PHARMACOLOGICAL AND NON-PHARMACOLOGICAL THERAPIES FOR THE RELIEF OF BREATHLESSNESS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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ABSTRACT

Rationale. Despite receiving triple inhalation therapy and undergoing pulmonary rehabilitation, approximately 50-90% of adults with chronic obstructive pulmonary disease (COPD) suffer from persistent and disabling breathlessness and exercise intolerance. Therefore, adjunct therapies targeted to improve exertional breathlessness and exercise tolerance are urgently needed to help optimize disease management and health outcomes in COPD. **Objective.** To evaluate the acute effects of abdominal binding (AB), immediate-release oral morphine, and inhaled vaporized cannabis on exertional breathlessness and exercise endurance in symptomatic adults with advanced COPD. These three therapies were selected to target different mechanistic pathways within the neurophysiological construct of breathlessness, including [1] the neuromuscular efficiency of the diaphragm (AB); [2] neural respiratory drive and central corollary discharge (immediate-release oral morphine and inhaled vaporized cannabis); and [3] neuromechanical coupling of the respiratory system (inhaled vaporized cannabis). **Methods.** Study 1*:* In a 'proof of principle study', we compared the effect AB (sufficient to increase end-expiratory gastric pressure (Pga,ee) by 6.6 ± 0.6 cmH_2O at rest) *vs.* control (unbound condition) on physiological and perceptual responses during a constant-load cardiopulmonary cycle exercise test (CPET) in 12 healthy non-obese men. Study 2*:* In a randomized crossover trial, we compared the effect of AB (sufficient to increase Pga,ee by 6.7±0.3 cmH_2O at rest) *vs.* control (unbound condition) on physiological and perceptual responses during a constant-load CPET in 20 adults with advanced COPD. Study 3: In a randomized crossover trial, we compared the effect of immediate-release oral morphine (0.1 mg/kg body weight) *vs.* placebo (diluted simple syrup) on physiological and perceptual responses during a constant-load CPET in 20 adults with advanced COPD and chronic breathlessness syndrome. Study 4*:* In a randomized crossover trial, we compared the effects of 35 mg of inhaled vaporized

cannabis (18.2% delta-9-tetrahydrocannabinol (THC), <0.1% cannabidiol (CBD)) *vs.* 35 mg of placebo (0.33% THC, <0.99% CBD) on physiological and perceptual responses during a constant-load CPET in 16 adults with advanced COPD. **Results.** Studies 1 and 2: Compared to the unbound condition, AB enhanced neuromuscular efficiency of the diaphragm during exercise, but had no effect on exertional breathlessness and exercise endurance in healthy adults and in adults with COPD. Study 3: Compared with placebo, oral morphine had statistically significant and clinically meaningful improvements on exertional breathlessness and exercise endurance in adults with advanced COPD and chronic breathlessness syndrome. These improvements were [1] accompanied by small decreases in minute ventilation, breathing frequency and neural respiratory drive; and [2] most pronounced in adults whose exercise capacity was limited primarily by intolerable breathlessness. Study 4: Compared to the control treatment, inhaled vaporized cannabis had no clinically meaningful positive or negative effect on any of the physiological and perceptual outcomes measured at rest and during CPET. **Conclusion.** Immediate-release oral morphine, but not AB or inhaled vaporized cannabis improved exertional breathlessness and exercise endurance in symptomatic adults with advanced COPD. The collective results of this thesis suggest that, after optimizing disease modifying therapy (i.e., inhaled bronchodilators and anti-inflammatory agents), adjunct therapies for the management of breathlessness and exercise tolerance in COPD should be targeted to alter [1] central neural processing of breathlessness and [2] neural respiratory drive and central corollary discharge.

RESUME

Fondement. En dépit de la triple thérapie par inhalation et de la réadaptation pulmonaire, environ 50 à 90% des adultes atteints de maladie pulmonaire obstructive chronique (MPOC) souffrent d'essoufflement persistant et invalidant et d'intolérance à l'exercice. Par conséquent, des traitements d'appoint destinés à améliorer l'essoufflement face à l'effort physique et la tolérance à l'exercice présentent une nécessité urgente afin d'aider l'optimisation de la gestion et des résultats thérapeutique liés à la MPOC. **Objectif.** Évaluer les effets aigus de la liaison abdominale (LA), de la morphine par voie orale à libération immédiate et de la vaporisation de cannabis inhalé sur l'essoufflement relié à l'effort physique et l'endurance à l'exercice chez l'adulte symptomatique atteint de la MPOC avancée. Ces trois thérapies ont été sélectionnées pour cibler différentes voies mécanistiques au sein de la construction neurophysiologique de l'essoufflement, incluant [1] l'efficacité neuromusculaire du diaphragme (LA); [2] la commande respiratoire neurale (CRN) et la décharge corollaire centrale (morphine par voie orale à libération immédiate et vaporisation de cannabis inhalé); [3] couplage neuromécanique du système respiratoire (vaporisation de cannabis inhalé). **Méthodologie.** Étude 1 : Dans une « étude de preuve de principe », nous avons comparé l'effet de la LA (suffisante pour augmenter la pression gastrique à la fin de l'expiration (Pga,ee) par 6.6 ± 0.6 cmH_2O au repos) par rapport à la condition de contrôle (condition non liée) sur les réponses physiologiques et perceptuelles lors d'un test d'exercice du cycle cardiopulmonaire à charge constante chez 12 hommes en bonne santé non obèses. Étude 2 : Dans un essaie transversal aléatoire, nous avons comparé l'effet de la LA (suffisante pour augmenter la pression gastrique à la fin de l'expiration (Pga,ee) par 6.6 ± 0.6 cmH_2O au repos) par rapport à la condition de contrôle (condition non liée) sur les réponses physiologiques et perceptuelles lors d'un test d'exercice du cycle cardiopulmonaire à charge constante chez 20 adultes atteints de la MPOC avancée. Étude 3 :

Dans un essaie transversal aléatoire, nous avons comparé l'effet de la morphine orale à libération immédiate (0.1 mg/kg de poids corporel) par rapport à un placebo (sirop simple dilué) sur les réponses physiologiques et perceptuelles lors d'un test d'exercice du cycle cardiopulmonaire à charge constante chez 20 adultes atteints de la MPOC avancée et du syndrome d'essoufflement chronique. Étude 4 : Dans un essaie transversal aléatoire nous avons comparé les effets de 35 mg de cannabis vaporisé par inhalation (18.2% delta-9-tetrahydrocannabinol (THC), <0,1% de cannabidiol (CBD)) contre 35 mg de placebo (0,33% de THC, <0,99% de CBD)) sur les réponses physiologiques et perceptuelles au cours d'un test d'exercice du cycle cardiopulmonaire à charge constante chez 16 adultes atteints de la MPOC avancée. **Résultats.** Études 1 et 2 : Par rapport à la condition non liée (condition contrôle), la LA a amélioré l'efficacité neuromusculaire du diaphragme pendant l'exercice, mais n'a eu aucun effet sur l'essoufflement face à l'effort physique et la tolérance à l'exercice chez l'adulte en bonne santé et chez l'adulte souffrant de la MPOC. Étude 3 : Comparativement au placebo, la morphine orale présentait des améliorations statistiquement significatives et d'importantes améliorations cliniques sur l'essoufflement face à l'effort physique et la tolérance à l'exercice chez les adultes atteints de la MPOC avancée et du syndrome d'essoufflement chronique. Ces améliorations étaient [1] accompagnées de légères diminutions de la ventilation minute, de la fréquence respiratoire et du CRN; et [2] plus prononcé chez les adultes dont la capacité d'exercice était limitée principalement par un essoufflement intolérable. Étude 4 : Par rapport au traitement de contrôle, le cannabis vaporisé par inhalation n'avait aucun effet positif ou négatif cliniquement significatif sur les résultats physiologiques et perceptuels mesurés au repos et pendant le test d'exercice du cycle cardiopulmonaire à charge constante. **Conclusion.** La morphine par voie orale à libération immédiate, mais pas la fixation abdominale ou l'inhalation de cannabis vaporisé, a amélioré l'essoufflement face à l'effort

physique et la tolérance à l'exercice chez les adultes symptomatiques atteints de la MPOC avancée. Les résultats collectifs de cette thèse suggèrent qu'après l'optimisation du traitement modificateur de la maladie (bronchodilatateurs inhalés et agents anti-inflammatoires), les thérapies complémentaires pour la gestion de l'essoufflement et de la tolérance à l'exercice dans la MPOC doivent être ciblées afin de modifier [1] le traitement neural central d'essoufflement et [2] CRN et décharge corollaire centrale.

LIST OF ABBREVIATIONS

[M3G]	Plasma concentrations of morphine-3-glucuronide
[M6G]	Plasma concentrations of morphine-6-glucuronide
[M]	Plasma concentrations of morphine
11-OH-THC	11-hydroxy-Δ^9-tetrahydrocannabinol
6MWT	6-minute walk test
AB	Abdominal binding
ACC	Anterior cingulate cortex
AMYG	Amygdala
Ax	Area of reactance
BDI	Baseline Dyspnoea Index
BiPAP	Bilevel positive airway pressure
Ca^{2+}	Calcium
Ca^{2+}_i	Intracellular calcium concentration
cAMP	Cyclic adenosine 3',5'-monophosphate
CAT	COPD Assessment Test
CB	Cerebellum
CB_1	Cannabinoid receptor type 1
CB_2	Cannabinoid receptor type 2
CBD	Cannabidiol
CO	Cardiac output
CO_2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
CPET	Cardiopulmonary cycle exercise test
CTRL	Control
CTS	Canadian thoracic society
D_LCO	Diffusing capacity of the lung for carbon monoxide
EELV	End-expiratory lung volume
EET	Exercise endurance time
EMGdi	Electromyogram of the diaphragm
EMGdi%max	EMGdi relative to maximum EMGdi
$FEF_{25-75\%}$	Forced expiratory flow between 25% and 75%
FEV_1	Forced expiratory volume in 1-sec
fMRI	Functional magnetic resonance imaging
FRC	Functional residual capacity
F_{res}	Resonant frequency

FVC	Forced vital capacity
f_R	Breathing frequency
GABA	*Gamma*-Aminobutyric acid
GOLD	Global Initiative for Obstructive Lung Disease
HADS	Hospital Anxiety and Depression Scale
HDACs	Histone deacetylases
HR	Heart rate
IC	Inspiratory capacity
ICS	Inhaled corticosteroids
IP_3	1,4,5-trisphosphate
IRV	Inspiratory reserve volume
LABA	Long-acting β_2-agonists
LAMA	Long-acting antimuscarinic agents
M1	Primary motor cortex
M_1; M_2; M_3	Muscarinic receptors type 1, 2 and 3, respectively
MCID	Minimally clinically important difference
MDT	Medial dorsal thalamus
mMRC	Modified Medical Research Council dyspnoea scale
MMSE	Mini-Mental State Exam
MO	Medulla oblongata
NR	Non-responder
O_2	Oxygen
OLVs	Operating lung volumes
ORSDS	Opioid-related symptom distress scale
P-V	Pressure-volume
P_aCO_2	Arterial partial pressure for CO_2
$P_{ac}CO_2$	Arterialized capillary partial pressure for CO_2
Pdi	Transdiaphragmatic pressure
PEF	Peak expiratory flow rate
Pes	Esophageal pressure
Pes%max	Esophageal pressure relative to maximum inspiratory pressure
PET_{CO_2}	End-expiratory partial pressure for CO_2
PFC	Prefrontal cortex
Pga	Intra-abdominal pressure
Pga,ee	End-expiratory gastric pressure
PI	Phosphodiesterase inhibitor
PPC	Posterior parietal cortex

PPO	Peak power output
PR	Pulmonary rehabilitation
pre-BötC	pre-Bötzinger complex
R	Responder
R_{20}	Resistance at 20 Hz
R_5	Resistance at 5 Hz
RV	Residual volume
S1	Primary somatosensory cortex
S2	Secondary somatosensory cortex
SABA	Short-acting β_2-agonists
SCI	Spinal cord injury
SMA	Supplementary motor area
S_PO_2	Oxygen saturation by pulse oximetry
sRaw	Specific airway resistance
SV	Stroke volume
THC	delta-9 (Δ^9)-tetrahydrocannabinol
THC-COOH	11-nor-9-carboxy-Δ^9-tetrahydrocannabinol
THCA	Trans-Δ^9-tetrahydrocannabinol-9-acid A
TLC	Total lung capacity
TRPM8	Transient receptor potential melastatin 8
VAS	Visual analogue scale
VC	Vital capacity
$\dot{V}CO_2$	Rate of CO_2 output
$\dot{V}_E$	Ventilation
$\dot{V}O_2$	Rate of O_2 consumption
VPT	Ventroposterior thalamus
V_T	Tidal volume
X_5	Reactance at 5 Hz

LIST OF TABLES AND FIGURES

Tables

Figures

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I am forever grateful to Dr. Dennis Jensen for giving me the opportunity to pursue my doctoral work in his lab. I first joined his lab as a Research Assistant with no knowledge of exercise physiology or COPD. He took a chance on me, and for that, I will always be thankful. His drive and passion for science has always inspired me. No matter the circumstance, he always took the time to meet with me to discuss my projects and life in general. From him, I learned that there is no such thing as impossible – anything can be achieved with a little hard work. Thank you, Dennis, for believing in me even when I didn't believe in myself.

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PREFACE

Adults with chronic obstructive pulmonary disease (COPD) suffer from severe and disabling exertional breathlessness and exercise intolerance (1-3). In my doctoral work, I sought to identify novel adjunct pharmacological and non-pharmacological therapies for the management of breathlessness and exercise intolerance in symptomatic adults with advanced COPD, particularly those suffering from chronic breathlessness syndrome (4). To this end, I undertook three clinical trials to evaluate the efficacy of three different therapies on the symptom of breathlessness and exercise tolerance in adults with COPD. The three therapies were: [1] abdominal binding; [2] immediate-release oral morphine; and [3] inhaled vaporized cannabis. Each of these therapies was intended to alter a unique pathway within the neurophysiological construct of breathlessness.

This thesis was prepared according to McGill University's regulations for a manuscript-based thesis. It consists of four published manuscripts, including [1] a proof-of principle study conducted in healthy adults to evaluate the effects of abdominal binding on diaphragmatic neuromuscular efficiency during exercise (5), and [2] three randomized clinical trials that sought to evaluate the efficacy and mechanisms of action of abdominal binding (6), immediate-release oral morphine (7), and inhaled vaporized cannabis (8) on exertional breathlessness and exercise endurance in symptomatic adults with moderate-to-very severe COPD. The work presented within this thesis has advanced our understanding of the neurophysiological mechanisms of breathlessness and exercise intolerance in adults with COPD, and over time, it is my hope that this work will serve to improve the health-related quality of life of those suffering from COPD.

Thesis composition and manuscript overview.

Thesis chapter: Title/ ***Manuscript title***	**Originality**	**Publication status**
Chapter 1: Introduction		
Chapter 2: Literature review		
Chapter 3: Objectives and hypotheses		
Chapter 4: Populations and study design		
Chapter 5: Manuscript 1 *Abdominal binding improves neuromuscular efficiency of the human diaphragm during exercise*	This randomized controlled trial was the first to evaluate the acute effects of abdominal binding on diaphragmatic neuromuscular efficiency, exertional breathlessness and exercise endurance in healthy adults.	**Published** in *Frontiers in Physiology.* **Doi:**10.3389/fphys.2017.00345
Chapter 6: Manuscript 2 *Effect of abdominal binding on diaphragmatic neuromuscular efficiency, exertional breathlessness and exercise endurance in chronic obstructive pulmonary disease*	This randomized controlled trial was the first to evaluate the acute effects of abdominal binding on diaphragmatic neuromuscular efficiency, exertional breathlessness and exercise endurance in adults with COPD.	**Published** in *Frontiers in Physiology.* **Doi:** 10.3389/fphys.2018.01618
Chapter 7: Manuscript 3 *Effect of morphine on breathlessness and exercise endurance in advanced COPD: a randomized crossover trial*	This randomized controlled trial was the most detailed and comprehensive study to evaluate the acute effects of immediate-release oral morphine *vs.* placebo on breathlessness, exercise endurance and neural respiratory drive in adults with advanced COPD and chronic breathlessness syndrome.	**Published** in *The European Respiratory Journal* and accompanied by an editorial. **Manuscript Doi:**10.1183/13993003.01235-2017 **Editorial Doi:** 10.1183/13993003.01865-2017
Chapter 8: Manuscript 4 *Effect of vaporized cannabis on exertional breathlessness and exercise endurance in advanced COPD: a randomized controlled trial*	This was the first randomized controlled trial to evaluate acute the effects of inhaled vaporized cannabis *vs.* placebo on pulmonary function, exertional breathlessness and exercise endurance in adults with advanced COPD.	**Published** in *Annals of the American Thoracic Society* and accompanied by an editorial. **Manuscript Doi:** 10.1513/AnnalsATS.201803-198OC **Editorial Doi:** 10.1513/AnnalsATS.201807-463ED
Chapter 9: General discussion		

Dissertation organization and overview

Chapter 1 includes a brief introduction of chronic obstructive pulmonary disease (COPD) and the general aims of this thesis.

