version of the Breathlessness Beliefs Questionnaire. Chron Respir Dis. 2011;8(1):11-9.
34. von Leupoldt A, Chan P-YS, Bradley MM, Lang PJ, Davenport PW. The impact of anxiety on the neural processing of respiratory sensations. Neuroimage. 2011;55(1):247-52.
35. Donzel-Raynaud C, Redolfi S, Arnulf I, Similowski T, Straus C. Abnormal respiratory-related evoked potentials in untreated awake patients with severe obstructive sleep apnoea syndrome. Clin Physiol Funct Imaging. 2009;29(1):10-7.
36. Von Leupoldt A, Vovk A, Bradley MM, Keil A, Lang PJ, Davenport PW. The impact of emotion on respiratory-related evoked potentials. Psychophysiology. 2010;47(3):579-86.
37. Webster KE, Colrain IM. The respiratory-related evoked potential: effects of attention and occlusion duration. Psychophysiology. 2000;37(3):310-8.
38. HARVER A, SQUIRES NK, BLOCH-SALISBURY E, KATKIN ES. Event-related potentials to airway occlusion in young and old subjects. Psychophysiology. 1995;32(2):121-9.
39. Sucec J, Herzog M, Van den Bergh O, Van Diest I, von Leupoldt A. The Effects of Repeated Dyspnea Exposure on Response Inhibition. Front Physiol. 2019;10:663.
40. von Leupoldt A, Brassen S, Baumann HJ, Klose H, Büchel C. Structural brain changes related to disease duration in patients with asthma. Eur Respir J. 2011;38(Suppl 55).
41. Troosters T, van der Molen T, Polkey M, Rabinovich RA, Vogiatzis I, Weisman I, et al. Improving physical activity in COPD: towards a new paradigm. Respir Res. 2013;14:115.
42. Pumar MI, Gray CR, Walsh JR, Yang IA, Rolls TA, Ward DL. Anxiety and depression-Important psychological comorbidities of COPD. J Thorac Dis. 2014;6(11):1615-31.

43. Willgoss TG, Yohannes AM. Anxiety disorders in patients with COPD: a systematic review. Respir Care. 2013;58(5):858-66.
44. Yohannes AM, Alexopoulos GS. Depression and anxiety in patients with COPD. European respiratory review : an official journal of the European Respiratory Society. 2014;23(133):345-9.
45. von Leupoldt A, Dahme B. Psychological aspects in the perception of dyspnea in obstructive pulmonary diseases. Respir Med. 2007;101(3):411-22.
46. Janssen DJ, Spruit MA, Leue C, Gijsen C, Hameleers H, Schols JM, et al. Symptoms of anxiety and depression in COPD patients entering pulmonary rehabilitation. Chron Respir Dis. 2010;7(3):147-57.
47. Harrison SL, Greening NJ, Williams JE, Morgan MD, Steiner MC, Singh SJ. Have we underestimated the efficacy of pulmonary rehabilitation in improving mood? Respir Med. 2012;106(6):838-44.
48. von Leupoldt A, Janssens T. Could targeting disease specific fear and anxiety improve COPD outcomes? Expert Rev Respir Med. 2016;10(8):835-7.
49. Reijnders T, Schuler M, Wittmann M, Jelusic D, Troosters T, Janssens W, et al. The impact of disease-specific fears on outcome measures of pulmonary rehabilitation in patients with COPD. Respir Med. 2019;146:87-95.

Chapter 8

General Discussion

8.1 General discussion and future directions

This Ph.D. thesis aimed to investigate potential physiological and psychological mechanisms that can help us to understand better, how IMT improves dyspnea sensation and exercise tolerance in patients with COPD. Chapter 2 provides an overview of several mechanisms underlying the relationship between respiratory muscle dysfunction, symptom perception, and exercise limitation. In chapter 4 a novel approach is introduced for analyzing EMGdi data, which addressed an important problem of ECG artifacts of the diaphragm EMG recording (EMGdi). Then in chapters 5-7 the three candidate mechanisms were investigated, namely neuroventilatory dissociation, muscle metaboreflex, and neural processing of respiratory sensations. With the preliminary results presented in this project, each of these mechanisms has the potential to contribute to improvements in dyspnea and exercise tolerance in response to IMT in patients with COPD in different ways. The summary of the findings from this project and the value added to the field are presented in table 8.1 below.

In Table 8.1, the findings have been categorized into two domains, those which confirm previous findings on the one hand and novel findings on the other hand. The plus and minus signs indicate whether the findings confirm (+) or challenge (-) pre-specified hypotheses.

Table 8.1 Summary of the findings from this thesis project

Chapter	Hypothesis (findings)	Confirmed what we know	Novel finding	References	Future direction
4 (semi-)automated EMG amplitude analysis method	• the newly developed semi-automated EMGdi analysis method is more time efficient and less prone to inter-rater variability • EMGdi amplitudes obtained with the semi-automated method agreed well with values obtained by one of the two manual assessors		+ +		validate this new method for other respiratory muscles and over a broad range of minute ventilation (i.e., during maximal cardiopulmonary exercise test)
5 neuroventilatory uncoupling	8 week of IMT can: • improve dyspnea sensation during exercise o reduction in dyspnea rating on Borg scale during exercise at iso-time (medium effect size) • reduce the neural respiratory drive to the diaphragm o Improve diaphragm function by reducing diaphragm activation (EMGdi/EMGdiMax%) (medium effect size) • reduce the neural respiratory drive to the extra-diaphragmatic respiratory muscles. o increase reliance to extra-diaphragmatic respiratory muscle (SCM) during exercise (small effect size) • improve activity related dyspnea in daily life o improve transitional dyspnea index (small effect size) o improve mMRC score (small effect size) • improve inspiratory muscle strength o increase MIP (large effect size) o Increase sniff,Pes (small effect size) o improve diaphragm function by improving diaphragm strength (sniff,Pdi) (medium effect size) • improve exercise capacity o Prolong endurance exercise duration (medium effect size)	 + + + + + + - + + + + + +	 - +	 (1-4) (1) (1-3) (1-4) (1) (1-4)	Larger sample size required to confirm findings

Chapter	Hypothesis/findings	Confirmed what we know	Novel finding	References	Future direction

6 muscle metaboreflex	8 week of IMT can
	• attenuate the respiratory muscle metaboreflex
	  ○ improvements in respiratory and locomotor muscle blood flow during exercise at iso-time
	  ○ reduce locomotor muscle fatigue
	• increase perfusion of both respiratory and locomotor muscles from iso-time to the new time to task failure of CWR cycling test
			
			-
			-
			
			-
			+
					Subjectively measure diaphragm and extra-diaphragmatic respiratory muscle fatigue to test the potential role of muscle metaboreflex in exercise limitation

7 neural processing of respiratory sensations	8 week of IMT can
	• reduce the affective unpleasantness of respiratory sensations, associated with the reduction in the amplitudes of the later RREP components (P2 and P3)
	  ○ reduce the affective unpleasantness of respiratory sensations response to short inspiratory occlusions during unloaded breathing (small effect size)
	  ○ associated with the reduction in P2 (medium effect size)
	  ○ associated with the reduction in P3 (small effect size)
	• reduce general anxiety levels associated with the changes in the later RREP component
	  ○ reduce general anxiety levels (small effect size)
	  ○ associated with the reduction in P2 (medium effect size)
	  ○ associated with the reduction in P3 (small effect size)
	• reduce dyspnea-specific fears, associated with the changes in the later RREP component
	  ○ reduce dyspnea-specific fears (small effect size)
	  ○ associated with the reduction in P2 (medium effect size)
	  ○ associated with the reduction in P3 (small effect size)
	• reduce the intensity of respiratory sensations in response to short inspiratory occlusions during unloaded breathing (small effect size)
			
			+
			
			+
			
			+
			-
			-
			
			-
			-
			-
			+
			
			+
			+
			-
			+
					Larger sample size required to confirm the findings

The findings from the thesis have been categorized into two domains, those which confirm previous findings and novel findings. The bold plus sign (**+**) indicated that the findings confirmed the pre-specified hypotheses with statistically significant, the plus sign (+) indicated that the findings were in the expected direction, the minus signs (-) indicate that the findings challenge the pre-specified hypotheses. Cohen's d effect sizes were presented: small effect sizes were defined as scores between 0.2 and 0.5, medium effect sizes as scores between 0.5 and 0.8 and large effect sizes were those greater than 0.8.

In **chapter 4**, we are convinced that our findings can contribute importantly to develop the field of continuously monitoring EMG signals during tidal breathing in different populations. The newly developed algorithm is more time-efficient and less prone to inter-rater variability compared to previously use manual analysis methods. It also agreed well with values obtained by one of the two manual assessors and can be used to evaluate changes in EMGdi amplitudes at rest and during CWR exercise in patients with COPD. However, several unresolved problems still need to be tackled in the future. First, EMG measurements can be performed not only on the diaphragm but also on other respiratory muscles. Some studies propose to supplement the measurement of respiratory neural drive using surface EMG assessments of other chest wall muscles (e.g., intercostal muscles, abdominal muscles, or neck muscles) (5, 6). The EMG signals of these muscles might also be contaminated with ECG artifacts. In this chapter, we validated an algorithm to be used with EMGdi signals, however, separate studies are needed to validate this new method for use during EMG recordings of other respiratory muscles. Second, in this study, the manual method and the semi-automated method were evaluated during the measurement of a CWR cycling test only. In the future these methods should also be evaluated over a broader range of minute ventilation (i.e., during maximal cardiopulmonary exercise test). Third, likewise, measurements of EMGdi are also performed in different populations for example in healthy individuals (5, 7), patients with obstructive sleep apnea (OSA)(8), and patients with diaphragm paralysis (9), and therefore further validation of using the semi-automated EMGdi analysis in other populations will be required.

In **chapter 5,** our findings were in line with many aspects of the effects of IMT in patients with COPD that had been observed in previous studies. However, a larger sample size will be required to confirm these findings with a greater degree of certainty. At this point, evidence is accumulating indicating that IMT can improve dyspnea sensation during exercise by reducing the neural respiratory drive (NRD) to the diaphragm. However, we did not find any reduction of the NRD to extra-diaphragmatic respiratory muscles during exercise hyperpnea in response to IMT. We rather observed an increased activation of the SCM muscle possibly contributing to unloading of the diaphragm during exercise.

We further believe that alternative approaches might be useful to identify the mechanisms leading to the reductions in EMGdi after 8 weeks of IMT. Whether the diaphragm contributes less due to the unloading by the other respiratory muscle or whether there was a neuronal adaptation leading to less motor unit recruitment of the diaphragm required could be evaluated by quantifying the relative contribution of the activation of each respiratory muscle in an overall activation index. This total inspiratory neural drive (IND) could be estimated by calculating the sum of the root mean square (RMS) of the maximum activation of each respiratory muscle (i.e., IND(RMS)= EMGdi(RMS) + EMGsca(RMS) + EMGscm(RMS) + EMGicm(RMS) + EMG 7th ICM (RMS)), and the partial contribution of each

respiratory muscle to the respiratory pump (i.e., %IND) as measured by its respective activation divided by the IND (e.g., EMGdi, %IND= EMGdi(RMS)/IND).

Another possibility of assessing relative changes in respiratory muscle function in response to IMT would be to evaluate the development of respiratory muscle fatigue during exercise hyperpnea before and after the training. Diaphragm fatigue can be assessed by using the magnetic phrenic nerve stimulation technique to evaluate the potentiated twitch transdiaphragmatic pressure before and after exercise. A decrease in potentiated twitch force after the task indicates the degree of diaphragm fatigue (10). The fatigue in the extra-diaphragmatic muscles, however, cannot be evaluated with the same technique. The development of fatigue in these muscles could be evaluated by analyzing the median frequency of the EMG power spectrum (11). A shift in the median frequency of the surface EMG power spectrum towards lower values is a well-known method of assessing muscle fatigue (12, 13).

In **chapter 6**, our findings were not in line with a priori formulated hypotheses. Our data did not provide any evidence that 8 weeks of IMT could attenuate the respiratory muscle metaboreflex. Neither improvements in respiratory and/or locomotor muscle blood flow during exercise at iso-time, nor reductions in locomotor muscle fatigue were observed. However, it is not clear whether IMT does actually not impact on the muscle metaboreflex or whether the methodology applied in this study could not capture signs of the muscle metaboreflex during the exercise at iso-time with sufficient precision. In future studies evaluating the effects of IMT on the muscle metaboreflex, the subjective measurement of respiratory muscle fatigue should be performed to confirm that respiratory muscles were stimulated to the point of fatigue during exercise. This could be done by non-volitional evaluation of diaphragm fatigue by phrenic nerve stimulation (10), or by performing medium frequency analysis of diaphragm and other extra-diaphragmatic respiratory muscles.

Another theory that needs to be considered is that central fatigue could influence the results of locomotor fatigue measurements. A number of factors affect muscle contraction and fatigue, for example, metabolic factors (e.g., hydrogen ions and lactate), changes in concentration of central neurotransmitters, blood flow, and oxygen (14). Arousal, motivation, attention, tolerance to discomfort and sensitivity to stress can each alter voluntary drive and modify central fatigue (15). To determine the contribution of central factors to fatigue, the twitch interpolated method has been used as a standard technique. This technique consists of stimulation of superimposing single twitches on a maximal voluntary contraction (MVC) to compare the superimposed response to the potentiated response obtained from the relaxed muscle (15, 16). In this study, we ensured that there is no influence of central fatigue in our results by performing the superimposing single twitch on a maximal voluntary

contraction (MVC) during the measurement of locomotor muscle fatigue. The absence of potentiated responses obtained on top of MVC in our results led us to believe that there was no important influence of central fatigue on our measurements.

The findings in **chapter 7**, do support part of the hypothesis that the reductions in the unpleasantness of respiratory sensations (next to the intensity), and dyspnea specific-fears in the IMT group were paralleled by changes in the central neural processing of these respiratory sensations (reductions in the later affect/cognition-related of respiratory-related evoked potentials (RREP) component P2). However, the same reduction was not observed in P3. We did not find any reduction in general anxiety levels associated with the changes in the later RREP component. This is in contrast with a previous study that found a significant reduction in general anxiety ($p<0.001$) after 6 months of pulmonary rehabilitation (17). These contrasting results might be related to the rather low baseline levels of general anxiety, which might have prevented stronger reductions over the course of intervention in the sense of a floor effect.