Chapter 2 provides a comprehensive [1] review on the mechanisms of breathlessness and exercise intolerance in adults with COPD, [2] summary of various pharmacological and non-pharmacological therapies available to adults with COPD, and [3] overview of the prevalence and burden of breathlessness and chronic breathlessness syndrome in adults with advanced COPD.

Chapter 3 includes the rationale, objective and hypotheses of this thesis.

Chapters 4-7 include the manuscripts for studies 1-4, respectively. Each chapter is organized as follows: preface, title page, abstract, introduction, materials and methods, results, discussion, acknowledgements, tables and figures.

Chapter 4 consists of a manuscript entitled "*Abdominal binding improves neuromuscular efficiency of the human diaphragm during exercise".* This was a "proof of principle" study conducted in healthy adults to determine the acute effects of abdominal binding on neuromuscular efficiency of the diaphragm, exertional breathlessness and exercise endurance. This manuscript was published in *Frontiers in Physiology* (5) (**Appendix I**).

Chapter 5 consists of a manuscript entitled "*Effect of abdominal binding on diaphragmatic neuromuscular efficiency, exertional breathlessness and exercise endurance in chronic obstructive*

pulmonary disease". This randomized controlled trial was designed based on the findings presented in **Chapter 4** and represents the first study to evaluate the acute effects of abdominal binding on diaphragmatic neuromuscular efficiency, exertional breathlessness and exercise endurance in adults with COPD. This manuscript was published in *Frontiers in Physiology* (6) (**Appendix II**).

Chapter 6 consists of a manuscript entitled "*Effect of morphine on breathlessness and exercise endurance in advanced COPD: a randomized crossover trial"*. This randomized controlled trial was the most detailed and comprehensive study to evaluate the efficacy and mechanisms of action of immediate-release oral morphine on exertional breathlessness and exercise endurance in adults with advanced COPD and chronic breathlessness syndrome. This manuscript was published in the *European Respiratory Journal* (7) (**Appendix III**) and accompanied by an editorial (9).

Chapter 7 consists of a manuscript entitled "*Effect of vaporized cannabis on exertional breathlessness and exercise endurance in advanced COPD: a randomized controlled trial"*. This was the first randomized controlled trial to evaluate the acute effects of inhaled vaporized cannabis on airway function, exertional breathlessness and exercise endurance in adults with advanced COPD. This manuscript was published in the *Annals of the American Thoracic Society* (8) (**Appendix IV**) and accompanied by an editorial (10).

Chapter 8 includes a summary of the manuscripts and a general discussion of the overall thesis.

Contributions of Co-authors

This thesis consists of four original research projects for which, I, Sara J. Abdallah, Ph.D. Candidate, under the supervision of Prof. Dennis Jensen, am responsible for.

With regards to **Study 1** (*Abdominal binding improves neuromuscular efficiency of the human diaphragm during exercise*), I analyzed the data and wrote the manuscript with critical input from my supervisor and co-authors.

With regards to **Study 2** ***(****Effect of abdominal binding on diaphragmatic neuromuscular efficiency, exertional breathlessness and exercise endurance in chronic obstructive pulmonary disease)*, I recruited study subjects; collected and analyzed data; and wrote the manuscript with critical input from my supervisor and co-authors.

With regards to **Study 3** (*Effect of morphine on breathlessness and exercise endurance in advanced COPD: a randomized crossover trial*), in collaboration with Prof. Dennis Jensen, and Dr. Jean Bourbeau, I generated the objectives and hypothesis for the study; designed the study protocol as well as the consent and case report forms; obtained regulatory approval from Health Canada and ethics approval from the Research Institute of the McGill University Health Centre; co-ordinated all aspects of the study; collected and analyzed data; and wrote the manuscript with critical input from my supervisor and co-authors.

With regards to **Study 4** (*Effect of vaporized cannabis on exertional breathlessness and exercise endurance in advanced COPD: a randomized controlled trial*), with critical input from Prof.

Dennis Jensen, I conceptualized the study; generated the objectives and hypothesis for the study; designed the study protocol as well as the consent and case report forms; obtained regulatory approval from Health Canada and ethics approval from the Research Institute of the McGill University Health Centre; co-ordinated all aspects of the study; collected and analyzed the data; and wrote the manuscript with critical input from my supervisor and co-authors.

Prof. Dennis Jensen was the principle investigator on **Studies 1-4**. Prof. Jensen oversaw all aspects of **Studies 1-4**, including: study conceptualization; study design and protocol development; attainment of regulatory approval from Health Canada (where required) and ethics approval from the Research Institute of the McGill University Health Centre; data analysis and interpretation; and organization and content of all manuscripts included in this thesis.

Dr. Jean Bourbeau was a member of my doctoral thesis committee and co-investigator on **Studies 1-4**. Specifically, Dr. Bourbeau served as the Qualified Investigator for **Studies 3 and 4**. Dr. Bourbeau contributed to the conceptualization of **Study 3**. Furthermore, Dr. Bourbeau participated in study design and protocol development of **Studies 1-4**; and provided critical feedback on all manuscripts presented in this thesis.

Dr. Benjamin M. Smith was a co-investigator on **Studies 1-4**. Dr. Smith participated in study design and protocol development of **Studies 1-4**; he served as the medical supervisor for **Studies 3 and 4**; and provided critical feedback on all manuscripts presented in this thesis.

Dr. Mark A. Ware was a co-investigator on **Study 4**, contributing his expertise to the design of **Study 4** and provided critical feedback on the manuscript.

Dr. Yuanming Luo was a collaborator on **Study 1** and provided critical feedback on the manuscript.

Pei Z Li is a biostatistician at the Montreal Chest Institute that assisted with the statistical analyses for **Studies 2**, **3 and 4**.

David S. Chan, **Robin Glicksman** and **Michelle Moore** are former undergraduate students, while **Cassandra T. Mendonca** and **Courtney Wilkinson-Maitland** are former graduate students of Prof. Jensen's Clinical Exercise & Respiratory Physiology Laboratory. David, Robin and Cassandra were responsible for collecting the data for **Study 1**. Courtney assisted in data collection for **Studies 2 and 3**, while Michelle assisted in data collection for **Study 4**.

Statement of originality

Adults living with chronic obstructive pulmonary disease (COPD) suffer from pervasive and disabling activity-related breathlessness and exercise intolerance (1-3). Despite intensive therapy with traditional pharmacological and non-pharmacological therapies (e.g., bronchodilators, pulmonary rehabilitation), many adults with COPD continue to suffer from exertional breathlessness and exercise intolerance (1-3). It follows, that adjunct therapies must be considered in order to adequately manage exertional breathlessness and exercise intolerance in adults with COPD. Therefore, the aim of this thesis was to explore the efficacy and physiological mechanisms of action of novel and/or poorly studied pharmacological and non-pharmacological therapies in the management of exertional breathlessness and exercise intolerance in adults with advanced COPD, many of who were receiving optimal disease modifying therapies. To this end, we designed three randomized clinical trials to evaluate the acute effect of [1] abdominal binding (AB), [2] immediate-release oral morphine and [3] inhaled vaporized cannabis on exertional breathlessness and exercise endurance in adults with COPD. A statement of originality for each of these studies is included below.

Study 1 (proof of principle study: abdominal binding in healthy adults).

Study 1 served as a "proof of principle" study to evaluate the acute effects of AB on diaphragmatic neuromuscular efficiency, exertional breathlessness and exercise endurance in healthy adults. This study was the first to evaluate the effects of AB on [1] neuromuscular coupling of the diaphragm and [2] cardiac, metabolic, ventilatory, breathing pattern, dynamic operating lung volume, diaphragm electromyogram (EMGdi), respiratory pressure and breathlessness responses during high-intensity, constant-load cycle exercise testing in healthy adults. We hypothesized that AB

would improve exertional breathlessness and exercise endurance in healthy adults by enhancing neuromuscular coupling of the diaphragm.

The main finding of **Study 1** was that AB sufficient to increase intra-abdominal pressures by 5-8 cmH_2O enhanced neuromuscular efficiency of the diaphragm during exercise in the absence of clinically meaningful improvements in exertional breathlessness and exercise endurance in healthy adults. We postulated that the lack of effect of enhanced neuromuscular efficiency of the diaphragm during exercise with AB on exertional breathlessness and exercise endurance was due, in part, to the fact that diaphragmatic neuromuscular inefficiency is not the proximate cause of exertional breathlessness and exercise intolerance in healthy adults. Nevertheless, this "proof of principle" study clearly demonstrated that AB can enhance neuromuscular efficiency of the diaphragm during exercise, and therefore provided a strong physiological rationale for the use of AB as a potentially effective means of alleviating breathlessness and improving exercise tolerance by enhancing neuromuscular efficiency of the diaphragm during exercise in COPD. This is important in as much as neuromuscular inefficiency of the diaphragm has been implicated as a mechanism of exertional breathlessness and exercise intolerance in adults with COPD (11-13).

Study 2 (non-pharmacological therapy: abdominal binding).

Based on the findings of **Study 1**, **Study 2** was designed to test the hypothesis that AB would improve exertional breathlessness and exercise endurance by enhancing neuromuscular efficiency of the diaphragm during exercise in adults with COPD. **Study 2** was the first adequately powered randomized clinical trial to evaluate the effects of AB on [1] neuromuscular coupling of the diaphragm and [2] cardiac, metabolic, ventilatory, breathing pattern, dynamic operating lung

volume, EMGdi, respiratory pressure and breathlessness responses during high-intensity, constant-load cycle exercise testing in adults with COPD.

The main finding of **Study 2** was that AB enhanced neuromuscular efficiency of the diaphragm in the absence of clinically significant improvements in exertional breathlessness and exercise endurance in adults with COPD. Specifically, the results of **Study 2** suggested that the etiology of exertional breathlessness in COPD is multifactorial, and that, in the absence of improvements in static and dynamic lung function, expiratory flow-generating capacity, ventilation, breathing pattern, and inspiratory reserve volume, isolated and acute improvements in diaphragmatic neuromuscular efficiency during exercise is unlikely to improve exertional breathlessness and exercise tolerance in adults with COPD. In other words, the results of **Study 2** suggested that, in order for a pharmacological and/or non-pharmacological therapy to effectively improve exertional breathlessness and/or exercise endurance in adults with COPD, it/they must [1] enhance respiratory mechanics, [2] alter central neural processing of breathlessness and/or [3] decrease inspiratory neural drive. To this end, the pharmacological therapy evaluated in **Study 3** was designed to reduce exertional breathlessness by targeting central neural processing of breathlessness and inspiratory neural drive.

Study 3 (pharmacological therapy: immediate-release oral morphine).

In **Study 3**, immediate-release oral morphine was selected as a pharmacotherapy as it was hypothesized to reduce exertional breathlessness and improve exercise tolerance by modulating central neural processing of breathlessness and decreasing inspiratory neural drive in symptomatic adults with COPD.

Study 3 represented the most detailed and comprehensive randomized, double-blind, placebo-controlled, cross-over trial to evaluate the efficacy and mechanisms of action of immediate-release oral morphine in reducing exertional breathlessness and improving exercise endurance in adults with advanced COPD and chronic breathlessness syndrome. Specifically, this was the first study to evaluate the acute effect of single-dose administration of immediate-release oral morphine *vs.* placebo on: [1] carbon dioxide (CO_2) retention; [2] opioid-related side effects; [3] pharmacokinetics of morphine and its metabolites; and [4] cardiac, metabolic, ventilatory, breathing pattern, dynamic operating lung volume, EMGdi, respiratory pressure and breathlessness responses during high-intensity, constant-load cycle exercise testing in advanced COPD.

The main finding of **Study 3** was that immediate-release oral morphine was associated with clinically meaningful improvements in exertional breathlessness and exercise tolerance in adults with advanced COPD and chronic breathlessness syndrome. We demonstrated that relief of breathlessness and improvements in exercise endurance following administration of oral morphine *vs.* placebo were associated with a decrease in minute ventilation and EMGdi. Furthermore, this study was the first to report on the variability of responsiveness to morphine therapy in adults with advanced COPD (i.e., individuals that exhibit opioid-induced relief of breathlessness *vs.* those that do not). We demonstrated that adults with COPD that stopped exercise due to intolerable breathlessness were more likely to experience morphine-induced relief of exertional breathlessness than those individuals that stopped exercise for reasons other than intolerable breathlessness. Therefore, this study demonstrated that the locus of symptom-limitation on laboratory-based cycle exercise testing may help to predict which adults with advanced COPD and chronic breathlessness

syndrome are most likely to achieve clinically meaningful improvements in exertional breathlessness and exercise endurance in response to morphine therapy.

The results of **Study 3** added to the growing body of evidence supporting the use of oral morphine for the management of breathlessness and exercise intolerance in adults with advanced COPD and chronic breathlessness syndrome (14). To our knowledge, this study was the first to formally introduce the notion of an "opioid responder" and "opioid non-responder" when referencing the effect of oral morphine on exertional breathlessness in symptomatic adults with advanced COPD. In reporting on differences between opioid responders and non-responders, **Study 3** provided a rationale for additional studies to identify genotypic and phenotypic characteristics of these two groups that may help to personalize symptom management with opioids.

Although the results of **Study 3** were positive, they did not preclude the need to identify additional therapies for the management of breathlessness and exercise intolerance in adults with COPD, particularly in light of the longstanding concerns about the use of opioids for relief of breathlessness (e.g., dose escalation, fear of adverse events, addiction) (15-17). To this end, **Study 4** was designed to explore the effects of a novel and alternative pharmacotherapy to morphine on exertional breathlessness and exercise endurance in adults with advanced COPD.

Study 4 (pharmacological therapy: inhaled vaporized cannabis).

Previous studies conducted in adults with asthma demonstrated a profound bronchodilator response to cannabis and its main cannabinoid constituent, delta-9 (Δ^9)-tetrahydrocannabinol (THC) (18-22). Therefore, in **Study 4**, cannabis was selected as the pharmacotherapy as it was

hypothesized to reduce exertional breathlessness and improve exercise endurance by enhancing static and dynamic airway function in adults with COPD.

Study 4 was the first randomized, double-blind, placebo-controlled, cross-over trial to evaluate the efficacy and physiological mechanisms of action of inhaled vaporized cannabis in improving exertional breathlessness and exercise endurance in symptomatic adults with advanced COPD. Specifically, this was the first study to evaluate the acute effect of single-dose administration of 35 mg of inhaled vaporized cannabis containing 18.2% THC *vs.* control on: [1] static and dynamic lung function; [2] cannabis-related side effects; [3] pharmacokinetics of inhaled vaporized cannabis; and [4] cardiac, metabolic, ventilatory, breathing pattern and breathlessness responses during high-intensity constant-load cycle exercise testing.

For the first time, this study demonstrated that inhaled vaporized cannabis *vs.* control did not have a clinically meaningful positive or negative effect on exertional breathlessness, exercise endurance or airway function in symptomatic adults with advanced COPD receiving dual or triple inhalation therapy for management of their underlying pulmonary pathophysiology. Nevertheless, the results of this study indicated that there is some heterogeneity in responsiveness to cannabis therapy, with some (25%) adults with COPD experiencing relief of breathlessness following inhalation of vaporized cannabis *vs.* control.

Therefore, in conducting this randomized clinical trial, we expanded our understanding of the effects of vaporized cannabis on airway function, exertional breathlessness and exercise endurance

in adults with advanced COPD. The results of this study demonstrated that further research is needed to determine the therapeutic potential (if any) of cannabis in this patient population.

Conclusion

In my doctoral work, I sought to explore novel and/or poorly studied adjunct pharmacological and non-pharmacological therapies for relief of exertional breathlessness and improved exercise tolerance in COPD. The work presented in this dissertation offers original insights into [1] the mechanisms of exertional breathlessness in COPD and [2] the efficacy and physiological mechanisms of action of AB, immediate-release oral morphine, and inhaled vaporized cannabis in reducing exertional breathlessness and improving exercise endurance in adults with moderate-to-very severe COPD who suffer from severe breathlessness despite (in most cases) receiving optimal disease-modifying treatments.