This is the first study that evaluated the association between subjective symptom scores (perceived sensation), and self-reported questionnaires (general anxiety measurement via the Hospital Anxiety and Depression Scale (HADS), and dyspnea-specific fear measurement via The Breathlessness Beliefs Questionnaire (BBQ)) with the neural processing of respiratory sensations in response to IMT. We observed a trend for a positive linear relationship between the reductions of the mean amplitude of P2 and the reduction of affective unpleasantness scores. Indeed, a larger sample size is needed to confirm these findings. Future research aspects might be to evaluate the mechanism of these associations. For example, to confirm that changes in dyspnea related fear could result in a reduction in P2 or vice versa. A different experimental design in which one of the variables of interest needs to be controlled in order to assess the changes in the other would be required to evaluate these aspects.

In this study, respiratory sensations were evaluated during short inspiratory occlusions at rest, which might not be representative of the factors triggering symptoms of breathlessness in patients during real-life situations. Several alternatives could be considered in the future to evaluate respiratory sensations. First, the measurements could be performed in more 'real-life' dyspnea stimulating situations. The RREP's elicited by occlusions could for example be evaluated during loaded breathing, cycling, or other daily activities. Second, other advanced technologies, for example, fMRI (Functional magnetic resonance imaging) could also be used in order to investigate the brain areas related to dyspnea perception, the relationship between brain activity, and dyspnea during exercise and in responses to the intervention (18-20).

8.2 Clinical implications

Due to hyperinflation in COPD, the diaphragm is exposed to a mechanical disadvantage and is forced to operate from a chronically shortened position. However, during exercise or physical activities when acute dynamic hyperinflation occurs, the adaptations of the diaphragm to chronic shortening can be quickly overwhelmed. The diaphragm is then forced to generate higher pressures (working against higher elastic and resistive loads) and simultaneously has to contract with increasing velocities while being further acutely shortened. This results in an acute reduction of pressure-generating capacity, leading to a growing disparity between the ventilatory load and the capacity of the respiratory muscles (neuroventilatory uncoupling) resulting in an increase in dyspnea. Due to the mechanical disadvantages induced by static hyperinflation, it was unclear whether diaphragm function can actually be improved in response to IMT.

The patients in our study presented with a high level of static hyperinflation and the absence of changes in static lung volumes pre-post intervention confirmed that IMT did not reduce the level of static hyperinflation. On the other hand, our results indicate that it is possible to improve diaphragm function at rest and during exercise by IMT in patients with COPD (improvements in sniff,Pdi and reductions in EMGdi/EMGdiMax%). In addition, a novel finding of this study showed that IMT not only can improve diaphragm function but also leads to more reliance on extra-diaphragm respiratory muscle (SCM). This is an indication that improving respiratory muscle capacity is not limited only to the diaphragm but also the contribution of extra-diaphragmatic inspiratory muscles.

Our results pointed into the direction that IMT as a stand-alone intervention could result in improvements in dyspnea sensation and exercise capacity (increased endurance cycling time). In practice, IMT is prescribed as an adjunct intervention during pulmonary rehabilitation program (PR). However, we could speculate that in some cases, IMT as a stand-alone might be beneficial as a “pre-rehabilitation” intervention. A duration of 4 to 8 weeks is required to achieve a reduction in dyspnea sensation due to muscle adaptation in response to IMT. Therefore, to prescribe IMT in advance before starting PR could be beneficial in preparing the patients for the PR program to, first, try to improve dyspnea sensation, reduce dyspnea-related fears and potentially motivate the patients to participate in a pulmonary rehabilitation program (in the case of less motivated patients). Besides, the reduction in dyspnea could provide a possibility to increase in physical activity (PA) (combine with coaching or educational program for PA) (21).

Previous studies found that IMT as an adjunct intervention during PR did not result in additional improvements in 6MWD (2, 3). However additional gains in endurance time and reductions in symptoms of dyspnea were observed during an endurance cycling test (secondary outcome) (4). In our

study, the reduction in dyspnea sensation in response to a stand-alone IMT program also can facilitate the improvement in endurance cycling. This findings suggest that incorporating IMT as a part of the routine PR program might be beneficial, especially to stimulate exercise progression during the symptom-based progression aerobic exercise.

In the near future, IMT devices could be more conveniently accessible by the patients and healthcare providers, for example, the device that can connect wirelessly to the mobile phone to provide spontaneous feedback. This could make the training more pleasant and perhaps increase the motivation of the patients in performing the training. It would also be helpful for the healthcare provider to be able to better monitor the training progression and supervise the patients in the long term in their home setting.

In this study, the potential improvements in dyspnea intensity, unpleasantness, dyspnea related-fears, and exercise tolerance in response to IMT did not translate to modify PA levels of the patients with COPD. Also, IMT did not result in a change in anxiety levels as has been expected. These results emphasize the importance of combining muscle conditioning strategies with interventions that encourage change in patients' behavior, for example, PA coaching program or psychological interventions (i.e., cognitive behavioural therapy (CBT)(22)) that could improve anxiety levels in these patients.

8.3 Overall conclusions

To conclude, the findings of this thesis project indicate that IMT is an intervention impacting on several physiological and psychological mechanisms that can potentially improve inspiratory muscle function, dyspnea sensation (not only intensity but also unpleasantness), exercise capacity, and dyspnea-related fears in patients with COPD. IMT could deliver a combined physiological and psychological effect. Improving respiratory muscle function indicated the physiological effect while a reduction of dyspnea-related fear indicated the psychological effect due to habituation in neural processing by providing repeated exposure to dyspnea sensation during the training. In the meantime, tailored multidisciplinary interventions will be required for the best outcomes in these patients.

References

1. Langer D, Ciavaglia C, Faisal A, Webb KA, Neder JA, Gosselink R, et al. Inspiratory muscle training reduces diaphragm activation and dyspnea during exercise in COPD. J Appl Physiol (1985). 2018;125(2):381-92.
2. Beaumont M, Forget P, Couturaud F, Reychler G. Effects of inspiratory muscle training in COPD patients: A systematic review and meta-analysis. Clin Respir J. 2018;12(7):2178-88.
3. Gosselink R, De Vos J, van den Heuvel SP, Segers J, Decramer M, Kwakkel G. Impact of inspiratory muscle training in patients with COPD: what is the evidence? Eur Respir J. 2011;37(2):416-25.
4. Charususin N, Gosselink R, Decramer M, Demeyer H, McConnell A, Saey D, et al. Randomised controlled trial of adjunctive inspiratory muscle training for patients with COPD. Thorax. 2018;73(10):942-50.
5. Reilly CC, Jolley CJ, Ward K, MacBean V, Moxham J, Rafferty GF. Neural respiratory drive measured during inspiratory threshold loading and acute hypercapnia in healthy individuals. Exp Physiol. 2013;98(7):1190-8.
6. MacBean V, Hughes C, Nicol G, Reilly CC, Rafferty GF. Measurement of neural respiratory drive via parasternal intercostal electromyography in healthy adult subjects. Physiol Meas. 2016;37(11):2050-63.
7. Jolley CJ, Luo YM, Steier J, Reilly C, Seymour J, Lunt A, et al. Neural respiratory drive in healthy subjects and in COPD. Eur Respir J. 2009;33(2):289-97.
8. Luo YM, Tang J, Jolley C, Steier J, Zhong NS, Moxham J, et al. Distinguishing obstructive from central sleep apnea events: diaphragm electromyogram and esophageal pressure compared. Chest. 2009;135(5):1133-41.
9. Caleffi Pereira M, Dacha S, Testelmans D, Gosselink R, Langer D. Assessing the effects of inspiratory muscle training in a patient with unilateral diaphragm dysfunction. Breathe. 2019;15(2):e90-e6.
10. Laveneziana P, Albuquerque A, Aliverti A, Babb T, Barreiro E, Dres M, et al. ERS Statement on Respiratory Muscle Testing at Rest and during Exercise. Eur Respir J. 2019:1801214.
11. Gross D, Grassino A, Ross WR, Macklem PT. Electromyogram pattern of diaphragmatic fatigue. J Appl Physiol Respir Environ Exerc Physiol. 1979;46(1):1-7.
12. Allison GT, Fujiwara T. The relationship between EMG median frequency and low frequency band amplitude changes at different levels of muscle capacity. Clin Biomech (Bristol, Avon). 2002;17(6):464-9.
13. Phinyomark A, Thongpanja S, Hu H, Phukpattaranont P, Limsakul C. The Usefulness of Mean and Median Frequencies in Electromyography Analysis. In: Naik GR, editor. Computational Intelligence in Electromyography Analysis - A Perspective on Current Applications and Future Challenges. Rijeka: InTech; 2012. p. Ch. 08.
14. Wan JJ, Qin Z, Wang PY, Sun Y, Liu X. Muscle fatigue: general understanding and treatment. Exp Mol Med. 2017;49(10):e384.
15. Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. Physiol Rev. 2001;81(4):1725-89.
16. Millet GY, Martin V, Martin A, Verges S. Electrical stimulation for testing neuromuscular function: from sport to pathology. Eur J Appl Physiol. 2011;111(10):2489-500.
17. von Leupoldt A, Taube K, Lehmann K, Fritzsche A, Magnussen H. The impact of anxiety and depression on outcomes of pulmonary rehabilitation in patients with COPD. Chest. 2011;140(3):730-6.
18. Stoeckel MC, Esser RW, Gamer M, Buchel C, von Leupoldt A. Brain mechanisms of short-term habituation and sensitization toward dyspnea. Front Psychol. 2015;6:748.