CHAPTER 1: INTRODUCTION

In adults with COPD, noxious particles (e.g., cigarette smoke) and gases are the preeminent stimulants responsible for the abnormal inflammatory response of the lungs and airways, including lung parenchymal destruction and mucus hypersecretion, with loss of lung elasticity and airway narrowing (23) that contribute to expiratory flow limitation, pulmonary gas trapping and lung hyperinflation (24-26). These pathophysiological manifestations of COPD are strongly associated with exertional breathlessness and exercise intolerance, which in turn contribute to loss of functional autonomy, a poor health-related quality of life and increased mortality (27-33). It follows that alleviating breathlessness and improving exercise tolerance are among the principal goals in the management of COPD (23, 34, 35). Nevertheless, effective medical management of COPD remains a major challenge for most physicians since traditional evidence-based pharmacotherapies (e.g., inhaled bronchodilators and anti-inflammatory molecules) produce only modest improvements in breathlessness and exercise tolerance (1, 3), particularly in patients with Global Initiative for Obstructive Lung Disease (GOLD) spirometric stage III (severe) and IV (very severe) COPD (23). Thus, research into the efficacy and physiological mechanisms of action of adjunct therapies targeted to relieve breathlessness and improve exercise tolerance in COPD is clinically important to patients, their families and physicians.

In COPD, loss of lung elastic recoil enables a greater contribution of the outward recoil forces of the chest wall to the overall pressure-volume (P-V) relationship of the respiratory system (25, 26, 36). Consequently, the relaxation volume of the respiratory system (i.e., functional residual capacity (FRC)) in COPD is elevated relative to healthy controls (25, 26, 36). This “static lung hyperinflation” increases the threshold load on the diaphragm leading to an inefficient respiratory

effort and an elevated work of breathing with a greater inspiratory pressure and inspiratory neural respiratory drive required for a given ventilation ($\dot{V}_E$) (25, 26, 36, 37). As an efference copy of the neural respiratory drive is projected centrally to various forebrain sensory areas, central corollary discharge is also increased (25, 26, 38-41), and central neural processing of breathlessness is altered in adults with COPD (42, 43).

During exercise, these pathophysiological abnormalities are amplified further as the rate of lung emptying decreases when $\dot{V}_E$ increases to meet metabolic demand (i.e., carbon dioxide production ($\dot{V}CO_2$)), resulting in "dynamic lung hyperinflation" (i.e., transient increase in end-expiratory lung volume above resting levels) (25, 26, 36). As exercise progresses, the inspiratory muscles (i.e., the diaphragm) shorten and weaken as end-inspiratory lung volume increases towards total lung capacity, resulting in restrictive mechanical constraints on tidal volume (V_T) expansion that manifest as increased neural respiratory drive and central corollary discharge (25, 26, 36).

Therefore, pathophysiological abnormalities exhibited in COPD include: [1] perturbed respiratory mechanics, [2] dynamic diaphragmatic neuromuscular inefficiency, [3] exaggerated neural respiratory drive and central corollary discharge, [4] neuromechanical uncoupling of the respiratory system, and [5] altered central neural processing of breathlessness (25, 26, 36). These pathophysiological abnormalities are mechanistically linked to abnormally high levels of breathlessness during exercise with attendant early exercise cessation in adults with COPD (43-46). It follows that any pharmacological and/or non-pharmacological therapy capable of mitigating any one or combination of these pathophysiological abnormalities has the potential to relieve breathlessness and improve exercise tolerance in adults with COPD. To this end, three randomized

clinical trials were conducted in this dissertation to determine the efficacy of one non-pharmacological and two pharmacological therapies in reducing exertional breathlessness and improving exercise endurance in COPD. Specifically, the therapies selected where intended to improve: [1] neuromuscular coupling of the diaphragm; [2] central neural processing of breathlessness; [3] neural respiratory drive and/or central corollary discharge; and [4] respiratory mechanics (and therefore neuromechanical coupling of the respiratory system).

CHAPTER 2: LITERATURE REVIEW

2.1 OVERVIEW OF THE BURDEN OF COPD

2.1.1 Definition

According to the Global Initiative for Obstructive Lung Disease (GOLD) 2018 report, COPD is a *"common, preventable and treatable disease that is 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"* (23). COPD is a term used to classify lung diseases characterized by chronic airflow limitation, including emphysema and chronic bronchitis. COPD is spirometrically-defined as a forced expiratory volume in 1-sec (FEV_1) to forced vital capacity (FVC) ratio of less than 70%. COPD patients are typically stratified into one of four spirometric grades as per the GOLD international guidelines (23): GOLD I (mild) = FEV_1 ≥80% predicted; GOLD II (moderate) = FEV_1 50-79% predicted; GOLD III (severe) = FEV_1 30-49% predicted; and GOLD IV (very severe) = FEV_1 <30% predicted (23).

2.1.2 Etiology

Inhaled cigarette smoke and other noxious particles and gases (e.g., methane) are the preeminent stimulants responsible for airway inflammation that contributes to chronic airflow limitation and the subsequent diagnosis of COPD (23). Recent evidence suggests that major air pollutants (e.g., carbon monoxide) may also contribute to the development of COPD (47-49).

2.1.3 Prevalence of COPD

According to the Global Burden of Disease study, 174.4 million individuals were affected by COPD in 2015 worldwide (49). In Canada, 4% of adults between 35-79 years of age self-reported being diagnosed with COPD from 2009-2010 (50), yet direct measures of lung function by the Canadian Obstructive Lung Disease Initiative indicated that 17% of Canadians met the spirometric criteria for at least mild COPD from 2005-2009 (51). Among major chronic illnesses in Canada, COPD accounts for the highest number of inpatient hospitalizations by volume, and is the 5th leading cause of emergency department visits for adults >65 years of age (52).

2.1.4 Burden of COPD

In 2015, the Global Burden of Disease study (49) estimated that the global number of years of life lost due to COPD were 51,803; years lived with disability due to COPD were 12,047; and COPD disability-adjusted life years were 63,850.

2.1.5 Morbidity

Morbidity estimates of COPD are based on physician visits, emergency room visits and hospitalization (23). Due to limited availability of data, estimates of morbidity are lacking (23). Available data suggests that morbidity from COPD increases with age and may be affected by various comorbid conditions (e.g., cardiovascular disease) related to smoking (23).

2.1.6 Mortality

COPD mortality rates have been steadily increasing over the years (23). This increase has been attributed to the escalating smoking epidemic, aging of the world's population, and reduced

mortality rates from other common chronic diseases (49). In 2015, it was estimated that 3.2 million people died from COPD, accounting for 5% of all deaths globally (49). In Canada, it was estimated that COPD accounted for 4.4% of all deaths in 2011. It is expected that the total deaths from COPD will increase by more than 30% in the next 10 years, becoming the 3rd leading cause of death worldwide by 2030 (as per the World Health Organization predictions (53)). According to GOLD guidelines, global mortality estimates of COPD should be interpreted with caution due to: under-recognition and under-diagnosis of COPD; variable definitions of COPD; and unknown reliability and accuracy with which COPD-related deaths are reported (23).

2.1.7 Summary

COPD is a leading cause of morbidity and mortality globally and in Canada (23). With the extended life span of the aging population, efforts should be made to ensure a good health-related quality of life for individuals living with COPD.

2.2 SYMPTOMS IN COPD

Adults with COPD experience a range of respiratory symptoms over the course of their disease, including: breathlessness at rest and on exertion; wheeze; chest tightness; and excess mucus production (23, 54). Among these respiratory symptoms, breathlessness on exertion is the most debilitating symptom experienced by this patient population (23, 55). Importantly, exertional breathlessness diminishes exercise tolerance in adults with COPD. This deterioration in exercise capacity restricts the type, intensity and duration of activities of daily life that adults with COPD can perform (32, 33, 50, 56). It is perhaps not surprising then, that adults with COPD consider

relief of breathlessness among the most important outcomes in the management of their disease (57).

The following sections will provide an overview of the symptoms of breathlessness and exercise intolerance in adults with COPD. A comprehensive discussion on the pathophysiological mechanisms of breathlessness and exercise intolerance in adults with COPD will follow.

2.3 BREATHLESSNESS IN COPD

2.3.1 Definition and burden of breathlessness in COPD

Breathlessness is the cardinal symptom of COPD, and one of the most distressing symptoms experienced by this patient population (23, 55). Breathlessness is defined as a *"subjective experience of breathing discomfort that consists of qualitatively distinct sensations that vary in intensity. The experience derives from interactions among multiple physiological, psychological, social, and environmental factors, and may induce secondary physiological and behavioral responses"* (55).

Breathlessness on exertion severely impairs adults with COPD from carrying out activities of daily life, rendering simple tasks such as running errands and doing household chores as burdensome and exacting. According to a 2011 Survey on Living with Chronic Disease in Canada (50), 21% of adults with COPD found that their breathing impacted their life *"quite a bit or extremely"*. Respondents also reported that their breathing caused *"a lot"* of difficulty in each of the following: participating in exercise/sports (31%); getting a good night's sleep (19%); doing chores (16%); running errands/shopping (16%); and participating in leisure activities (15%) (50). Over time and

with disease progression, the restrictive effect of breathlessness on the ability to carry out activities of daily life deprives individuals of their health-related quality of life and vitality, while slowly bereaving them of their autonomy (27, 56, 58). As a result, breathlessness contributes to the established extra-pulmonary manifestations of COPD, including anxiety, depression (58-63), and cardiovascular (64, 65) and peripheral locomotor muscle deconditioning (66-68). Importantly, breathlessness is strongly and positively correlated with increased morbidity and mortality in adults with COPD (1, 2, 29).

2.3.2 Qualities of breathlessness

Breathlessness perception is comprised of both a sensory and affective dimension (26, 69-73). The sensory components of breathlessness encompass intensity, quality (i.e., air hunger/unsatisfied inspiration, work/effort of breathing, chest tightness) and time course (26, 69-73). The affective dimension encompasses unpleasantness and emotional impact of breathlessness (26, 69-73).

Sensory dimensions

The sensory qualities of breathlessness can broadly be classified into three distinct respiratory sensations: [1] air hunger or unsatisfied inspiration; [2] work/effort of breathing; and [3] chest tightness (26, 69-73). Each of these sensory qualities can vary in intensity and time course. The distinction between sensory qualities is made by verbal descriptors that are volunteered and/or selected by patients following a breathlessness stimuli (e.g., exercise) (26, 70, 71).

The sensation of air hunger or unsatisfied inspiration is often used by patients to describe the sense of an uncomfortable urge to breathe and is associated with descriptors such as *"I am starved for*

air" and *"I cannot get enough air in"* (26, 69-73). Banzett, *et al.* (74, 75) and others (76, 77) have demonstrated that air hunger can be [1] induced by increasing end-expiratory PCO_2 (PET_{CO_2}) at a constant $\dot{V}_E$ or by decreasing tidal volume (V_T) at a constant PET_{CO_2}; and [2] alleviated by increasing V_T at a constant PET_{CO_2}. More recent studies have also demonstrated that air hunger or unsatisfied inspiration can be induced by increasing neural respiratory drive in the setting of abnormal restrictive constraints on V_T (46, 78, 79).

The work/effort sensation of breathlessness is pervasive in both health and disease, and associated with descriptors like *"breathing is difficult"*, *"breathing requires more work"* or *"breathing requires effort"* (26, 69-73). Stimuli that increase the load on the respiratory muscles tend to evoke the sense of increased work/effort of breathing in adults with COPD (26, 70).

The sensation of chest tightness is commonly described as the *"chest is constricted"* and the *"chest feels tight"*, which can be induced by bronchoconstriction (26, 69-73) and is most commonly associated with asthma.

Affective dimensions

The affective dimensions of breathlessness are associated with breathlessness-related anxiety/fear and are often reported as the unpleasantness of the sensory quality (70). Banzett*, et al.* (75) demonstrated that at similar sensory intensities, air hunger is more unpleasant than work/effort of breathing.

2.3.3 Summary

Breathlessness is a complex, multidimensional symptom that imposes a substantial burden on adults with COPD. Therefore, efforts should be made to adequately manage the symptom of breathlessness in order to improve health-related quality of life in COPD.

2.4 EXERCISE INTOLERANCE IN COPD

2.4.1 Definition of a diminished functional capacity in COPD

Functional capacity – or exercise capacity – represents an individual's maximal ability to perform work (80, 81). In adults with COPD, functional capacity is often measured during a symptom-limited cycle/treadmill exercise test and/or a self-paced walking test and is typically reported as exercise endurance time and/or peak rate of O_2 consumption ($\dot{V}O_2$peak) (80, 81). Measures of functional capacity in adults with COPD provide meaningful information regarding the individual's cardiorespiratory fitness (81).

In adults with mild-to-very severe COPD, exercise tolerance is significantly reduced when compared to age-matched healthy controls (32, 33, 56, 82). This diminished exercise capacity restricts adults with COPD from carrying out activities of daily life, thus contributing to increased levels of anxiety and depression and a poor health-related quality of life. Indeed, established measures of functional capacity including $\dot{V}O_2$peak and exercise endurance time are negatively correlated with COPD disease severity (2), breathlessness (83), physical activity levels (e.g., steps per day) (56), health-related quality of life (84, 85), health status (86) and self-efficacy (87).

2.4.2 Summary

Adults with COPD have diminished exercise tolerance compared to age-matched healthy controls. In order to improve health-related quality of life and health status in adults with COPD, efforts should be made to improve exercise tolerance.

2.5 NEUROPHYSIOLOIGCAL MODEL OF BREATHLESSNESS

Breathlessness is the result of perturbed neurophysiological processes that alter normal respiration and/or central neural processing of breathlessness. The neurophysiological underpinnings of breathlessness are complex and multifactorial and consist of a delicate interplay between: [1] various brain centers implicated in the neural processing of breathlessness; [2] central neural respiratory drive and corollary discharge; [3] sensory feedback information from lung and chest wall receptors; [4] neuromechanical uncoupling of the respiratory system; and [5] psychological factors. In the following discussion, the role of each of these aforementioned factors in the neurophysiological construct of breathlessness will be considered.

In an effort to present a comprehensive neurophysiological model of breathlessness, experimental evidence from studies conducted in healthy subjects, and in subjects with various pulmonary illnesses, including COPD, will be considered. Where appropriate, differences in the neuromodulation of breathlessness in health *vs.* COPD will be discussed.

2.5.1 Brain centers implicated in the neural processing of breathlessness

Using high-resolution imaging techniques such functional magnetic resonance imaging (fMRI), several investigators have demonstrated that multiple brain centers (identified below) are important in the neural processing of breathlessness (42, 43, 88-94).

Using mechanically ventilated healthy volunteers, Banzett, *et al.* (88) and Evans, *et al.* (91) induced epochs of breathlessness (specifically "air hunger") by restraining V_T (~0.6 L) under a constant hypercapnic background, while altering with epochs of higher V_T (~1.2 L) to alleviate breathlessness. In both studies, the air hunger stimuli were localized to the anterior insula, anterior cingulate cortex, operculum, cerebellum, amygdala, thalamus and basal ganglia. Peiffer, *et al.* (93, 94) and von Leupoldt, *et al.* (95-97) conducted several fMRI studies using external resistive loads to induce breathlessness in healthy volunteers. Subjective ratings of "increased work and effort of breathing" in these studies were associated with neural activations in the right anterior insula, premotor cortex, anterior cerebellum and right amygdala. Brannan, *et al.* (89) demonstrated a network of activation in the pons, midbrain and various limbic and paralimbic regions following acute periods of hypercapnia in healthy subjects. Pattinson, *et al.* (98) reported that breath-holding (a breathlessness inducing stimuli that increases neural respiratory drive *via* progressive hypoxic-hypercapnia) increases activation in the insula, operculum, anterior cingulate cortex, prefrontal and motor cortices in healthy individuals.

Similarly, fMRI studies conducted in adults with COPD demonstrated that breathlessness induced by inspiratory resistive loading and/or "dyspnea word cues" was associated with increased activation in the pons, amygdala, insula, anterior cingulate cortex, operculum, thalamus, and motor

and sensory cortices (99). Collectively, neuroimaging studies performed to date implicate a cortico-limbic-cerebellar circuitry in mediating the sensation of breathlessness in health and COPD (42, 43, 88-94).

2.5.2 Central processing of breathlessness

At present, two distinct yet overlapping neural pathways have been implicated in the cortical processing of breathlessness (**Figure 2.1**) (26, 41, 90, 100). The first pathway is seemingly related to the affective (unpleasantness) component of breathlessness as it is postulated to relay information from sensory afferent nerves in the lungs and airways to the brainstem respiratory centers (**Figure 2.1**) where the neural signal may ascend to various sensory areas, including the amygdala, insula and anterior cingulate cortex (26, 41, 90, 100). The second pathway likely conveys information on the work/effort of breathing as it is proposed to relay sensory information arising from the respiratory muscles to the brainstem respiratory network, where the neural signal is subsequently transmitted to the ventroposterior thalamus (with possible projections to the insula) and somatosensory cortices (**Figure 2.1**) (26, 41, 90, 100). Both pathways include final projections to the supplementary, primary and secondary motor cortices, from where efferent motor command projects to the medulla/pons and/or respiratory muscles (26, 41, 90, 100). Finally, an efferent copy (i.e., central corollary discharge) of the motor cortex and brainstem respiratory output is relayed to various forebrain sensory areas, most notably the insula (26, 41, 90, 100).