19. Herigstad M, Faull OK, Hayen A, Evans E, Hardinge FM, Wiech K, et al. Treating breathlessness via the brain: changes in brain activity over a course of pulmonary rehabilitation. Eur Respir J. 2017;50(3).
20. Herigstad M, Hayen A, Evans E, Hardinge FM, Davies RJ, Wiech K, et al. Dyspnea-related cues engage the prefrontal cortex: evidence from functional brain imaging in COPD. Chest. 2015;148(4):953-61.
21. O'Connor C, Lawson R, Waterhouse J, Mills GH. Is inspiratory muscle training (IMT) an acceptable treatment option for people with chronic obstructive pulmonary disease (COPD) who have declined pulmonary rehabilitation (PR) and can IMT enhance PR uptake? A single-group prepost feasibility study in a home-based setting. BMJ open. 2019;9(8):e028507-e.
22. Heslop-Marshall K, Baker C, Carrick-Sen D, Newton J, Echevarria C, Stenton C, et al. Randomised controlled trial of cognitive behavioural therapy in COPD. ERJ Open Research. 2018;4(4):00094-2018.

Summary

Shortness of breath (dyspnea) during exercise is an important symptom in patients with COPD. Dyspnea is regarded as a multidimensional sensation. Its perception is frequently subdivided into three different dimensions. The first domain deals with the sensory-perceptual aspect of dyspnea perception (i.e., what breathing feels like) including intensity and sensory quality of the perceived symptom. The second component relates to the degree of affective distress that is involved with symptom perception (i.e., how distressing breathing discomfort feels). Finally, also the impact or burden on daily functioning that is caused by dyspnea needs to be considered (i.e., how symptoms impact on activity limitations and participation restrictions as well as on perceived quality of life and health status).

The sensation of dyspnea can withhold patients from performing physical activities including exercise contributing to further deconditioning of respiratory and limb muscles, which in turn aggravate exertional dyspnea symptoms. This activity limitations caused by shortness of breath will eventually impact on patients' quality of life. Reducing exertional dyspnea symptoms in patents with COPD is, therefore, an important treatment goal within the rehabilitation. Inspiratory muscle training (IMT) is one of the treatments aiming to reduce dyspnea and improving exercise tolerance in these patients. In international treatment guidelines, IMT is a recommended treatment for patients who present with inspiratory muscle weakness and exertional dyspnea symptoms. IMT can improve the inspiratory muscle function (strength and endurance), dyspnea, exercise capacity, and quality of life in patients with COPD. Several biological and psychological mechanisms (e.g., neuro-ventilatory dissociation, respiratory muscle metaboreflex, dyspnea related fears and neural processing of respiratory sensations) might contribute to explain the effects of IMT on dyspnea and exercise capacity, however, these mechanisms are not yet well understood. Despite of a strong theoretical rationale and an abundance of clinical research supporting the effects of IMT, mechanistic studies investigating the physiological and psychological adaptations that might occur with training are sparse. Therefore, the focus of this Ph.D. project is to investigate in-depth some of these candidate mechanisms to be able to better understand the position of IMT in the treatment of dyspnea and impaired exercise performance in COPD patients.

This Ph.D. project highlights the role of inspiratory muscle weakness in sensory (especially intensity) and affective (unpleasantness) perception of dyspnea in patients with COPD. Patients with respiratory muscle weakness (lower than predicted normal respiratory muscle strength) participated in the randomized, controlled study in which they were assigned to participate in either a high-intensity training program for their respiratory muscles or a "sham" training intervention. We investigated several key mechanisms including changes in 'neuro-ventilatory dissociation', the potential role of the

'muscle metaboreflex', and changes in 'neural processing of respiratory sensations' that had previously been hypothesized to be potentially related to dyspnea reduction in response to IMT.

Chapter 2 provides background information on several physiological mechanisms linking respiratory muscle dysfunction to dyspnea and exercise limitation. The chapter also includes an overview of available treatments and potential mechanisms that can help to better explain and understand the effects of these interventions.

Chapter 4 is focusing on the electromyogram analysis method that was used for analyzing the data collected in chapter 4. The electromyogram (EMG) of the diaphragm was recorded, however, these signals contained electrocardiogram (ECG) artifacts. To remove these ECG artifacts out of the recorded EMG signals, in an interfaculty collaboration with the department of Electrical Engineering (ESAT), KU Leuven, we developed an algorithm using commercially available software (LABVIEW) that enables us to semi-automatically remove ECG artifacts from the EMG recordings. We evaluated whether this new method was reliable, had a good agreement with the typically used manual removal methods and whether it could be used to assess changes of the signal in response to intervention. We also evaluated the degree of agreement among the two raters using the manual method. The newly developed method could also reduce the analyzing time by more than half of the manual removal method. In addition, the semi-automated method was less prone to the variability (inconsistency) and can be used to evaluate changes in response to an intervention. We concluded that the semi-automated ECG artifact removal method for diaphragm EMG analyses would be helpful to eliminate sources of inconsistency that were observed between different raters when applying the manual method. This new method offers a more objective approach for analyzing EMG signal while requiring less analyzing time. We propose this method to be a new standard for objective diaphragm EMG analyses in the future.

In **chapter 5** the effects of 8 weeks IMT or sham training on dyspnea, respiratory muscle function at rest and during exercise and neuroventilatory coupling were evaluated. We found that eight weeks of IMT was able to improve the capacity of the respiratory muscles (i.e., improvements in strength and endurance). Patients were able to cycle longer, and the dyspnea intensity was reduced. At the same time, similar loads were imposed on the respiratory muscles during exercise (i.e., no changes in minute ventilation and similar pressures that had to be generated by the respiratory muscles during breathing). For these similar demands that were imposed on the respiratory muscles during exercise, the activation of the diaphragm was reduced while the activation of the extra-diaphragmatic respiratory muscles was not changed in ratio. However, we found that together with less activation of the diaphragm, the sternocleidomastoid (neck muscle) was activated more. Therefore, we concluded

that IMT tended to improve inspiratory muscle strength and endurance exercise performance, reduced symptoms of leg discomfort and dyspnea potentially due to a combination of the improvement in the capacity of the diaphragm (increased in pressure-generating capacity of the diaphragm and decreased diaphragm EMG) and more reliance on sternocleidomastoid muscle.

The aim of **chapter 6** was to investigate the effects of 8 weeks IMT on the changes in respiratory and leg muscle blood flow and leg muscle fatigue during cycling exercise. Respiratory and leg muscle blood flow and oxygenation were evaluated before and after eight weeks of IMT training. We found no changes at iso-time in blood flow and oxygenation of all muscles measured compared to pre-training. Leg muscle fatigue also was not changed at iso-time. This indicates that the extent of respiratory muscle work and activation observed at isotime, while triggering intolerable symptoms, was not yet having a major impact on limiting leg muscle perfusion and oxygenation. Otherwise, the observed reductions in diaphragm activation after training should not only have resulted in reducing symptoms but also in reducing the degree of leg muscle fatigue at iso-time. However, due to further increase in blood flow and preserved oxygenation level during the prolonged duration of cycling (5 min) at post-measurement suggests that IMT could potentially attenuate the so-called metaboreflex by reallocation of available blood flow from other sources to the working muscles.

In **chapter 7**, we focused on the perception of affective aspects related to dyspnea in patients with COPD. The effect of 8 weeks IMT on changes in the unpleasantness component of respiratory perception in relation to parallel changes in the brain signals related to respiratory sensations was assessed. The brain signals were recorded using non-invasive measurements of Respiratory-Related Evoked Potentials (RREPs) in the electroencephalogram (EEG). We found that in response to 8 weeks IMT, there was a trend for a reduction in the central neural processing of inspiratory occlusions (RREP component P2) in association with a trend for reductions in ratings of perceived intensity and affective unpleasantness. We also found a trend for a reduction in dyspnea-specific fear measured with the Breathlessness Beliefs Questionnaire after eight weeks of IMT. We concluded that IMT could potentially reduce respiratory sensation, both intensity and affective unpleasantness, which might relate to a habituation process in the neural, affect-related processing of respiratory sensations.

In conclusion, this Ph.D. thesis offers a comprehensive evaluation of some previously unexplored physiological and psychological mechanisms that might help to explain the improvements in exertional dyspnea perception and functional capacity after IMT in patients with COPD. Detailed recommendations for further research building on these initial findings is provided in the individual chapters and in the general discussion.

Samenvatting

Kortademigheid (dyspnoe) tijdens inspanning is een belangrijk symptoom van patiënten met COPD. Dyspneu wordt beschouwd als een multidimensionale ervaring. De perceptie van kortademigheid wordt vaak onderverdeeld in drie verschillende subcategorieën. Het eerste domein omvat de sensorisch perceptuele aspecten van de gewaarwording van dyspneu inclusief de sensorische kwaliteit van de ervaren sensatie. The tweede domein omvat de emotionele stress die met de perceptie gepaard gaat. Ten laatste moet ook de invloed van de symptomen op beperkingen in activiteiten en sociale interacties in rekening gebracht worden.

Het ervaren van kortademigheid kan patiënten weerhouden om aan dagelijkse fysieke activiteiten deel te nemen. Hierdoor verslechterd de spierfunctie verder, waardoor ventilatoire nood tijdens inspanningen toeneemt en symptomen over tijd verergeren. De beperking in activiteiten die uit deze neerwaartse spiraal van deconditionering en kortademigheid resulteren zullen uiteindelijk een belangrijke negatieve impact op de levenskwaliteit van deze patiënten hebben. Het verminderen van symptomen van dyspneu is daarom een belangrijk behandeldoel bij deze patiënten. Respiratoire spiertraining is een van de behandelopties die beschikbaar is om symptomen van dyspneu te verminderen en die kan helpen om het inspanningsvermogen van deze patiënten te verbeteren. In internationale behandelrichtlijnen wordt respiratoire spiertraining aanbevolen als een behandeloptie voor patiënten met respiratoire spierzwakte en symptomen van dyspneu. De training kan helpen om de ademspierfunctie (kracht en uithouding), dyspneu, en inspanningsvermogen bij deze patiënten te verbeteren. Het wordt verondersteld dat de effecten van respiratoire spiertraining door verschillende biologische en psychologische factoren tot stand komen. Terwijl de klinische effecten van de interventie uitvoerig onderzocht en beschreven zijn is er weinig mechanistisch onderzoek naar onderliggende fysiologische en psychologische adaptaties uitgevoerd. Daarom was de focus van de studies die deel uitmaken van deze proefschrift om enkele potentiele mechanismen die de effecten van ademspiertraining zouden kunnen verklaren in meer detail te bestuderen.

De studies die in het kader van deze proefschrift uitgevoerd zijn benadrukken de impact van ademspiertraining op de perceptie van intensiteit en onaangenaamheid van kortademigheid bij patiënten met COPD. Patiënten met respiratoire spierzwakte namen hiervoor deel aan een gerandomiseerde, gecontroleerde studie waarin zij per toeval toegewezen werden aan een interventiegroep (ademspiertraining) of een controlegroep die een niet werkzame interventie ontving. We bestudeerden enkele mechanismen die op basis van theoretische overwegingen verondersteld werden om potentieel een bijdrage aan de effecten van ademspiertraining op symptomen van dyspneu en inspanningsvermogen te hebben.

In hoofdstuk 2 wordt achtergrondinformatie over verschillende physiologische en psychologische mechanismen samengevat die de effecten van ademspiertraining op symptomen van dyspneu en inspanningsvermogen mogelijks kunnen verklaren.

In hoofdstuk 4 ligt de focus op technische aspecten tijdens de analyses van het diafragma electromyogram. Diafragmaactivatie die met behulp van het electromyogram gekwantificeerd kan worden wordt als een surrogaat voor 'respiratory neural drive' gebruikt en is een belangrijke parameter om de effecten van ademspiertraining op het mechanisme van 'neuroventilatoire dissociatie' te bestuderen (zie resultaten hoofdstuk 5). In dit hoofdstuk introduceren we een zelf ontwikkelde, semi-geautomatiseerde analysemethode om ECG artefacten uit deze EMG signalen te verwijderen. We konden aantonen dat deze nieuwe methode minder tijdsintensief is dan eerder gebruikte manuele analysemethoden en bovendien de mate van interbeoordelaarvariabiliteit tijdens de analyses kon verminderen.

In **hoofdstuk 5** werden de effecten van ademspiertraining op dyspneu, respiratoire spierfunctie en neuroventilatoire dissociatie tijdens inspanning bestudeerd. Ondanks een kleine steekproef konden eerdere bevindingen van verbeteringen in respiratoire spierfunctie, dyspneu en inspanningsvermogen gereproduceerd worden. Ook kon net als in voorgaande studies aangetoond worden dat activatie van het diafragma verminderd was na training. Voor een meer omvattende analyse van neuroventilatoire dissociatie en 'neural respiratory drive' te kunnen maken werd in deze studie ook de activatie van andere inspiratoire spieren bestudeerd die naast het diafragma voor al tijdens inspanning geactiveerd worden. Uit deze analyses bleek dat deze in tegenstelling tot het diafragma na de interventie tijdens inspanning niet minder geactiveerd worden. In het geval van de m. sternocleidomastoideus vonden we zelfs een verhoogde activatie na ademspiertraining. Op basis van onze bevindingen concludeerden wij dat de afname in diafragma activatie tijdens inspanning na ademspiertraining mogelijks uit een combinatie van verbeteringen in diafragmafunctie en een toegenomen bijdrage van de niet diafragmale inspiratoire spieren tijdens inspanningsademhaling resulteert.