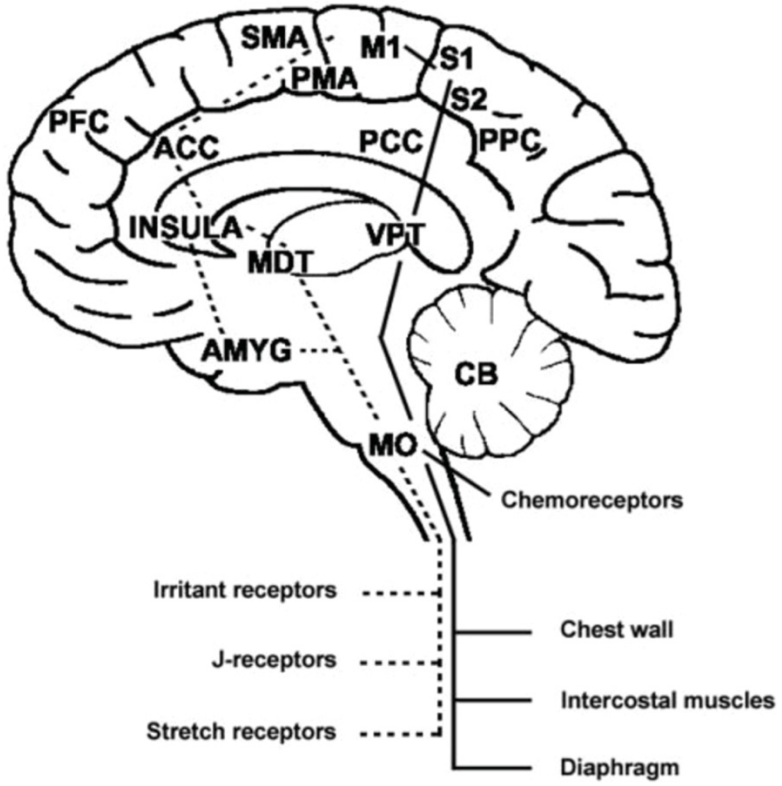

Figure 2.1. Cortical areas and neural pathways implicated in the sensation of breathlessness. See text for detailed discussion of the affective (dashed line) and sensory (solid line) pathways implicated in the sensation of breathlessness. ACC=anterior cingulate cortex; AMYG=amygdala; CB=cerebellum; M1=primary motor cortex; MDT=medial dorsal thalamus; MO=medulla oblongata; PFC=prefrontal cortex; PPC=posterior parietal cortex; S1=primary somatosensory cortex; S2=secondary somatosensory cortex; SMA=supplementary motor area; VPT=ventroposterior thalamus. From (100) with permission.

In accordance with these neural pathways, it can be hypothesized that any stimuli capable of altering brainstem chemoreceptor activity; peripheral sensory receptor activity (e.g., lung, airway and chest wall afferents); and/or motor cortex/brainstem respiratory output may alter the sensation of breathlessness, with the quality, intensity and unpleasantness of breathlessness being dictated by the receptors and/or brain centers being activated or deactivated (26, 41, 90, 100).

2.5.3 Central neural respiratory drive and central corollary discharge

The neural respiratory drive required to elicit a given $\dot{V}_E$ is produced in the brainstem respiratory neural network (26, 37, 41, 101). This neural respiratory drive activates the respiratory muscles, most notably the diaphragm (26, 37, 41, 101). Experimentally, neural respiratory drive may be reported as the electromyogram of the diaphragm (EMGdi), i.e., the neural drive required to activate the diaphragm during inspiration (26, 37, 101).

Central corollary discharge refers to the neural output from the brainstem respiratory neural network and/or motor cortex to the forebrain sensory areas (26, 37, 41, 101, 102). These efferent inputs encode a neurological copy of the outgoing efferent command to the respiratory muscles (26, 37-41). As such, central corollary discharge keeps the forebrain sensory areas aware of the outgoing neural respiratory drive (26, 37-41). According to the central corollary discharge hypothesis, breathlessness increases when central corollary discharge increases (26, 37, 41). Evidence in support of the central corollary discharge hypothesis was provided by Banzett*, et al.* (74) who evaluated the effects of increasing inspired PCO_2 (i.e., an air hunger stimuli) in four mechanically ventilated tracheostomized quadriplegic subjects whose respiratory muscles were paralyzed. By controlling $\dot{V}_E$ and inhibiting afferent feedback from respiratory muscles, Banzett*, et al.* (74) were effectively measuring the contribution of neural respiratory drive and presumably also central corollary discharge to the perception of breathlessness (specifically air hunger) elicited by the hypercapnic stimuli. Quadriplegic subjects in this study reported air hunger in the absence of a change in $\dot{V}_E$, when $P_{ET}CO_2$ increased by ~8-13 mmHg above resting levels (74). Based on this experimental evidence, it was postulated that the sense of breathlessness induced by the air hunger stimuli was the result of increased neural respiratory drive that was perceived by increased

central corollary discharge. This hypothesis was further supported by Shea, *et al.* (103) who demonstrated that patients with central congenital hypoventilation syndrome, who lack a central ventilatory chemoreflex response to CO_2, fail to report any respiratory discomfort during CO_2 inhalation and/or voluntary breath-holding.

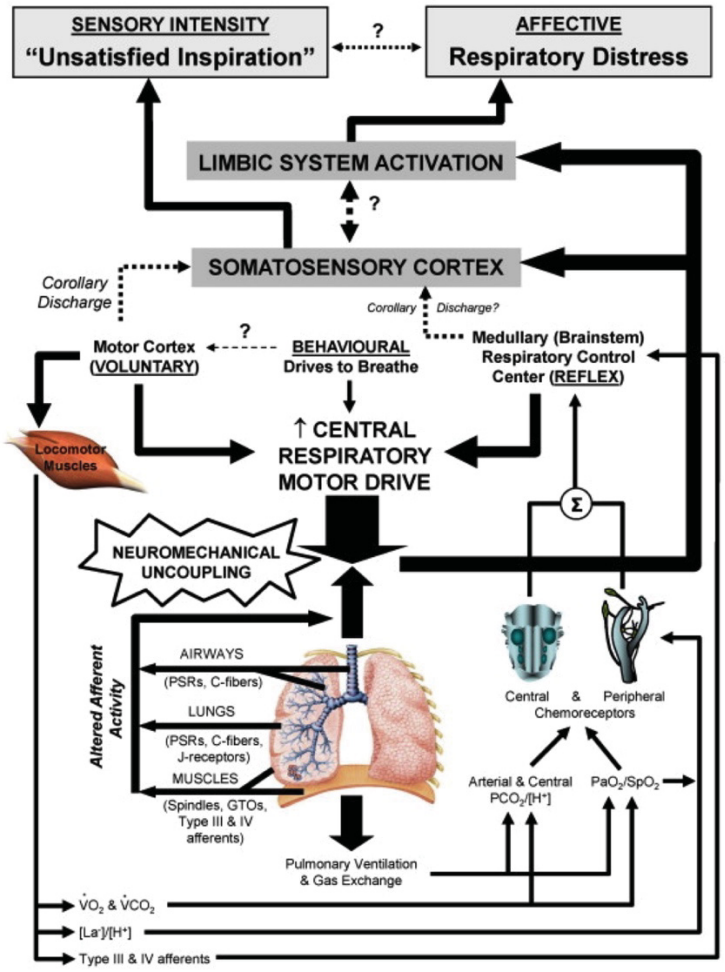

Figure 2.2. Proposed neurophysiological mechanisms of breathlessness. See text for details. From (104) with permission.

2.5.4 Sensory receptors

Sensory receptors implicated in the neuromodulation of breathlessness include: central and peripheral chemoreceptors; vagal receptors; chest wall receptors; and phrenic afferents. Central

and peripheral chemoreceptors are stimulated by stimuli implicated in the sensation of breathlessness, including: arterial blood pH, CO_2 and oxygen (O_2) (26, 105, 106). Vagal, chest wall and phrenic afferent receptors relay sensory information from the lungs and respiratory muscles to various brain centers, including the respiratory control center (**Figure 2.1** and **Figure 2.2**). Afferent signals arising from these receptors may alter the outgoing neural respiratory drive and/or $\dot{V}_E$, with a concomitant change in breathlessness (**Figure 2.2**). The contribution of these sensory receptors to the etiology of breathlessness will be discussed below.

Chemoreceptors

The retrotrapezoid nucleus and pre-Bötzinger complex (pre-BötC) are central chemoreceptors located in the ventrolateral medulla, the respiratory brain center that contains excitatory neurons regulated by CO_2/pH and input from the peripheral chemoreceptors (107-109). The carotid bodies, located at the bifurcation of the common carotid artery (110), are considered to be the principle peripheral chemoreceptors. When stimulated, the carotid bodies release neurotransmitters onto the glossopharyngeal nerve, which relays sensory activity to the brainstem neural network (**Figure 2.3**) (110).

The mechanisms underlying hypoxia (i.e., a decrease in O_2)- and hypercapnia (i.e., an increase in PCO_2)-induced sensations of breathlessness are poorly understood, but thought to be associated with increased neural respiratory drive and central corollary discharge (111-113). For example, Moosavi*, et al.* (112) elicited air hunger in healthy subjects when end-tidal PO_2 was experimentally decreased below 60 mmHg in the presence and/or absence of a change in end-tidal PCO_2 and/or $\dot{V}_E$. Chronos*, et al.* (111) reported that breathlessness induced by hypoxia during exercise in

healthy subjects did not correlate with changes in $\dot{V}_E$. Collectively, these results suggested that stimulation of central and/or peripheral chemoreceptors may trigger a chemotransduction cascade that alters neural respiratory drive and central corollary discharge leading to the sensations of breathlessness, particularly the sensation of air hunger (111-113).

Vagal receptors

The vagus nerve innervates upper airway and lung parenchymal receptors, including: upper airway receptors; slowly adapting pulmonary stretch receptors; rapidly adapting pulmonary stretch receptors; and bronchopulmonary C-fibers (**Figure 2.3**) (114-117). The vagus nerve relays sensory information from the upper airways and lungs to various brain centers that are integral in the processing of breathlessness (**Figure 2.1**, **Figure 2.2** and **Figure 2.3**) (114-117). Therefore, any stimuli that can excite and/or inhibit vagal afferent feedback may contribute to the sensation of breathlessness (114-119).

Upper airway receptors

Upper airway receptors include transient receptor potential melastating 8 (TRPM8) channels, which are [1] expressed in the trigeminal and vagal afferent neurons and [2] stimulated by cold temperatures and L-menthol (120). A role for the upper airway receptors in mediating the sensation of breathlessness has been identified in both health and COPD (121-124) .

Schwartzstein*, et al.* (123) demonstrated that cold air directed against the cheeks of healthy individuals decreased breathlessness induced by hypercapnia and inspiratory resistive loading without causing a significant reduction in $\dot{V}_E$. Similarly, Spence, *et al.* (124) demonstrated that

compared to breathing room air, breathing cold air reduced exertional breathlessness in adults with COPD. In contrast to the findings by Schwartzstein, *et al.* (123), however, Spence, *et al.* (124) reported that breathlessness at any given $\dot{V}_E$ was not different between conditions (cold air vs. room air), thus suggesting that relief of exertional breathlessness following the application of cold air was the result of a decrease in neural respiratory drive and presumably also central corollary discharge secondary to altered upper airway receptor activity (124, 125).

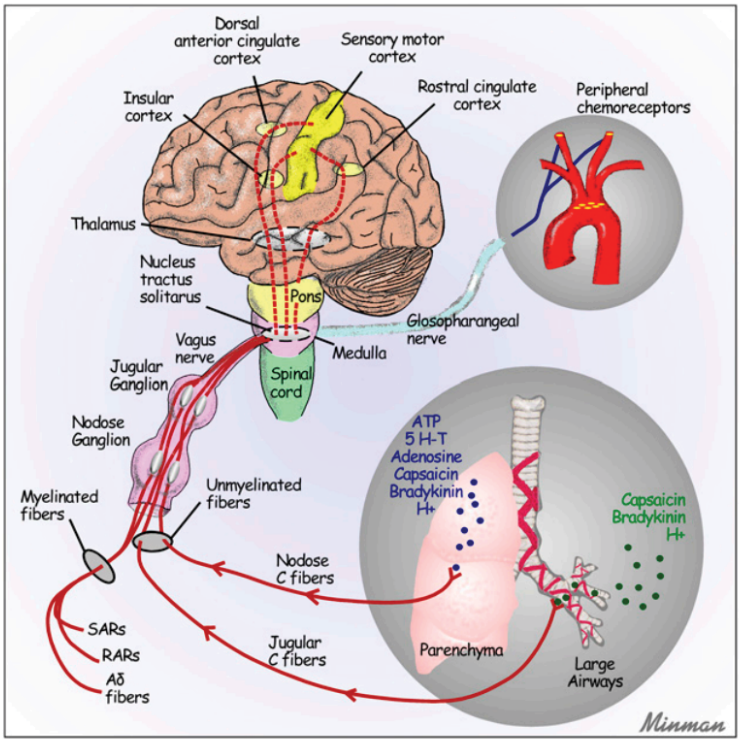

Figure 2.3. Schematic representation of vagal receptors and peripheral chemoreceptors to the central nervous system. RAR=rapidly adapting receptor; SAR=slowly adapting receptor. From (126) with permission.

The effect of L-menthol on the perception of breathlessness has also been investigated in healthy adults. For example, a placebo controlled study by Nishino, *et al.* (122) demonstrated that nasal inhalation of L-menthol decreased breathlessness induced by loaded breathing. Similarly, Kanezaki and Ebihara (121) reported relief of breathlessness during constant-load cycle exercise

testing following inhalation of L-menthol *vs.* placebo. In both studies, relief of breathlessness with L-menthol was not associated with a change in breathing pattern and/or $\dot{V}_E$.

Based on the collective results of these studies, it is feasible to suggest that stimulation of upper airway receptors may alleviate breathlessness by [1] altering central neural processing of breathlessness by stimulating TRPM8 channels on trigeminal and/or vagal afferents, which relay sensory information to the thalamus and cortex and/or [2] decreasing neural respiratory drive and presumably central corollary discharge. Additional studies are required to better understand the role of upper airway receptors in mediating the sensation of breathlessness.

Slowly adapting pulmonary stretch receptors

Slowly adapting pulmonary stretch receptors are mechanosensors found in the smooth muscle along the airways (114). Slowly adapting pulmonary stretch receptors are most vigorously stimulated by an increase in lung volume. Activation of slowly adapting pulmonary stretch receptors elicits an inhibitory effect on the central nervous system leading to the cessation of inspiration and the facilitation of expiration (i.e., Hering-Breuer reflex) (114-118, 127). In two separate studies, Flume, *et al.* (128, 129) performed a series of maximal voluntary breath-hold maneuvers in healthy subjects and in patients with bilateral lung transplants. Study subjects were required to complete two maximal voluntary breath-hold maneuvers: the first breath-hold was performed under normal conditions (breathing room-air), while the second breath-hold was performed immediately following the breaking point of the first breath-hold, but after rebreathing a gas mixture containing 7.5% CO_2 and 8.2% O_2 (128, 129). It was hypothesized that rebreathing the hypoxic-hypercapnic gas mixture would intensify the sensation of breathlessness by

stimulating central and peripheral chemoreceptors (i.e., by further increasing neural respiratory drive and central corollary discharge), and therefore abolish the ability of study subjects to perform a second breath-hold (128, 129). In contrast to this hypothesis, Flume, *et al.* (129) reported that healthy subjects were: [1] able to perform a second breath-hold with an average duration of ~17 sec, and [2] that respiratory distress decreased rapidly from a visual analogue scale score of ~5 to ~1 while rebreathing the hypoxic-hypercapnic gas mixture, despite worsening of blood gases (and possibly increasing neural respiratory drive and central corollary discharge). From these results it was postulated that the act of rebreathing (i.e., changing V_T) altered the mechanical properties of the lungs, thus stimulating slowly adapting pulmonary stretch receptors and provoking a decrease in breathlessness (129). This hypothesis was further supported by the observation that relief of breathlessness in response to rebreathing a hypoxic-hypercapnic gas mixture was blunted in lung transplant recipients, when compared to healthy controls (128). In other words, diminished afferent sensory feedback from slowly adapting pulmonary stretch receptors in lung transplant recipients may have decreased the efficacy of rebreathing on relief of breathlessness (128). These results have been substantiated by numerous investigators that have demonstrated a positive relationship between increasing V_T (and therefore slowly adapting pulmonary stretch receptors activity) and relief of breathlessness (91, 130-132). Collectively, these studies suggest that slowly adapting pulmonary stretch receptors may ameliorate breathlessness by decreasing neural respiratory drive and central corollary discharge. By contrast, abnormal restrictive constraints on V_T expansion (and therefore slowly adapting pulmonary stretch receptors activation) may intensify ratings of breathlessness at any given $\dot{V}_E$ by withdrawal of vagal afferent inhibition of neural respiratory drive and central corollary discharge.

Rapidly adapting pulmonary stretch receptors

Rapidly adapting pulmonary stretch receptors are mechanosensors found in the smooth muscle along the airways (114). Rapidly adapting pulmonary stretch receptors are stimulated by increases in lung volume, inspired flow rate, and the rate of change of airway pressure (114). Recent evidence suggests that rapidly adapting pulmonary stretch receptors also respond to chemical irritants, such as ammonia and cigarette smoke (114). Augmented rapidly adapting pulmonary stretch receptors activity has been implicated in the neuromodulation of breathlessness, predominantly through indirect evidence obtained from animal studies (133-135). For example, Dallak, *et al*. (133) demonstrated that a rabbit model of pulmonary emphysema exhibits greater rapidly adapting pulmonary stretch receptor activity when compared to control (wild-type) rabbits. This augmented rapidly adapting pulmonary stretch receptors activity was associated with elevated phrenic nerve activity and therefore neural respiratory drive and presumably also central corollary discharge. On the basis of this experimental evidence, it has been postulated that the heightened sensation of breathlessness in adults with COPD may be due, at least in part, to an elevated neural respiratory drive (and attendant central corollary discharge) secondary to augmented rapidly adapting pulmonary stretch receptors activity (133).

Bronchopulmonary C-fibers

Bronchopulmonary C-fibers are chemical sensitive receptors distributed from the trachea to the lung periphery (114). Bronchopulmonary C-fibers are excited by a variety of endogenous and exogenous substances, including bradykinin, capsaicin, reactive oxygen species (ROS), H^+ and lactic acid (114). Stimulation of bronchopulmonary C-fibers triggers a rapid shallow breathing pattern and bronchoconstriction of smooth muscle in the trachea and the intrathoracic airways

(114, 115, 127). As with slowly adapting pulmonary stretch receptors and rapidly adapting pulmonary stretch receptors, bronchopulmonary C-fibers have been implicated in modulating the sensation of breathlessness, although direct evidence is limited. In two separate placebo controlled studies, Burki, *et al.* (136, 137) demonstrated that intravenous administration of adenosine, a bronchopulmonary C-fiber stimulant, induced the sensation of breathlessness in healthy and asthmatic adults. In a subsequent placebo controlled study, Burki, *et al.* (138) reported an attenuated breathlessness response to intravenous injection of adenosine in healthy adults pre-treated with inhaled lidocaine - an anaesthetic that inhibits the activity of pulmonary afferents. In a separate placebo controlled study, Taguchi*, et al.* (139) demonstrated that inhalation of prostaglandin E_2, a bronchopulmonary C-fiber stimulant, worsened the perception of breathlessness at any given work load, $\dot{V}_E$ and $\dot{V}O_2$ during exercise in healthy adults. Collectively, these results provide evidence in support of a role of bronchopulmonary C-fibers in mediating the sensation of breathlessness.

Chest wall receptors

Afferent signals from mechanoreceptors in muscle spindles, golgi tendon organs and joints in respiratory muscles may contribute to the sensation of breathlessness (**Figure 2.1** and **Figure 2.2**) (26, 140). Sensory information from respiratory muscles is relayed to the brainstem respiratory center and supra-brainstem regions *via* type I, II, III and IV afferents (26, 140-143). Chest wall receptor activity is modulated by hyperinflation, bronchoconstriction and/or mechanical stimulation, all of which may alter the sensation of breathlessness (26, 140).

In an eloquent study, Remmers (144) demonstrated that activation of chest wall receptors by either chest compression, intercostal muscle stretch and/or rib vibrations inhibited phrenic nerve activity (and therefore neural respiratory drive) in anaesthetized, vagotomised and mechanically ventilated dogs and cats. In the same study, Remmers (144) found that hypercapnia - a central and peripheral chemoreceptor stimulant that increases neural respiratory drive - amplified the amount of chest wall receptor activity required to inhibit phrenic nerve activity. Therefore, chest wall receptors may alleviate breathlessness by decreasing neural respiratory drive and presumably central corollary discharge.

The contribution of respiratory muscle afferents to the sensation of breathlessness has been evaluated during chest wall vibration in health and COPD (145-147). Manning, *et al.* (147) and Edo, *et al.* (145) demonstrated that, in healthy adults, in-phase intercostal vibration during the respiratory cycle significantly reduced breathlessness elicited by a combination of hypercapnia and inspiratory resistive loading. Similarly, Sibuya*, et al.* (148) demonstrated that, in adults with COPD, in-phase vibration decreased breathlessness at rest. Conversely, out-of-phase vibrations worsened breathlessness perception in healthy adults, and in adults with COPD (146, 148). Presumably, in-phase vibrations, in contrast to out-of-phase, decreases breathlessness by [1] facilitating inspiration, [2] increasing chest wall receptor activity, and [3] decreasing neural respiratory drive and central corollary discharge.

Phrenic afferents

Type III and IV afferents respond to mechanical (e.g., stretch) and chemical (e.g., lactic acid) stimuli arising from the exercising muscle, respectively (149-153). Once stimulated, type III and

IV impulses travel to the dorsal horn of the spinal cord to various brain centers (149-153). Therefore, sensory feedback arising from skeletal muscle mechano- and metaboreceptors may alter the sensation of breathlessness by modulating neural respiratory drive and central corollary discharge.

On exertion, when the diaphragm muscle is vigorously contracting to support the increased metabolic and ventilatory demands, type III and IV phrenic afferents are preferentially activated, leading to an increase in neural respiratory drive and presumably central corollary discharge with attendant increases in breathlessness (143, 154). Importantly, however, as reported by Ward, *et al.* (155) the increase in the sense of respiratory effort experienced by healthy adults during diaphragmatic fatigue is likely mediated by an amplified perception of an overall increase in central respiratory motor output (i.e., to the diaphragm, rib cage, sternocleidomastoid), and not to an isolated increase in neural respiratory drive to the diaphragm (elicited in part, by stimulation of type III and IV phrenic afferents), *per se*.

2.5.5 Neuromechanical uncoupling of the respiratory system

As eluded to above, feedback input from the respiratory sensors to the forebrain sensory areas provide information on the respiratory effort (e.g., appropriateness of lung stretch, pressure and flow generation and respiratory muscle tension development) achieved in response to the outgoing neural respiratory drive and attendant central corollary discharge (26, 104, 140). According to the theory of neuromechanical uncoupling, breathlessness ensues when the mechanical and muscular responses of the respiratory system are not appropriately matched to the prevailing level of outgoing efferent motor command (26, 104, 140).

Neuromechanical uncoupling is evaluated by examining the ratio of neural respiratory drive (e.g., EMGdi relative to maximum EMGdi (EMGdi%max)) or respiratory muscle effort (e.g., tidal esophageal pressure (Pes) relative to maximum inspiratory pressure (Pes%max)) to thoracic volume displacement (e.g., V_T expressed as a percentage of predicted vital capacity (VC)) during exercise (25, 36, 37, 156). During exercise in healthy adults, mechanical adaptations of the respiratory system enable V_T to expand along the linear, compliant portion of the respiratory systems sigmoid P-V curve (**Figure 2.4**) (refer to *Section 2.6.1* below) (25, 26, 36, 37). As a result, neuromechanical uncoupling is relatively preserved as the mechanical and muscular responses of the respiratory system are appropriately matched to the outgoing efferent motor command. During exercise in adults with COPD, respiratory mechanical abnormalities (i.e., static and dynamic lung hyperinflation) restrict V_T expansion to the upper alinear, non-compliant portion of the respiratory systems sigmoid P-V curve where a greater neural respiratory drive (and respiratory muscle effort) is required to achieve any given change in V_T (**Figure 2.4**) (157-164). Consequently, adults with COPD experience marked neuromechanical uncoupling with a concomitant increase in breathlessness, especially when compared to healthy adults (158-165).

2.5.6 Psychological processes

Breathlessness is a subjective experience shaped by various psychological processes, including: environmental cues; learned associations (e.g., climbing stairs triggers breathlessness); previous experiences; personality; and emotional status (166). Each of these psychological processes may, in part, account for the inter-individual variability in the perception of breathlessness. The neurological processes mediating the interaction between a given psychological state and the sensation of breathlessness are currently unknown.

Negative affect may increase breathlessness induced by exercise and/or by inspiratory resistive loading in healthy subjects (166, 167). von Leupoldt, *et al*. (167) reported that a negative emotional state elicited by viewing standardized emotional pictures increased breathlessness induced by inspiratory resistive loaded breathing with a concomitant increase in the neural activation of the right insula and right amygdala. Similarly, Aldhafeeri, *et al.* (168) found that, compared to viewing neutral images, viewing negative images increased neural activity in the hippocampus, amygdala and visual cortex. Based on these observations, it has been postulated that negative affect may modulate breathlessness by altering neural signalling between various brain centers.

Adults with COPD, who have a high prevalence of anxiety and depression, and who experience repeated bouts of breathlessness (58-63) may exhibit structural-functional modifications in brain regions implicated in the neuromodulation of breathlessness. That is, breathlessness-related brain centers in these individuals may become sensitized to negative affect such that a given breathlessness stimuli may elicit a greater sensation of breathlessness relative to healthy adults. Indeed, Esser, *et al.* (42) demonstrated that, compared to healthy control subjects, adults with COPD exhibiter higher activation in the bilateral hippocampus and right amygdala (brain regions implicated in the neuroprocessing of fear and aversive stimuli) during anticipation of resistive-load induced breathlessness. Furthermore, neural activation of the left hippocampus during breathlessness anticipation was positively correlated with measures of anxiety and depression in adults with COPD (42). These results suggested an inter-relationship between negative affect and brain activity during anticipation of breathlessness, which may contribute to worse breathlessness

perception in adults with COPD *vs.* healthy adults (42). Additional studies are required to elucidate the neurocircuitry between emotional state and breathlessness.

2.5.7 Summary

The neurophysiological model of breathlessness presented in **Section 2.5** reflects the complex, and multifactorial nature of the symptom of breathlessness. According to this neurophysiological construct, therapies intended to reduce breathlessness in health and COPD must serve to: [1] alter central neural processing of breathlessness; [2] decrease neural respiratory drive and central corollary discharge; [3] alter sensory feedback from lung, airway and chest wall receptors; [4] decrease neuromechanical uncoupling; [5] decrease negative affect; or [6] any combination thereof.

2.6 MECHANISMS OF EXERTIONAL BREATHLESSNESS AND EXERCISE INTOLERANCE IN COPD

In **Section 2.5**, the neurophysiological model of breathlessness presented was based on experimental evidence from a collection of neurophysiological and behavioural studies that [1] were conducted in health and COPD and [2] used various stimuli to induce breathlessness (e.g., breath-holding, hypoxic-hypercapnic gas mixture). In the following section, the neural, respiratory, peripheral muscular and cardiovascular factors that modulate breathlessness *during exercise* and that limit exercise performance in adults with COPD will be considered. In moving forward, a brief overview on the mechanisms of exertional breathlessness and exercise intolerance in healthy adults will be presented so as to provide the reader with a frame of reference to

appreciate the pathophysiological mechanisms of breathlessness and exercise intolerance in COPD.

2.6.1 Overview on the mechanisms of exertional breathlessness and exercise intolerance in healthy adults

At the onset of exercise in healthy adults, recruitment of expiratory muscles allows end-expiratory lung volume to decrease while enabling V_T to expand on the most linear and compliant portion of the respiratory system's sigmoid P-V curve (**Figure 2.4**) (169). These respiratory mechanical adaptations, in combination with enhanced ventilation-perfusion ($\dot{V}/Q$) relationships, allow for an efficient breathing pattern that minimizes the work and oxygen cost of breathing (169, 170). Under these circumstances, the respiratory system remains neuromechanically coupled (i.e., neural respiratory drive is adequately matched to ventilatory effort), as breathlessness intensity ratings increase in direct proportion to increases in neural respiratory drive and presumably central corollary discharge (37, 169). Therefore, in healthy adults, the proximate source of breathlessness is believed to be the awareness of increased neural respiratory drive (or diaphragm electrical activation, i.e., EMGdi%max) as sensed *via* increased central corollary discharge (171-173). Typically, leg discomfort and not intolerable breathlessness, is cited as the main reason for exercise cessation in healthy adults (83, 174).

2.6.2 Mechanisms of exertional breathlessness and exercise intolerance in adults with COPD

Compared to healthy individuals, adults with COPD report higher intensity and unpleasantness ratings of exertional breathlessness, and exhibit a diminished exercise capacity as a result of any

one or combination of: [1] respiratory mechanical abnormalities; [2] diaphragmatic neuromuscular inefficiency; [3] $\dot{V}/Q$ mismatching; [4] neuromechanical uncoupling ; [5] an exaggerated neural respiratory drive and central corollary discharge; [6] cardiovascular limitations; and [7] peripheral muscle abnormalities (25, 26, 36, 37, 41, 81, 169, 170, 175). In the following discussion, the contribution of each of these pathophysiological variables to the etiology of exertional breathlessness and exercise intolerance in adults with COPD will be considered.

2.6.2.1 Respiratory mechanical abnormalities

Due to loss of lung elastic recoil and insufficient expiratory time, adults with COPD experience dynamic lung hyperinflation during exercise (158-165). Consequently, end-expiratory lung volume increases as end-inspiratory lung volume approaches total lung capacity, leading to a decrease in both the inspiratory capacity and inspiratory reserve volume from rest to peak exercise (158-165). Initially, dynamic lung hyperinflation is beneficial as it tethers the airways open, decreasing airway resistance (minimizing expiratory flow limitation) and enabling adults with COPD to increase $\dot{V}_E$ (158-165). However, as exercise progresses, the positive effects of dynamic lung hyperinflation are quickly negated as V_T expansion becomes confined to the upper alinear, non-compliant and mechanically disadvantageous portion of the respiratory systems sigmoid P-V curve (**Figure 2.4**) (158-165). In turn, V_T becomes mechanically constrained, and further increases in $\dot{V}_E$ become largely dependent on increased breathing frequency (f_R). These respiratory mechanical abnormalities contribute to the symptom of breathlessness during exercise (158-165).

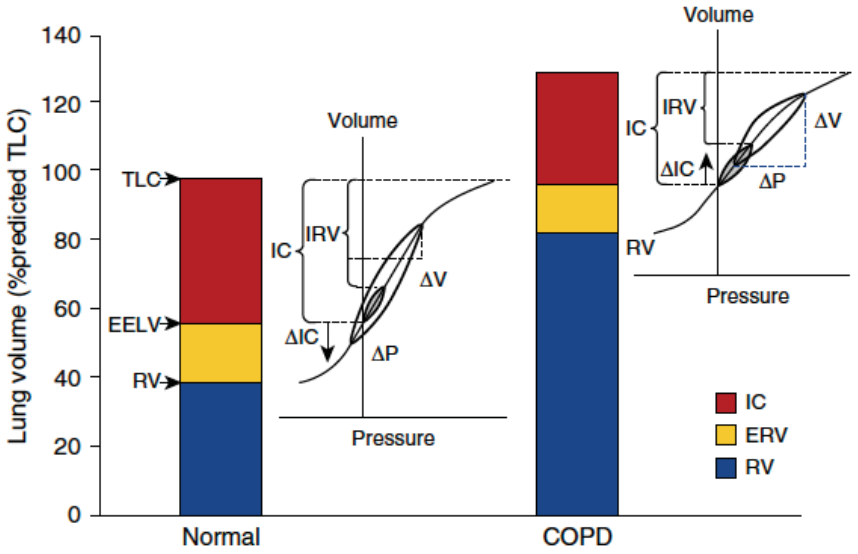

Figure 2.4. Resting lung volumes in patients with COPD and age-matched healthy controls. Pressure-volume (P-V) curves of the respiratory system are shown at rest (filled area) and exercise (open area). EELV, end-expiratory lung volume; ERV, expiratory reserve volume; IC, inspiratory capacity; IRV, inspiratory reserve volume; RV, residual volume; TLC, total lung capacity; ΔIC, change in IC from rest to exercise; Δ*P*, change in pleural pressure during a tidal breath while exercising; ΔV, change in volume during tidal breathing while exercising. From (36) with permission.

2.6.2.2 Diaphragmatic neuromuscular inefficiency

Hyperinflation in adults with COPD [1] shortens the diaphragm and compromise its length-tension relationship, [2] increases the radius of curvature of the diaphragm, and [3] decreases the diaphragm's area of apposition to the rib cage (176-178). Collectively, these changes decrease transdiaphragmatic pressure (Pdi) generating capacity and necessitate a high level of neural respiratory drive to support any given $\dot{V}_E$, particularly during exercise when dynamic lung hyperinflation further shortens and weakens the diaphragm (37, 179). This has been demonstrated by Sinderby*, et al.* (179), who reported that during progressive exercise, Pdi increased from 9.4 cmH_2O at rest to a plateau of 13 cmH_2O early in exercise, despite a progressive increase in EMGdi%max from 24% at rest to 81% at end-exercise (**Figure 2.5**). Presumably, during exercise in adults with COPD, dynamic lung hyperinflation impairs the diaphragms pressure-generating

capacity (i.e., Pdi) which in turn promotes a high level of neural respiratory drive (i.e., EMGdi) and central corollary discharge in an effort to increase $\dot{V}_E$ to meet metabolic demand (37, 179). This diaphragmatic neuromuscular inefficiency – defined as an elevated EMGdi/Pdi ratio – has been mechanistically linked to the heightened perception of exertional breathlessness and impaired exercise tolerance in COPD *vs.* health (12, 13).