In **hoofdstuk 6** bestudeerden we de effecten van ademspiertraining op veranderingen in doorbloeding en oxygenatie van zowel respiratoire spieren als beenspieren. Geen veranderingen op een gestandaardiseerd moment tijden inspanning warden geobserveerd na de interventieperiode. Veranderingen in de mate van beenspiervermoeidheid werden ook niet gemeten. In tegenstelling tot onze initiële hypothesen suggereren deze data dat, onder de bestudeerde omstandigheden en ondanks het ontstaan van ondragelijke symptomen van kortademigheid die het voor patiënten onmogelijk maakte om de inspanning verder te zetten, de mate van respiratoire spierarbeid op dit

moment nog geen belangrijke impact op de verdeling van bloedstroom tussen respiratoire en beenspieren en het ontstaan van beenspiervermoeidheid heeft gehad.

In **hoofdstuk 7** werden de effecten van ademspiertraining op affective aspecten dyspneuperceptie bij patiënten met COPD verder onderzocht. De mate van onaangenaamheid tijdens het ervaren van korte respiratoire occlusies werden samen met de centrale neurale verwerking van respiratoire stimuli bestudeerd. De zogenaamde 'Respiratory-Related Evoked Potentials (RREPs)' die in respons tot deze kort occlusies optreden werden hiervoor met behulp van elektro-encefalogram (EEG) metingen in kaart gebracht. Naast vermindering van de subjectieve onaangenaamheid van de ervaren stimuli na de interventieperiode vonden we ook een verandering in de neurale verwerking van deze sensaties. Deze data zijn suggestief voor desensitisatie mechanismen in respons tot ademspiertraining in de neurale, affect-gerelateerd verwerking van respiratoire sensaties.

In conclusie wordt in deze proefschrift een omvattende evaluatie van enkele hiervoor onbeschreven fysiologische en psychologische gepresenteerd die een bijdrage kunnen leveren om de effecten van ademspiertraining op inspanningsvermogen en dyspneuperceptie beter te begrijpen. Voortbouwend op deze exploratieve analyses gaat het belangrijk zijn om deze initiële observaties in een grotere steekproef te valideren. Gedetailleerde aanbevelingen voor vervolgonderzoek worden in de individuele hoofdstukken en in de algemene discussie gegeven.

Appositions

1. The language barrier is not as potent as the mind barrier. Once the mind is open, even with different languages, communication is possible.

2. In the circumstance of limited facility and budget, for example in community hospitals in Thailand, pulmonary rehabilitation can be effectively performed using simple and inexpensive equipment and measurements.

3. Dyspnea is a multidimensional sensation. To succeed in dyspnea management, approaches to all dimensions are necessary.

Scientific acknowledgement

Study 1: Respiratory muscle function and exercise limitation in patients with chronic obstructive pulmonary disease: a review

Published: Expert Rev Respir Med. 2018;12(1):67-79

Noppawan Charususin, Sauwaluk Dacha, Rik Gosselink, Marc Decramer, Andreas Von Leupoldt, Thomas Reijnders, Zafeiris Louvaris & Daniel Langer

NC, SD, RG, and DL contributed to the conception and design of the review. NC and SD wrote the first draft of the manuscript. TR, ZL, and, AvL wrote sections of the manuscript. RG, DL, and MD supervised equally. All authors contributed to the manuscript revision, and read and approved the submitted version.

Study 2: Comparison between manual and (semi-)automated analyses of esophageal diaphragm electromyography during endurance cycling in patients with COPD

Published: Front Physiol. 2019;10(885)

Sauwaluk Dacha, Luc Janssens, Antenor Rodrigues, Zafeiris Louvaris, Lotte Janssens, Rik Gosselink, & Daniel Langer

SD, RG, and DL contributed to the conception and design of the study. SD, ZL, DL and LoJ contributed to the acquisition. LuJ developed the analysis algorithm. SD and AR performed the data analysis. SD organized the database, performed the statistical analysis, and wrote the first draft of the manuscript. LuJ, AR, RG, and DL wrote sections of the manuscript. All authors contributed to the manuscript revision, and read and approved the submitted version.

Aspects of this manuscript have been presented at the European Respiratory Society (ERS) International Congress 2018, Paris, France, September 16–19, 2018 and also published as a congress abstract in the supplement material of the European Respiratory Journal 2018 52: Suppl. 62, PA1714. doi:10.1183/13993003.congress-2018.PA1714;
https://erj.ersjournals.com/content/52/suppl_62/PA1714.

Study 3: Effects of inspiratory muscle training on dyspnea and respiratory muscle function at rest and during exercise in patients with COPD – preliminary results

Sauwaluk Dacha, Antenor Rodrigues, Zafeiris Louvaris, Lotte Janssens, Rik Gosselink, & Daniel Langer

SD, RG, and DL contributed to the conception and design of the study. SD, AR, ZL, DL and LoJ contributed to the acquisition. SD and AR performed the data analysis. SD organized the database, performed the statistical analysis, and wrote the first draft of the manuscript. RG and DL supervised equally.

Aspects of this manuscript have been accepted to be presented at the European Respiratory Society (ERS) International Congress 2019, Madrid, Spain, September 28– October 2, 2019

Study 4: Impact of inspiratory muscle training on respiratory and locomotor muscle perfusion, oxygenation and locomotor muscle fatigue during exercise in patients with COPD – preliminary results

Sauwaluk Dacha, Zafeiris Louvaris, Lotte Janssens, Rik Gosselink, Daniel Langer

SD, ZL, RG, and DL contributed to the conception and design of the study. SD, ZL, DL, and LoJ contributed to the acquisition. SD and ZL performed the data analysis. SD organized the database, performed the statistical analysis, and wrote the first draft of the manuscript. RG and DL supervised equally.

Study 5: Impact of inspiratory muscle training on the perception and neural processing of respiratory sensations in COPD– preliminary results

Sauwaluk Dacha, Thomas Reijnders, Ysys Denutte, Andreas von Leupoldt, Rik Gosselink, & Daniel Langer

SD, TR, AvL, RG, and DL contributed to the conception and design of the study. SD, DL, TR and YD contributed to the acquisition. SD and TR performed the data analysis. SD organized the database, performed the statistical analysis, and wrote the first draft of the manuscript. AvL, RG and DL supervised equally.

We wish to thank Prof. Dr. Wim Janssens and Prof. Dr. Thierry Troosters for their support and constructive criticism. We also thank our colleagues in the Respiratory Rehabilitation and Respiratory Division, University Hospital Leuven, Belgium, for their invaluable support during the recruitment, screening for candidates and perform clinical assessments.

Personal contribution

In this thesis project, I contributed to the concept, execution, analysis, and reporting of all studies. I took part in writing the first draft of the review in study 1, split the work with NC. In study 2, I contributed to developing the semi-automated method with my supervisor and LuJ. I handled all the data and split the work with AR in analyzing the data with the manual method. I performed the semi-automated analysis and all the statistical analysis in this study.