2.6.2.3 Ventilatory Efficiency

Adults with COPD exhibit poor ventilatory efficiency, commonly defined as an abnormally high ratio of $\dot{V}_E$ to rate of CO_2 production during exercise (i.e., $\dot{V}_E/\dot{V}CO_2$) (170, 175, 180). In healthy adults, $\dot{V}_E/\dot{V}CO_2$ initially decreases until $\dot{V}_E$ increases to compensate for lactic acidosis. As a result, the $\dot{V}_E/\dot{V}CO_2$ nadir is typically reached at the lactate threshold (170, 175, 180). In contrast, pulmonary-mechanical abnormalities (e.g., a large dead space ventilation ($\dot{V}_D$)) in adults with COPD contribute to a high $\dot{V}_E/\dot{V}CO_2$ nadir, which is often achieved before the respiratory compensation point, particularly in severe-to-very severe COPD (170, 175, 180). Therefore, ventilatory inefficiency (i.e., abnormally high $\dot{V}_E/\dot{V}CO_2$ nadir, intercept and/or slope) appears to be [1] a marker of increased ventilatory requirements and neural respiratory drive during exercise in COPD; [2] associated with exertional breathlessness and exercise intolerance in COPD; and [3] an independent predictor of mortality in COPD (170, 175, 180).

2.6.2.4 Neuromechanical uncoupling of the respiratory system

During exercise in adults with COPD, dynamic lung hyperinflation mechanically constraints V_T expansion and reduces the functional capacity of the diaphragm, thereby limiting the ventilatory output that can be produced by the respiratory system (25, 26, 36, 37, 169). V_T and Pdi plateau

early in exercise despite a progressive increase in neural respiratory drive (i.e., EMGdi%max), particularly in moderate-to-severe COPD (179). Neural respiratory drive continues to increase in an effort to increase $\dot{V}_E$ and meet metabolic demand (156, 181). The resulting mismatch between neural respiratory drive and respiratory effort -i.e., neuromechanical uncoupling - (45, 156, 181) (defined as an increase in EMGdi%max/V_T%VC_{pred} or Pes%max/V_T%VC_{pred}) has been mechanistically linked to a heightened perception of exertional breathlessness (i.e., unsatisfied inspiration) (81, 182) and exercise intolerance (44-46) in adults with COPD.

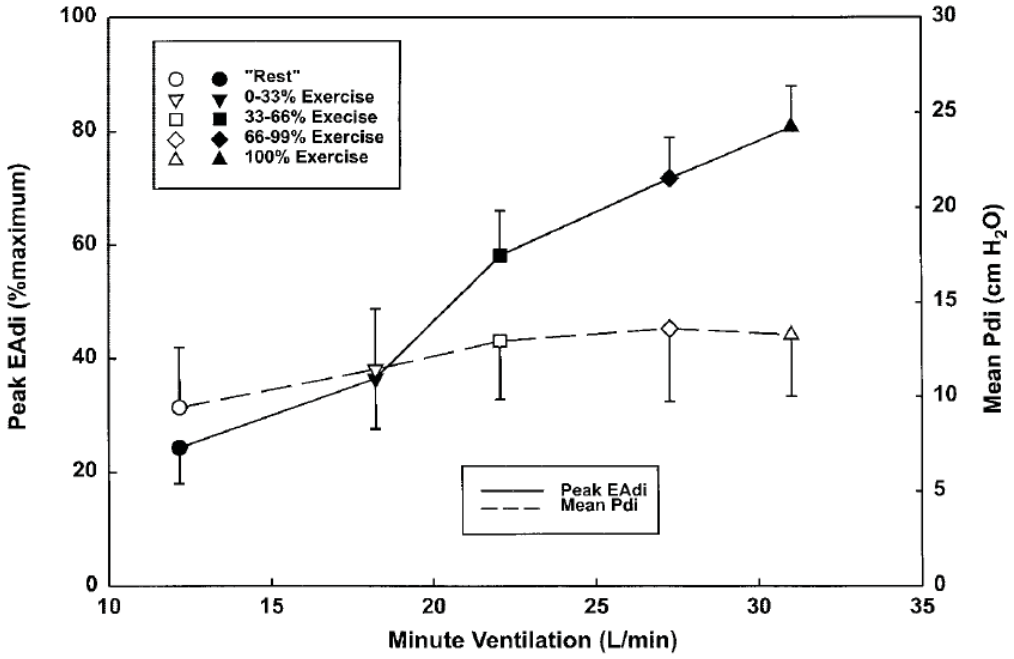

Figure 2.5. Group mean values of peak diaphragm electrical activity (EAdi, closed symbols) and mean transdiaphragmatic pressure (Pdi, open symbols) plotted against minute ventilation during resting breathing (circles) and during 0-33% (triangle with base up), 33-66% (squares), 66-99% (diamond) and 100% (triangle with base down) of exercise time. Values are mean ± SD. Adapted from (179).

2.6.2.5 Neural respiratory drive and central corollary discharge

As alluded to above, neural respiratory drive increases during progressive maximal exercise in adults with COPD (45, 156, 181). Numerous investigators have demonstrated a correlation

between increasing neural respiratory drive (i.e., EMGdi%max) and worsening breathlessness ratings during exercise (45, 156, 181). As discussed in *Section 2.5.3* above, a neurological copy (i.e., central corollary discharge) of the outgoing neural respiratory drive is thought to be relayed to forebrain sensory areas. Therefore, during exercise, central corollary discharge is likely increasing in direct proportion to the increasing levels of neural respiratory drive, and may be responsible for, in part, the heightened perception of exertional breathlessness in adults with COPD.

2.6.2.6 Cardiovascular limitations

Heart rate is elevated, while stroke volume and cardiac output are reduced during exercise in adults with COPD, when compared to age-matched healthy controls (183-186). The altered cardiovascular response during exercise in adults with COPD has been attributed to the deleterious effects of dynamic lung hyperinflation on intrathoracic pressures (e.g., elevated tidal Pes swings relative to healthy controls), and to some extent impaired sympathetic activation of the heart (158, 164, 187). Elevated pulmonary arterial pressure and intra-abdominal (gastric) pressure (Pga) have been shown to impede venous return to the right ventricle and decrease right ventricular preload (158, 164, 187). Right ventricular afterload in adults with *vs.* without COPD is increased as a result of elevated mean intra-thoracic pressures and left ventricular end-diastolic pressures in combination with high alveolar pressure (158, 187). During progressive exercise in COPD, as intra-thoracic pressure swings increase, right ventricular preload may continue to increase as left ventricular afterload increases, thus compromising stroke volume and possibly CO. Therefore, the heart rate, cardiac output and stroke volume achieved by adults with COPD at end-exercise may

reflect [1] an impaired cardiovascular response to exercise and/or [2] early exercise cessation due to the intolerable symptoms, most notable of which is breathlessness.

2.6.2.7 Peripheral muscular abnormalities

In addition to the pathological abnormalities of the lungs, COPD patients exhibit limb muscle dysfunction as a consequence of: systemic inflammation; malnutrition; oxidative stress; hypoxemia; hypercapnia; long-term corticosteroid use; and/or deconditioning due to participation in abnormally low levels of physical activity (66). Despite large inter-individual heterogeneity, limb muscle dysfunction, particularly of the lower extremities (i.e., quadriceps), is usually a reflection of any one, or combination of: muscle weakness; muscle atrophy; fiber type shifting; decreased oxidative capacity and/or mitochondrial dysfunction (66). These pathological abnormalities of the limb muscle may decrease the ability of the muscle to take up and utilize O_2 for adenosine triphosphate synthesis. Importantly, peripheral muscle dysfunction may manifest as an increased sensation of leg discomfort during exercise, which may curtail exercise capacity in adults with COPD (66, 188-190). For example, Butcher, *et al.* (188) and others (191, 192) demonstrated that a sub-population of COPD patients report intolerable leg discomfort, and not breathlessness, as the primary reason for stopping exercise.

2.6.2.8 Summary

Factors mediating exertional breathlessness and exercise intolerance in adults with COPD are complex and multifactorial. Nevertheless, any pharmacological and/or non-pharmacological intervention capable of: [1] improving static and dynamic lung function (i.e., decreasing the load on the diaphragm and improving respiratory mechanics); [2] enhancing diaphragmatic

neuromuscular efficiency; [3] decreasing neural respiratory drive and central corollary discharge; [4] enhancing neuromechanical coupling of the respiratory system; [5] improving cardiovascular function; [6] improving peripheral muscular strength and endurance; or [7] any combination thereof, has the potential to improve exertional breathlessness and exercise tolerance in adults with COPD. This neurophysiological construct of breathlessness provides therapeutic targets for relief of breathlessness in COPD, which will be discussed in the following sections.

2.7 MANAGEMENT OF BREATHLESSNESS AND EXERCISE INTOLERANCE IN COPD

The following sections will provide an overview of the pharmacological and non-pharmacological therapies currently available for the management of breathlessness and exercise intolerance in adults with COPD. For each therapy, the physiological mechanisms of action mediating relief of exertional breathlessness and improvements in exercise tolerance in adults with COPD are discussed. So as to limit the length of this discussion, only the most commonly prescribed and well-studied therapies are reviewed.

2.8 PHARMACOLOGICAL THERAPIES

2.8.1 Bronchodilators

Inhaled bronchodilators are the mainstay in the clinical management of COPD. There exist two main classes of bronchodilators: β_2-adrenoreceptros agonists (β_2-agonists) and antimuscarinic agents (23).

Pharmacological mechanisms

β_2-agonists bind to β_2-adrenergic receptors on the airway smooth muscle, activating cyclic adenosine 3',5'-monophosphate (cAMP) and increasing protein kinase A (193). Protein kinase A, in turn, phosphorylates and inactivates the myosin light chain kinase, precluding it from interacting with, and phosphorylating the myosin light chain. Furthermore, protein kinase A activates myosin light-chain phosphatase, which dephosphorylates the regulatory light chain of myosin II (193-195). This dephosphorylation reaction induces airway smooth muscle relaxation and bronchial dilatation in COPD (193-195). There are two classes of β_2-agonists: short-acting and long-acting β_2-agonists. The duration of efficacy for short-acting β_2-agonists and long-acting β_2-agonists is 4-6 and ≥12 hours, respectively (196, 197).

Antimuscarinic agents counteract the hyperactive parasympathetic tone of the airways in COPD by binding to muscarinic receptors on the airway smooth muscle (i.e., usually M_3 receptors, although antimuscarinic agents might be non-selective and bind to M_1 and M_2 receptors) (193-195). This binding inactivates phospholipase C, decreases the production of inositol 1,4,5-trisphosphate (IP_3), and reduces intracellular calcium concentration ($Ca^{2+}{}_i$) (193-195). The reduced $Ca^{2+}{}_i$, decreases myosin light chain kinase activity, and by extension, myosin light chain activity, thereby promoting airway smooth muscle relaxation (i.e., mitigating bronchoconstriction) and reducing the extent of expiratory flow limitation (193-195).

Effect on exertional breathlessness and exercise performance

In a recent systematic review and meta-analysis, Di Marco, *et al*. (198) reported that, on average, long-acting bronchodilators (long-acting β_2-agonists , long-acting antimuscarinic agents or long

acting β_2-agonists /long-acting antimuscarinic agents) decrease isotime (the highest equivalent two-minute time point completed during each of the constant-load exercise testing visits) breathlessness intensity ratings by 0.41 Borg units (95% CI ranges from -0.56 to -0.27) and increase endurance time by 67-sec (95% CI ranges from 55 to 79) during high-intensity constant-load exercise testing. The efficacy of long-acting bronchodilators on breathlessness and exercise endurance was similar between cycling and walking exercise modalities (198).

Bronchodilators decrease airway smooth muscle tone and improve static and dynamic lung function in adults with COPD (44, 45, 198-202) *via* the pharmacological pathways discussed above. By decreasing both static and dynamic end-expiratory lung volume, bronchodilators effectively: [1] unload the respiratory system and increase the force generating capacity of the diaphragm by placing it on a more optimal point of its length-tension relationship; [2] shift V_T expansion to a more compliant portion of the respiratory systems sigmoid P-V curve; and [3] enable greater V_T expansion in a setting of a reduced neural respiratory drive (44, 199-203). Collectively, these physiological changes enhance neuromechanical coupling of the respiratory system and delay the onset of critical mechanical constraints on V_T expansion (44, 199-203). Relief of exertional breathlessness following bronchodilator therapy is believed to be a consequence of this enhanced neuromechanical coupling (44, 200-202). Indeed, studies evaluating the efficacy of bronchodilator therapy in adults with COPD have demonstrated a correlation between relief of exertional breathlessness and indices of improved neuromechanical uncoupling (45, 198, 200-202). As breathlessness is the proximate cause of exercise limitation in COPD, decreasing breathlessness during submaximal exercise prolongs exercise tolerance and delays the time to reach the point of symptom-limiation.

GOLD Guidelines

Bronchodilator therapy is patient-specific and dependent on multiple factors including: the degree of airflow limitation (e.g., FEV_1); severity of breathlessness assessed using the modified Medical Research Council dyspnea scale; frequency of exacerbations; and the patient's responsiveness to treatment (23). GOLD clinical practice guidelines advocate initiating pharmacological therapy with a short-acting bronchodilator (short-acting β_2-agonists or short-acting antimuscarinic agents). With increasing breathlessness, exercise intolerance and/or poor health status, use of a long-acting β_2-agonist or long-acting anti-muscarinic agents is recommended (23). Dual therapy may be considered in the absence of symptomatic improvement with monotherapy (i.e., in the setting of persistent breathlessness) and are usually preferred to increasing the dose of a single agent (**Figure 2.6**).

Summary

In summary, bronchodilators [1] enhance neuromechanical coupling of the respiratory system and [2] delay the onset of mechanical constraints on V_T expansion, leading to [3] relief of exertional breathlessness with concomitant [4] improvement in exercise tolerance in adults with COPD. Nevertheless, the reported mean changes in exertional breathlessness (-0.41 Borg units) and exercise endurance time (+67-sec) following long-acting bronchodilator therapy are far from the MCID of ≥1 Borg unit and 101-sec (204) for breathlessness and exercise endurance time (205), respectively (198). As chronic breathlessness often remains troublesome despite optimal treatment, additional management options are required even if the effects of bronchodilators would have induced relief of breathlessness that exceeded the MCID of ≥1 Borg unit.

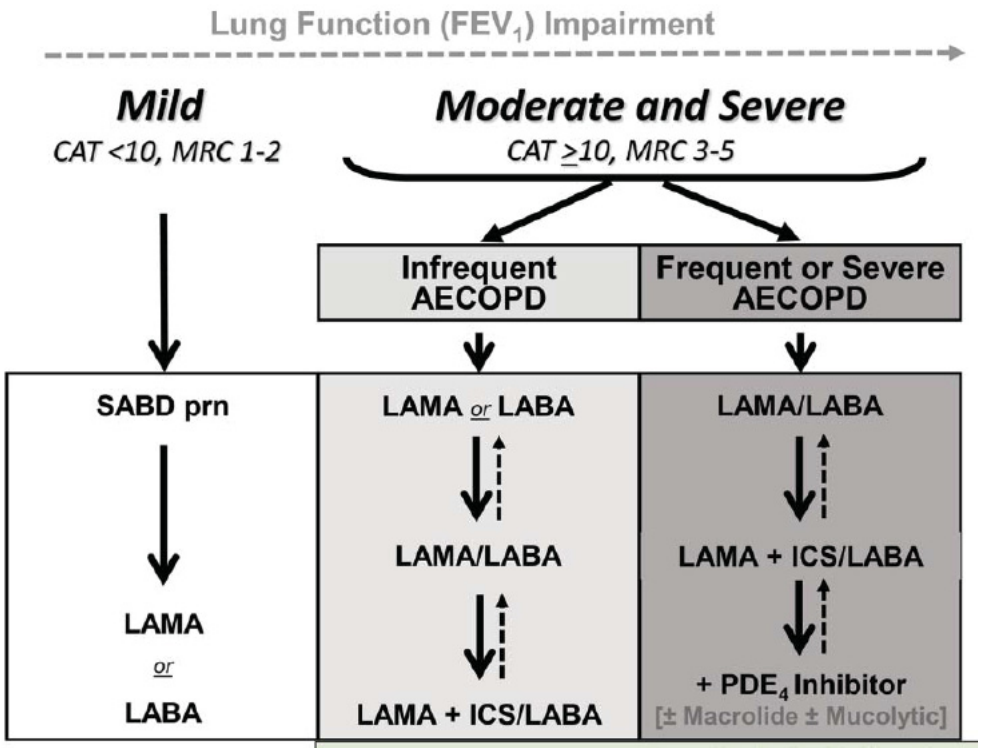

Figure 2.6. COPD pharmacotherapy. AECOPD: acute exacerbation in COPD; ICS: inhaled corticosteroid; LABA: long-acting β2-agonists; LAMA: long-acting antimuscarinic agent; PDE4: phosphodiesterase-4. From (34) with permission.

2.8.2 Methylxanthines

Methylxanthines are a class of oral bronchodilators that are derived from xanthines (23). Theophylline (dimethylxanthine) is the most commonly used methylxanthine in the management of COPD (23).

Pharmacological mechanisms

The mechanisms of action of methylxanthines are multifactorial and include: non-selective inhibition of phosphodiesterase; antagonism of adenosine receptors on the surface of airway smooth muscle; and deacetylation of histones involved in the transcription of inflammatory genes (206, 207). As non-selective phosphodiesterase inhibitors, methylxanthines increase cAMP and

protein kinase A. In turn, protein kinase A inactivates myosin light chain kinase precluding it from interacting with myosin light chain, leading to airway smooth muscle relaxation and bronchial dilatation (208). As adenosine receptor antagonists, methylxanthines are postulated to induce bronchodilation by inhibiting adenylate cyclase and increasing cAMP in airway smooth muscle (208-210). Finally, some methylxanthines (e.g., theophylline) activate histone deacetylases (HDACs), which deacetylate core histones and supress the transcription of inflammatory genes (208). These anti-inflammatory actions of methylxanthines may be significant in the prevention of exacerbations in patients with moderate-to-very severe COPD (34).

Effect on exertional breathlessness and exercise performance

Relief of exertional breathlessness and improvements in exercise capacity following methylxanthine therapy have been reported in some (211-215), but not all studies (211, 216). The mechanisms of methylxanthine-induced relief of breathlessness are not fully understood, but are thought to be the consequence of bronchodilation and/or enhanced respiratory muscle (i.e., diaphragm) function.

Methylxanthines induce bronchodilatation and improve spirometric measures of lung function (e.g,. FEV_1) in COPD *via* the pharmacological pathways discussed above (212-215, 217, 218). Although the effects of methylxanthines on dynamic lung function have not been thoroughly investigated, a placebo controlled study by Voduc, *et al.* (219) demonstrated small improvements in inspiratory capacity during exercise at isotime in 11 adults with COPD after treatment with theophylline. Therefore, it is possible that methylxanthines may improve exertional breathlessness and exercise endurance by unloading the respiratory system and enhancing neuromechanical

coupling. However, it is possible that mechanisms other than bronchodilatation (e.g., enhanced respiratory muscle function) may be important (220), particularly as some studies have reported relief of exertional breathlessness following methylxanthine therapy in the absence of significant improvements in lung function (211, 213, 221).

Murciano*, et al.* (213) reported a significant improvement in maximal voluntary Pdi (measured during a Mueller maneuver) following 30 days of theophylline therapy *vs.* placebo that did not correlate with changes in FEV_1, FVC or FRC, thus implicating an effect of theophylline on diaphragmatic strength independent of changes in lung volume. In a subsequent study, Murciano*, et al.* (214) demonstrated that two months of theophylline *vs.* placebo therapy enhanced respiratory muscle function (i.e., decreased the ratio of inspiratory pleural pressure to maximal pleural pressure during quiet breathing) in adults with COPD; these physiological changes correlated with a decrease in ratings of breathlessness during activities of daily living (100- visual analogue scale). It is possible that, by enhancing the contractility of the diaphragm, chronic administration of methylxanthines may decrease the neural respiratory drive required to generate a given Pdi at rest and on exertion in COPD. In turn, this enhanced neuromuscular coupling of the diaphragm may alleviate breathlessness by [1] reducing neural respiratory drive and presumably also central corollary discharge and/or [2] enhancing neuromechanical coupling of the respiratory system (i.e., if Pdi increases and EMGdi%max decreases, then EMGdi%max:V_T/VC_{pred} may decrease, assuming no effect of methylxanthine on lung volumes and breathing pattern). Although these hypotheses are feasible, it is important to note that some studies have failed to demonstrate a positive effect of methylxanthine therapy on respiratory muscle function (222, 223).

As alluded to above, the effect of methylxanthines on exercise capacity in adults with COPD are variable (211, 212, 215-217). Enhanced exercise capacity following methylxanthine therapy has been reported in the absence and presence of improved static lung function, respiratory muscle function and/or exertional breathlessness (211, 212, 215-217). Given this variability in the effects of methylxanthine on physiological and perceptual responses to exercise, it is difficult to draw definitive conclusions regarding the mechanisms mediating methylxanthine-induced improvement of exercise performance. Differences in the dose, type and duration of methylxanthines used across studies may account for the variable effects on lung function, respiratory muscle function, breathlessness, and exercise capacity. Additional studies are required to elucidate the mechanisms mediating relief of breathlessness and improvements in exercise capacity following methylxanthine therapy.

GOLD Guidelines

Methylxanthine therapy is patient-specific and dependent on the clinical course of COPD. In stable COPD, the GOLD practice guidelines state that theophylline may exert a small bronchodilator effect that may be associated with modest relief of breathlessness (23).

Summary

Methylxanthines may [1] enhance neuromechanical coupling of the respiratory system *via* bronchodilatation and [2] augment the capacity of the respiratory muscles (i.e., enhance neuromuscular coupling of the diaphragm), possibly leading to [3] relief of exertional breathlessness with attendant [4] improvement in exercise capacity in adults with COPD. Nevertheless, due to insufficient evidence, methylxanthines are not readily recommended for the

management of breathlessness and exercise intolerance in adults with stable COPD (34). Consequently, additional therapies are required to achieve symptomatic relief of breathlessness and to improve exercise capacity in adults with COPD.

2.8.3 Inhaled corticosteroids

Inhaled corticosteroids are a class of anti-inflammatory drugs used for the management of COPD-associated pulmonary inflammation (23). Inhaled corticosteroids may be used alone or in combination with long-acting bronchodilator therapy (23).

Pharmacological mechanisms

Inhaled corticosteroids mediate their anti-inflammatory effects by recruiting HDAC2 upon binding to glucocorticoid receptors (224, 225). HDAC2 inhibits histone acetylation and prevents the transcription of pro-inflammatory genes, thereby reducing airway inflammation (224-226). Presumably, relief of breathlessness following inhaled corticosteroid therapy in adults with COPD is due, in part, to reduced airway inflammation. However, cigarette smoke and oxidative stress in adults with COPD reduces HDAC2 expression, thus impeding the anti-inflammatory actions of inhaled corticosteroid therapy (224, 225, 227, 228).

Effect on exertional breathlessness and exercise performance

The effects of inhaled corticosteroids on exertional breathlessness and exercise performance have been evaluated in a small number of studies using various measures of breathlessness and exercise modalities (229-232). Systematic reviews on the topic have failed to demonstrate a positive or

negative effect of inhaled corticosteroids on exertional breathlessness and exercise performance, largely due to the limited data available (233).

In a double-blind randomized, parallel group, placebo controlled trial, Bourbeau*, et al.* (229) demonstrated that 6-months of inhaled corticosteroid therapy (inhaled budesonide) had no significant effects on spirometric measures of lung function (e.g., FEV_1), exercise performance or exertional breathlessness (measured at the end of the 6-minute walk test (6MWT)). In a similar study, Paggiaro*, et al.* (232) reported that, compared to placebo, 6-months of inhaled corticosteroid therapy (inhaled fluticasone propionate) significantly improved spirometric measures of lung function and exercise performance (6MWT), with no concomitant effects on exertional breathlessness (measured at the end of the 6MWT). Collectively, the studies by Bourbeau*, et al.* (229) and Paggiaro*, et al.* (232) failed to demonstrate a beneficial effect of inhaled corticosteroid *vs.* placebo on exertional breathlessness in adults with COPD, as evaluated using the 6MWT, which is a relatively insensitive test for the assessment of relief of breathlessness following therapy in adults with COPD (234).

Using more sensitive tests of exercise tolerance and exertional breathlessness (i.e., constant-load cycle exercise testing), later studies sought to evaluate the effects of inhaled corticosteroid in combination with long-acting β_2-agonists (i.e., inhaled corticosteroid/long-acting β_2-agonists) on exertional breathlessness and exercise performance in adults with COPD. In a randomized, double-blind, placebo controlled, parallel group study, O'Donnell*, et al.* (235) demonstrated that an 8-week intervention of inhaled corticosteroid/long-acting β_2-agonists therapy (fluticasone proprionate/salmeterol) significantly improved static and dynamic lung function (e.g., isotime

inspiratory capacity was increased by 0.13-0.20 L) and exercise tolerance (e.g., exercise endurance time measured during a constant-load cycle exercise test at 75% of peak power output (PPO) increased by 132-sec) in the absence of a significant effect on isotime breathlessness. Improvements in exercise endurance time correlated with increases in resting inspiratory capacity following inhaled corticosteroid/long-acting β_2-agonists *vs.* placebo therapy. In contrast to these findings, a placebo controlled trial by Guenette*, et al.* (231) reported that a 6-week inhaled corticosteroid/long-acting β_2-agonists intervention (fluticasone proprionate/salmeterol) significantly improved static and dynamic airway function and neuromechanical coupling of the respiratory system at rest and throughout exercise. Despite these improvements, inhaled corticosteroid/long-acting β_2-agonists *vs*. placebo did not have a significant effect on exertional breathlessness or exercise endurance. Similar studies have demonstrated a beneficial effect of inhaled corticosteroid/long-acting β_2-agonists *vs.* long-acting β_2-agonists alone (236) and/or inhaled corticosteroid as adjunct therapy to established maintenance long-acting bronchodilator therapy (230) on: spirometric measures of lung function; static and dynamic lung volumes; and exercise performance. These conflicting results suggest that additional studies are required to elucidate the effects of inhaled corticosteroid with or without long-acting bronchodilator therapy on exertional breathlessness and exercise performance in adults with COPD.

GOLD Guidelines

Currently, GOLD recommends against treatment with inhaled corticosteroid monotherapy in adults with stable COPD (23). In patients with moderate-to-very severe COPD, inhaled corticosteroid combined with long-acting β_2-agonists is considered superior to the individual

components in improving FEV_1 and health status (23). Finally, triple inhalation therapy (i.e., inhaled corticosteroid/long-acting antimuscarinic agents/ long-acting β_2-agonists) is more effective than inhaled corticosteroid/long-acting β_2-agonists or long-acting antimuscarinic agents monotherapy in improving lung function, symptoms and health status (23).

Summary

Inhaled corticosteroid monotherapy is not associated with improved exertional breathlessness and/or exercise capacity in adults with COPD. Compared to placebo or long-acting β_2-agonists therapy, inhaled corticosteroid / long-acting β_2-agonists therapy may have some beneficial effects on exercise capacity in adults with COPD; however, the impact of inhaled corticosteroid / long-acting β_2-agonists therapy on exertional breathlessness remains equivocal. Consequently, additional therapies are required to achieve symptomatic relief of exertional breathlessness and improvements in exercise capacity in adults with COPD.

2.8.4 Phosphodiesterase-4 (PDE4) inhibitors

Phosphodiesterase type 4 inhibitors are a class of oral anti-inflammatory drugs. Roflumilast and cilomilast are the most commonly used phosphodiesterase type 4 inhibitors in the management of COPD (237, 238).

Pharmacological mechanisms

PDE_4 inhibitors interfere with cAMP metabolism in smooth muscle and inflammatory cells (239, 240). Accumulation of cAMP increases the activity of protein kinase A leading to the

phosphorylation of various proteins (239, 240). In turn, this elevated phosphorylation is postulated to [1] induce airway smooth muscle relaxation and bronchial dilatation and [2] decrease in inflammatory cell activity (e.g., cytokines) in adults with COPD (239, 240).

Effect on exertional breathlessness and exercise performance

In a recent systematic review and meta-analysis, Chong*, et al.* (237) reported that, on average, PDE_4 inhibitors have little impact on exertional breathlessness and exercise tolerance in adults with COPD: the mean differences in exertional breathlessness and 6-min walk distance (exercise tolerance) were -0.19 Borg 0-10 scale units at the end of the 6MWT (95% CI ranges from -0.33 to -0.05) and +2.09 m (95% CI ranges from -7.39 to 11.57), respectively. These improvements are much lower than the MCIDs of ≥1 Borg 0-10 scale units (204) and 30 m (241), respectively.

Studies demonstrating relief of exertional breathlessness following PDE_4 inhibitor therapy have failed to elucidate the physiological mechanisms of action mediating this effect. Single-dose administration of PDE_4 inhibitors are not associated with bronchodilatation in adults with COPD (242). Nevertheless, significant improvements in pre-and post-bronchodilator FEV_1 have been reported following long-term treatment with PDE_4 inhibitors (237, 238); these improvements are postulated to be due to the anti-inflammatory effects of PDE_4 inhibitors (e.g., reduced mucosal edema) (243). Therefore, it is conceivable that relief of exertional breathlessness (albeit to a small magnitude, if at all) following PDE_4 inhibitor therapy is the result of altered sensory afferent input to various breathlessness brain centers secondary to improvements in airway mucosal inflammation (243). Although this hypothesis is feasible, the collective results of clinical studies conducted to date suggest that the beneficial effects of PDE_4 inhibitors is likely restricted to the

management of exacerbations, and not to improved exertional breathlessness and/or exercise tolerance, in adults with COPD (23).

GOLD guidelines

GOLD recommends PDE_4 inhibitors to prevent exacerbations in patients with severe-to-very severe COPD, chronic bronchitis and a history of at least one exacerbation (23).

Summary

PDE_4 inhibitors reduce airway inflammation and decrease the severity and frequency of exacerbations, but have minimal effects on exertional breathlessness and exercise capacity in adults with COPD. To this end, additional therapies are required to reduce exertional breathlessness and improve exercise intolerance in adults with COPD.

2.8.5 Anxiolytics and Antidepressants

The role of various anxiolytics (e.g., benzodiazepines and buspirone) and antidepressants (e.g., tricyclic antidepressants and selective serotonin-reuptake inhibitors) has been evaluated in COPD (244-255).

Pharmacological mechanisms

Benzodiazepines enhance the effects of the natural brain chemical, *gamma*-Aminobutyric acid (GABA) on GABA receptors (256). This leads to neuronal cell hyperpolarization and a decrease in neuronal cell activity within the central nervous system (256). Through various mechanisms, buspirones, tricyclic antidepressants and SSRIs increase the activity of serotonin receptors on

postsynaptic cells. Stimulation of these cellular transduction cascades result in anxiolytic and antidepressant properties (256).

Effect on exertional breathlessness and exercise performance

Although some studies have demonstrated a beneficial effect of anxiolytics/benzodiazepines (246, 249, 253, 255), the majority of available evidence suggests that these therapies are ineffective in the management of exertional breathlessness and exercise intolerance in symptomatic adults with COPD (244, 245, 247, 248, 252, 254-256). Indeed, a recent systematic review and meta-analysis revealed that benzodiazepines have no statistically significant effect on breathlessness in adults with COPD (251): the estimated standardised mean difference in exertional breathlessness was just -0.12 (95% CI ranges from -0.52 to 0.29), while the effect of benzodiazepines on exercise tolerance was not reported.

Studies reporting relief of exertional breathlessness following anxiolytic/benzodiazepine therapy have failed to elucidate the physiological mechanisms of action mediating these effects. Nevertheless, it has been suggested that anxiolytics/benzodiazepines may ameliorate breathlessness secondary to their effects on anxiety/depression (i.e., by improving negative affect, perception of breathlessness may decrease) (54, 257). Alternatively, it has been hypothesized that anxiolytics/benzodiazepines may reduce exertional breathlessness by decreasing neural respiratory drive and presumably also central corollary discharge. In support of this hypothesis, some studies have reported a decrease in ventilatory and mouth occlusion pressure responses to CO_2 following anxiolytic/benzodiazepine therapy in adults with COPD (249, 250).

GOLD guidelines

GOLD recommends against the use of anxiolytics/benzodiazepines for the management of breathlessness in adults with advanced COPD (54). Additional studies are required to assess the effectiveness of these therapies in the management of exertional breathlessness and exercise intolerance in adults with COPD.

Summary

There is some evidence to suggest that anxiolytics/benzodiazepines may reduce exertional breathlessness in adults with COPD by [1] improving negative affect and/or [2] decreasing neural respiratory drive and the attendant central corollary discharge. Nevertheless, the collective results of randomized controlled trials conducted to date do not support a beneficial effect of anxiolytics/benzodiazepines in the management of breathlessness and exercise intolerance in adults with COPD (251). Therefore, the therapeutic potential of alternative therapies in the management of exertional breathlessness and exercise intolerance in adults with COPD should be investigated.