For study 3 to 5, I took part in the recruitment process of the participants with the help from my supervisors and colleagues from UZ Leuven. I performed some of the clinical assessments (inspiratory muscle strength and endurance measurements) and delivered an inspiratory muscle training program to all the patients in the study. I also took part in the experimental measurements (cycling test, balance test, miniBes test, quadriceps twitch force measurement, and EMG measurement) with the help with my supervisors, AR, ZL, and LoJ. I handled most of the data in study 3 and 4, including performing all the statistical analysis. ZL provided me with the results from NIRS and central hemodynamic. In study 5, I organized the appointments with TR and YD for them to perform the EEG measurements for the patients. TR performed the EEG data analysis, and I took the results to perform the statistical analysis.

Conflict of interest statement

This project has been supported by the following specific funding sources: Research Foundation - Flanders (FWO) grant GOA4516N and KU Leuven - University of Leuven grant C22/15/035).

AvL is supported by the 'Asthenes' long-term structural funding grant (#METH/15/011) by the Flemish Government, Belgium, and by a research grant (STRT/13/002) from the Research Fund KU Leuven, Belgium.

TR is supported by the Flemish Government, Belgium, and by a research grant (STRT/13/002) from the Research Fund KU Leuven, Belgium.

ZL is the recipient of a European Respiratory Society Long-Term Research fellowship number LTRF 2016-6686 and a postdoctoral fellow of Research Foundation - Flanders (FWO) (12U5618N).

AR is supported by the Coordination for the Improvement of Higher Education Personnel (CAPES), Brazil (88881.188754/2018-01).

SD is supported by Chiang Mai University's scholarship, Chiang Mai, Thailand (Ref. No. 6392(7)/0807).

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Personal acknowledgments

I would like to acknowledge Professor Luc Sels, rector of the Katholieke Universiteit Leuven, and Professor Christophe Delecluse, dean of the Faculty of Movement and Rehabilitation Sciences of our university, for the opportunity to develop my Ph.D. at this institution.

I would also like to acknowledge Professor Niwes Nantachit, President of Chiang Mai University, and Professor Sakorn Pornprasert, dean of the Faculty of Associated Medical Sciences of Chiang Mai University, for the opportunity to pursue my Ph.D. by permitting my study leave and providing the essential financial support.

This thesis and doctoral degree that I earned could not be possible without an enormous amount of help and support from many people. I would like to thank my advisory committee, Professor Thierry Troosters, Professor Wim Janssens, Professor Ilse Van Diest, Professor Sabine Verschueren, and Professor W. Darlene Reid who guided me through these four years with your constructive criticism, valuable suggestions and recommendations. I would also like to thank my promotors and co-promotor Professor Rik Gosselink, Professor Daniel Langer and Professor Andreas Von Leupoldt for your guidance and support. I have learned a lot from you.

To professor Gosselink, it was an honor to receive the opportunity to come here and join the team, thank you for your kindness always. To Daniel, you are a big part of the equation to make this achievement possible for me. Thank you for your enormous help, guidance, and for being patient with me. It was a pleasure to have a chance to work with you. A special thanks for Professor Luc Janssens, Professor Lotte Janssens, Thomas, and Ysys who are part of the collaboration in this project. It was a pleasure to have been working with you all.

I would like to extend my gratitude to all the patients who participated in the studies. Thank you for your scarify and for your kindness and goodwill to help in the clinical trials. Thanks for your patience with my broken Dutch. I am very grateful.

To the former dean of the faculty of Associated Medical Sciences of Chiang Mai University Professor Wasna Sirirungsi, thank you for supporting me with anything I needed, especially when I encountered problems with the scholarship and visa applications. Also big thanks to Professor Orawan Prasartwuth, and Professor Samatchai Chamnongkichthe, former and present head of the physical therapy department, who always support me professionally and personally. Thanks to all of my colleagues in the physical therapy department, especially those in the cardio-respiratory unit whom all these years taking the workload off me, so I can come here and study. Your love, support, and encouragement help a lot to get me going, and I hope I made you proud. Also, a big thanks to the administrative and

financial team, who help sorted out many paper works and made sure I receive the scholarship on time every six months.

To my family, my super mom who is a hero for me, for her, “everything is possible,” she herself got her Ph.D. in 2006, and if mom can do it! So do you. For dad, who is a quiet cheerleader in the family, he doesn’t talk much, but you know he always got your back! Thanks mom and dad for your unconditional love, for raising me in such a way that makes me a strong and independent person. I am lucky to be your child. To my two brothers, Tucha and Freshy! I am sorry I am not always there for you. I have been away too much when you are growing up, but I hope you know that I always love you and anything you ever need, I will make it happen. Thanks always for your love and support. Also, thanks to grandma, uncles, aunties, and cousins, who always support me and take care of my family when I am away, I am very appreciated it. Besties, you are my favorite cousin, thanks for helping me with those statistics! Best of luck for your Ph.D. as well.

To my two favorite girls Nathalie and Stephanie, seeing your smiles can already make my day. Thank you Felicia and Joe for letting me be a part of the family and for the love and support that you always send my way no matter how far apart we are.

Som, Thanks a lot for helping me from the first day I arrived in Belgium, for helping me through the culture shock, for everything you did for me. I really appreciated it.

Fernanda! I couldn’t make it without you, thanks for your support, your friendship, those countless hours we spent together, watching movies, cooking, eating or do something fun. Thanks for always be there for me, especially to listen to me whining about my life! We will meet again soon! A trip to Brazil is on the very top of my bucket list!

Zafeiris! Thanks for your support, for the work that we have done together, I learn a lot from you. Also, thanks for your friendship through all these years and counting! Thanks for all the bananas you brought for me to keep my sugar level up! It wouldn’t survive these days without those bananas!!!

Cristina! Thanks for always be there for me, for always supporting me, thanks for the fun time we spent together and thanks for believing in me (sometimes more than I believe in myself)! We will, for sure, be in touch!

Matthias! It was fun to have you around, thanks for the good time we spent together, best of luck to you for your bright future.

To Marine, Astrid and Heleen, thanks for your friendship and for being such good colleagues. I also wish you all the best!

To Abby, Weili, Cintia thanks for your friendship, thanks for your support, especially the last few months of my Ph.D., having you around helps a lot.

For the “upstairs people” Marko, Karen, Kristina, Carolien, Sofie, Neel, Siva, Birger, Tom, Tobias, Jef, Manosij, Nirosha..…. Thanks for including me for those activities, every Christmas dinners, and parties, even though we did not see each other often, but I really appreciated your friendships.

Veronica, Lode, Ilse, Iris, Nele, Miek, Beike, Bart and the rest of “the blue team” thanks for your help to guide me around in the clinic, to help to recruit patients, deal with KWS and for those fun lunches in the sun or lunch hour walks. I really enjoy my time with you guys. Vero! Thanks for your kindness and for the good time we spent together in those ERS congresses.

Thank you, Geert, Karen, Kristine, May, Stephanie, and the rest of my colleagues in the Pneumology unit, UZ Leuven, without your help, the study could not be successful.

Anita and Eline, thanks for your help with all the paper works and administrative work you do for us.

Jo and Nina! You two are my favorite doctors! Thanks for your help at work and also your friendships!

To Top, Tum, Ben and the rest of my Thai friends who became family, having you guys here made the life much more pleasant. Thanks for sharing the good time, for your love, support, and encouragements.

To all my friends from Dutch classes Amanda, Shengyun, Emilije, Ella, Elise, Nina, Moniquita…and all the international friends that I met here Raquel, Cinthia, Eleonora, Jiyeon, Alaide, Aline, Andrea (s), Karla, Yves, Mariana, Erika, Nur, Debjani….. we share the struggle, we share the similarity, we made foreign place feels like home. Thanks for your friendship, for the fun time hanging out together. Good luck with your future! We will keep in touch.

Thank you, Kim, Nut, Pat, my PNS-square! Thanks for your love and support. Thank you, P’Aorn for always listening to me whining and give me support and encouragement. I feel so lucky to have you all around.

For all the friends from all over the world that impossible for me to list all the names, my aupair friends, university friends, high school friends, or even elementary school friends! Thank you for your support, for all the messages asking about how I am doing, for always sending love and care my way. I really appreciated it.

I always live my life with no regret. I have lived, I have done things I wanted to do. This chapter is closing beautifully, and I am ready for the next one! THANK YOU!

Curriculum Vitae

Personal information

Sauwaluk Dacha

Date of Birth: 16 September 1982

Place of Birth: Nakhonsawan, Thailand

Nationality: Thai

Education/Degree

06/2011	Master of Health Service Administration, Strayer University, VA, United States
03/2004	Bachelor of Science (Physical Therapy), Chiang Mai University, Chiang Mai, Thailand

Employment

10/2012-Present	Lecturer at Department of Physical Therapy, Chiang Mai University, Chiang Mai, Thailand
06/2008-03/2009	Physical Therapist at Department of Physical Therapy, Sawanpracharak Hospital, Nakhonsawan, Thailand
04/2004-02/2006	Physical Therapist at Department of Physical Therapy, Srisawan Hospital, Nakhonsawan, Thailand

Training and Courses

04/2017	ERS (European Respiratory Society) pulmonary rehabilitation course, Athens, Greece
03/2017	Spike 2 program; CED (Cambridge Electronic Design Limited), Cambridge, United Kingdom

Publications

1. **Dacha S**, Janssens L, Rodrigues A, Louvaris Z, Janssens L, Gosselink R, et al. Comparison Between Manual and (Semi-)Automated Analyses of Esophageal Diaphragm Electromyography During Endurance Cycling in Patients With COPD. Front Physiol. 2019;10(885).
2. Charususin N, **Dacha S**, Gosselink R, Decramer M, Von Leupoldt A, Reijnders T, et al. Respiratory muscle function and exercise limitation in patients with chronic obstructive pulmonary disease: a review. Expert Rev Respir Med. 2017:1-13.
3. Langer D, Ciavaglia C, Faisal A, Webb KA, Neder JA, Gosselink R, **Dacha S**, Topalovic M, Ivanova A, O'Donnell D. E. Inspiratory muscle training reduces diaphragm activation and dyspnea during exercise in COPD. J Appl Physiol (1985). 2018;125(2):381-92.
4. Caleffi Pereira M, **Dacha S**, Testelmans D, Gosselink R, Langer D. Assessing the effects of inspiratory muscle training in a patient with unilateral diaphragm dysfunction. Breathe. 2019;15(2):e90-e6.
5. Radtke T, Crook S, Kaltsakas G, Louvaris Z, Berton D, Urquhart S. D, Kampouras K, Rabinovich A. R, Verges S, Kontopidis D, Boyd J, Tonia T, Langer D, De Brandt J, Goërtz V, Burtin C, Spruit A. M, Braeken C. W. D, **Dacha S**, Franssen M. E. F, Laveneziana P, Eber E, Troosters T, Neder J.A, Puhan A. M, Casaburi R, Vogiatzis* I, Hebestreit H. (2019). ERS Statement on Standardisation of Cardiopulmonary Exercise Testing in Chronic Lung Diseases. *European Respiratory Review*. Accepted Jun 2019, 16.

Participation at international meetings

1. **Dacha S**, Langer D, Ciavaglia C, Webb K, Preston M, O'Donnell DE. Effect of Inspiratory Muscle Training on Respiratory Muscle Function and Diaphragm Activation in Patients with COPD. A109. HIGHLIGHTS IN PULMONARY REHABILITATION: ILD, ADJUNCTS, AND INSPIRATORY MUSCLE TRAINING. American Thoracic Society International Conference Abstracts: American Thoracic Society; 2017. p. A2860-A.
2. **Dacha S**, Langer D, Ciavaglia C, Webb K, Preston M, O'Donnell DE. Effect of inspiratory muscle training (IMT) on static and dynamic respiratory muscle function in patients with COPD. Eur Respir J. 2017;50(suppl 61):OA2923.
3. **Dacha S**, Janssens L, Louvaris Z, Janssens L, Gosselink R, Langer D. Comparison between manual and automated analyses of esophageal diaphragm electromyography during endurance cycling in patients with COPD. Eur Respir J. 2018;52(suppl 62):PA1714.
4. Janssens L, Langer D, **Dacha S**, Louvaris Z, Brumagne S, Goossens N, et al. Inspiratory muscle training decreases ankle proprioceptive use during balance control in patients with COPD. Eur Respir J. 2018;52(suppl 62):PA1708.
5. Louvaris Z, **Dacha S**, Janssens L, Gosselink R, Vogiatzis I, Langer D. Inspiratory muscle effort, perfusion and oxygenation responses to inspiratory muscle training (IMT) with Tapered Flow Resistive Loading (TFRL) and Normocapnic Hyperpnea (NH) in COPD. Eur Respir J. 2018;52(suppl 62):OA3634.
6. **Dacha S**, Louvaris Z, Janssens L, Testelmans D, Gosselink R, Langer D. Effect of an Inspiratory Muscle Training (IMT) Program on Respiratory Muscle Function, Symptoms of Dyspnea, Respiratory Muscle Activation and Tissue Oxygen Delivery During Exercise Breathing in a Patient with Idiopathic Unilateral Diaphragmatic Paralysis: A Case Report. B60. PULMONARY REHABILITATION: GENERAL. American Thoracic Society International Conference Abstracts: American Thoracic Society; 2019. p. A3744-A.

Supervision of Master students

2015-2017

1. Elien Myngheer and Mirte Van den Mooter: Effects of inspiratory muscle training on shortness of breath (dyspnea) in patients with COPD.
2. Simon D'Hoore and Anouk Lamberts: Lung volume specificity of two different inspiratory muscle training protocols.
3. David Schieffelers and Laura Smits: Effects of inspiratory muscle training on exercise capacity, blood flow distribution and peripheral muscle fatigue in COPD.

2016-2018

1. Hanne Geeraerts and Elise van der Linden: Effects of inspiratory muscle training on blood flow distribution between respiratory and locomotor muscles during exercise in patients with COPD.
2. Sebastiaan Thyvelen and Willem Van Damme: Effects of inspiratory muscle training on dyspnea and respiratory muscle activity in patients with COPD.
3. Ine Mortelmans and Christine Oppedijk: Prevalentie van spierdysfunctie en symptomen van dyspneu bij borstkankerpatiënten.

2017-2019

1. Liesbeth Vanhaverbeke and Latisha Decoene: Effects of inspiratory muscle training on exertional breathlessness in patients with unilateral diaphragm paralysis.
2. Elke Piot and Eva Staes: Effects of inspiratory muscle training on shortness of breath (dyspnea) in patients with COPD.

2018-2020

1. Anouk Fertin and Amber Beersaerts :Effects of inspiratory muscle training on the symptoms of dyspnoea in patients with chronic obstructive pulmonary disease.