2.8.6 Nebulized Furosemide

Furosemide is a powerful loop diuretic recently considered as an adjunct therapy in the management of breathlessness and exercise intolerance in adults with COPD. The therapeutic potential of inhaled nebulized furosemide in reducing breathlessness has been investigated in healthy and asthmatic adults (258), and individuals with cancer (259-268), with only two studies conducted in adults with COPD (269, 270).

Pharmacological mechanisms

The cellular transduction cascades activated in response to inhaled nebulized furosemide remain to be elucidated. It has been postulated that the activity of slowly adapting pulmonary stretch receptors, rapidly adapting pulmonary stretch receptors and/or bronchopulmonary C-fibers may be modified following the administration of inhaled nebulized furosemide (260, 262, 271). Sudo, *et al.* (271) demonstrated that slowly adapting pulmonary stretch receptors are sensitized and rapidly adapting pulmonary stretch receptors are desensitized in anesthetized rats following the inhalation of furosemide. Presumably, this altered slowly adapting pulmonary stretch receptors and rapidly adapting pulmonary stretch receptors activity is relayed to the cardiorespiratory centers by the vagus nerve, where it may inhibit neural respiratory drive and central corollary discharge (133, 262, 272). Furthermore, inhaled nebulized furosemide may induce bronchodilatation secondary to its effects on slowly adapting pulmonary stretch receptors and rapidly adapting pulmonary stretch receptors (i.e., reduced parasympathetic outflow and cholinergic tone of the airway smooth muscle) (269, 270).

Effect on exertional breathlessness and exercise performance

The acute effects of inhaled nebulized furosemide on exertional breathlessness and exercise capacity in adults with COPD has been evaluated by Ong*, et al.* (270) and Jensen*, et al.* (269) during a constant-load cardiopulmonary cycle exercise test. Ong*, et al.* (270) reported significant bronchodilation (i.e., an increase in FEV_1 and FVC) and improvement in isotime breathlessness (i.e., an 8.7 mm decrease in ratings of breathlessness on a 100-mm visual analogue scale) after single-dose inhalation of furosemide (40 mg) *vs.* 0.9% saline placebo. Despite these improvements, exercise capacity and respiratory variables at isotime were not different between

treatments. Jensen*, et al.* (269) demonstrated that, compared to placebo, single-dose inhalation of nebulized furosemide (40 mg) significantly: improved dynamic lung function (i.e., increased dynamic inspiratory capacity at isotime by 120 ml); decreased isotime breathlessness by 0.9 Borg 0-10 scale units; and increased exercise endurance time by 1.65 min in adults with COPD. Most of the variance in the furosemide-induced changes in isotime breathlessness were explained by concomitant increases in inspiratory time, mean tidal expiratory flow rate, and V_T expansion. Improvements in exercise endurance time significantly correlated with the decrease in isotime breathlessness. These results suggest that inhaled nebulized furosemide may reduce exertional breathlessness by altering the activity of slowly adapting pulmonary stretch receptors and rapidly adapting pulmonary stretch receptors. Additional studies are required to appreciate the effects (both short- and long-term) and mechanisms of action of inhaled nebulized furosemide on exertional breathlessness and exercise capacity in adults with COPD.

GOLD guidelines

Currently, GOLD does not hold a position on the use of inhaled nebulized furosemide in adults with COPD (23).

Summary

Based on the limited number of randomized controlled trials in adults with COPD, inhaled nebulized furosemide may alleviate exertional breathlessness by altering SAR and RAR activity with concomitant effects on dynamic lung function (i.e., bronchodilatation) and V_T expansion. Improvements in exercise capacity following single-dose inhalation of nebulized furosemide have been linked to improvements in breathlessness during sub-maximal exercise. It is important to

note, however, that despite the promising results of Ong*, et al.* (270) and Jensen*, et al.* (269), evidence in support of inhaled nebulized furosemide in the management of breathlessness in healthy and asthmatic adults, and individuals with cancer is equivocal (261, 263-268). Consequently, alternative therapies for the management of exertional breathlessness and exercise intolerance in adults with COPD should be investigated.

2.9 NON-PHARMACOLOGICAL THERAPIES

2.9.1. Pulmonary rehabilitation

Pulmonary rehabilitation (PR) is a comprehensive multidisciplinary approach to COPD management that consists of exercise training, psychological, behavioral and disease education, and nutritional counselling (273, 274). Patients are typically enrolled in the PR program for a period of 6-12 weeks where they complete 30-40 min of aerobic and resistance training 2-3 times per week (273, 274). During the psychological and behavioral education sessions, COPD patients are introduced to self-management techniques meant to facilitate smoking cessation; identify and treat acute exacerbations; increase daytime physical activity levels; improve body composition; and promote psychosocial health (273, 274).

Effect on exertional breathlessness and exercise performance

In a recent systematic review and meta-analysis, McCarthy*, et al.* (275) reported that compared to usual care, PR significantly improves maximal cycle exercise capacity by 6.77 watts (95% CI 1.89 – 11.65). Furthermore, PR is associated with clinically meaningful improvements in isotime breathlessness (i.e., decrease in breathlessness by ≥1 Borg unit) and exercise endurance time (i.e.,

increase in exercise endurance time during a constant-load cycle exercise test by more 101-sec) (276, 277).

Exercise training in adults with moderate-to-very severe COPD reverses muscle fibre remodelling and increases the cross-sectional area and capillarization of peripheral locomotor muscles, such as the vastus lateralis (278-281). These morphological and structural changes enhance the strength, endurance and oxidative capacity of the peripheral muscles (278-282). During exercise, these improvements lead to enhanced O_2 uptake and utilization by the peripheral locomotor muscle (283). In turn, ventilatory requirements decrease at any given sub-maximal power output following PR compared to baseline (284-290). Therefore, relief of exertional breathlessness following PR is achieved, at least in part, by a decrease in neural respiratory drive and presumably central corollary discharge.

Recently, Herigstad*, et al.* (92) reported that the activity of various brain centers implicated in the perception of breathlessness (e.g. anterior insular cortex, anterior cingulate cortex) during a breathlessness-related word cue task (e.g., asking the patient how breathlessness they might feel while walking up a hill) was significantly reduced following PR compared to baseline in adults with mild-to-moderate COPD. Furthermore, baseline activity in brain centers implicated in the generation of priors (i.e., neural representations of previous experiences) was negatively correlated with improvements in breathlessness-anxiety ratings. Collectively, these results suggested that PR may alleviate exertional breathlessness by decreasing negative affect (e.g., anxiety) and altering central neural processing of breathlessness (92), alone (277) or in combination with the aforementioned effects on metabolic and ventilatory demands of physical activity.

Improvements in exercise tolerance following PR are associated with enhanced peripheral locomotor muscle function (strength and endurance) and relief of isotime breathlessness and leg discomfort (284-288, 290). Presumably, enhanced peripheral locomotor muscle function improves the muscles ability to perform external work, thus enabling adults with COPD to maintain exercise for a longer period of time in the setting of lower sub-maximal exertional symptoms (i.e., delay the onset of intolerable symptoms of breathlessness and leg discomfort).

GOLD guidelines

GOLD strongly recommends that patients with moderate, severe and very severe COPD participate in PR (23).

Summary

PR [1] improves peripheral locomotor muscle function, [2] decreases neural respiratory drive by decreasing metabolic and ventilatory demands, and [3] alters central neural processing of breathlessness leading to [4] relief of exertional breathlessness and [5] enhanced exercise tolerance in adults with COPD. Nevertheless, in the absence of long-term maintenance strategies, adherence to exercise training quickly decreases following participation in a standard outpatient PR program (291, 292). As a result, the established health benefits of PR begin to diminish 6-12 months post PR and are virtually abolished within 24 months (291, 292). Finally, PR resources are limited, and as a result, not all patients with COPD have access to PR (293, 294). Therefore, additional therapies for the management of breathlessness and exercise intolerance in adults with COPD are needed.

2.9.2. Hyperoxia

Hyperoxia (i.e., supplemental oxygen) is primarily considered for the management of hypoxemia in adults with COPD (295, 296).

Effect on exertional breathlessness and exercise performance

In a systematic review evaluating the short-term efficacy of hyperoxia from single-assessment studies, Bradley*, et al.* (295) demonstrated that, on average, hyperoxia decreases isotime breathlessness intensity by 1.15 Borg 0-10 scale units (95% CI ranges from -1.65 to -0.66) and increases exercise endurance time by 2.71 min (95% CI ranges from 1.96 to 3.56). The magnitudes of these improvements are clinically meaningful in as much as they exceed their respective MCIDs (204, 205).

The mechanisms by which hyperoxia relieves exertional breathlessness in adults with COPD are complex and multifactorial. Compared to room air, hyperoxia significantly reduces isotime $\dot{V}_E$ by an average of 3.58 L/min (95% CI ranges from -4.85 to -2.31) (295). This decrease in $\dot{V}_E$ is achieved by a reduction in f_R in the setting of a relatively unchanged V_T. Mechanisms mediating the decrease in $\dot{V}_E$ during exercise are debated. It has been postulated that hyperoxia may directly blunt peripheral chemoreceptor activity with a concomitant decrease in central respiratory motor drive (i.e., loss of the hypoxic stimulus to breathe). Alternatively, hyperoxia could delay the onset of metabolic acidosis (and therefore decrease group III/IV locomotor sensory muscle afferent feedback to the brainstem respiratory center (297)) resulting in a decrease in $\dot{V}_E$ throughout exercise (298-304). Irrespective of the underlying mechanisms, numerous studies have demonstrated that during exercise with hyperoxia *vs.* room air, breathlessness and $\dot{V}_E$ decrease

proportionally, that is, breathlessness-$\dot{V}_E$ slopes are preserved during exercise whilst breathing hyperoxic *vs.* normoxic gas (302, 303). Therefore, relief of exertional breathlessness following hyperoxia may be attributable, at least in part, to a decrease in neural respiratory drive and central corollary discharge. In support of this hypothesis, Schaeffer, *et al.* (173) recently demonstrated significant reductions in EMGdi%max and improvements in neuromechanical uncoupling during constant-load cycle exercise testing at isotime with hyperoxia *vs.* room air in patients with fibrotic interstitial lung disease.

In conjunction with its positive effect on $\dot{V}_E$-time slopes during exercise, hyperoxia has been shown to significantly delay dynamic lung hyperinflation (i.e., increase inspiratory capacity and inspiratory reserve volume at standardized submaximal time points during exercise) in adults with COPD (298-304). Possible mechanisms of this delay include: [1] a decrease in $\dot{V}_E$ at any given level of expiratory flow limitation and/or [2] altered breathing pattern (i.e., prolongation of expiratory time and decreased f_R) at any given $\dot{V}_E$. These improvements in dynamic operating lung volumes as well as in the pattern and timing of breathing are likely to reduce elastic threshold loading on the diaphragm with concomitant positive effects on neuromechanical coupling of the respiratory system (298-304). Collectively, these mechanical changes translate into relief of exertional breathlessness in adults with COPD.

Enhanced exercise tolerance during hyperoxia therapy is associated with improvements in: isotime breathlessness; dynamic operating lung volumes (i.e., increase in inspiratory capacity and inspiratory reserve volume); and O_2 delivery to the peripheral locomotor muscles (299, 300, 302, 303). Presumably, by delaying the onset of intolerable breathlessness (primarily) and leg

discomfort (secondarily), hyperoxia enables adults with COPD to maintain exercise for a longer period of time. Furthermore, during exercise with hyperoxia, O_2 delivery to the exercising muscles (e.g., quadriceps) is enhanced in the absence of appreciable change in blood flow to the locomotor muscle in mildly hypoxemic adults with COPD (300, 305). This altered O_2 delivery augments muscle metabolism with attendant positive effects on the exercising muscles ability to increase work capacity (300).

GOLD guidelines

GOLD does not recommend long-term oxygen therapy in patients with stable COPD and resting or exercise-induced moderate desaturation (23). Long-term oxygen therapy should be considered in patients with severe resting arterial hypoxemia (PaO_2 < 55 mmHg) (23).

Summary

During exercise, hyperoxia may: [1] decrease neural respiratory drive and presumably also central corollary discharge; [2] improve dynamic ventilatory mechanics; and [3] augment O_2 supply to the peripheral locomotor muscles. These physiological changes reduce exertional breathlessness and enhance exercise tolerance in adults with COPD. Nevertheless, oxygen therapy is not recommended for non-hypoxemic adults with COPD. As such, alternative therapies for the management of breathlessness and exercise intolerance in adults with COPD should be investigated.

2.9.3. Heliox

Heliox therapy consists of breathing a gas mixture composed of helium and O_2; for example, 79% helium and 21% O_2 (306, 307). The O_2 concentration in heliox gas is not greater than that typically found in air (306, 307).

Effect on exertional breathlessness and exercise performance

Available evidence suggests that heliox decreases exertional breathlessness and improves exercise tolerance in adults with COPD (299, 306, 308-313). Because heliox is a lower density gas than room air, it: [1] reduces turbulent flow of air within the tracheobronchial tree; and [2] increases maximum expiratory flow generating capacity and enhances the rate of lung emptying (i.e., decreases expiratory flow limitation) with an attendant decreases in end-expiratory lung volume (increase in inspiratory capacity) and end-inspiratory lung volume (increase in inspiratory reserve volume) at rest and throughout exercise. In addition, compared to room air, heliox increases $\dot{V}_E$ at isotime by ~16% (299, 306, 308-313). This is achieved by an increase in V_T in the setting of a relatively unchanged f_R (299, 306, 308-313). Collectively, these effects of heliox on respiratory mechanics reduce the overall work of breathing (despite the increased $\dot{V}_E$), leading to a decrease in exertional breathlessness in adults with COPD.

It has been postulated that, by improving laminar airflow and minimizing both expiratory flow limitation and the work of breathing, heliox promotes the redistribution of blood flow from the respiratory muscles to the peripheral locomotor muscles (311). This hypothesis has recently been refuted by Louvaris, *et al.* (311) and others (305, 308, 314), who demonstrated that heliox improves blood flow to both peripheral (quadriceps) and respiratory (abdomen and intercostal muscles)

muscles. That is, heliox does not induce blood flow-redistribution, *per se*, but rather it augments blood flow to working muscles (both respiratory and locomotor), leading to increased systemic O_2 delivery (311). This enhanced blood flow during exercise with heliox is proposed to be the result of augmented cardiac output secondary to improved respiratory mechanics (305, 308, 311, 314).

Enhanced exercise tolerance during heliox has been correlated with improvements in peak expiratory flow; increased inspiratory capacity during exercise at isotime (i.e., decreased dynamic lung hyperinflation); decreased breathlessness during exercise at isotime; and decreased total work of breathing (309, 312). These correlative analyses support the hypothesis that, by enhancing respiratory mechanics and delaying the onset of intolerable breathlessness, heliox increases exercise tolerance in adults with COPD. In addition, by increasing O_2 availability to the peripheral locomotor muscles (*via* enhanced cardiac output and limb blood flow), heliox therapy enhances the skeletal muscle's ability to perform external work (with decreased leg discomfort), thus further contributing to improvements in exercise tolerance.

GOLD guidelines

Currently, GOLD does not hold a position on the use of heliox in adults with COPD (23).

Summary

By improving breathing mechanics, decreasing the work of breathing, and increasing respiratory and peripheral locomotor muscle blood flow (O_2 delivery), heliox improves exertional breathlessness and exercise tolerance in adults with COPD. Nevertheless, administration of heliox is expensive and cumbersome (i.e., not readily available within a community setting). Therefore,

alternative therapies for the management of breathlessness and exercise intolerance in adults with COPD should be investigated.

2.9.4. Non-invasive ventilation

Non-invasive ventilatory support is a form of mechanical ventilation that delivers positive pressure during the respiratory cycle (307). A mouthpiece, nasal prongs, or a facemask can be used to deliver non-invasive ventilatory support. There exist several modes of mechanical ventilation for the delivery of non-invasive ventilatory support, including: bi-level positive airway pressure; pressure support ventilation; and proportional assist ventilation (307).

Effect on exertional breathlessness and exercise performance

A systematic review by van 't Hul*, et al.* (315) demonstrated that acute administration of non-invasive ventilatory support during exercise improves exertional breathlessness in COPD. The average weighted improvement in breathlessness ratings at isotime was 2 Borg 0-10 scale units, while the average weighted improvement in exercise endurance time was 3.3 min. The magnitudes of these improvements are clinically meaningful in as much as they exceed their respective MCIDs (204, 205).

During exercise in adults with COPD, dynamic lung hyperinflation increases the positive elastic recoil pressure at end-expiration (316-319). In turn, the inspiratory muscles (e.g., diaphragm) must generate a force sufficient enough to overcome the positive elastic recoil in order to generate inspiratory flow. By applying mild air pressure to keep the airways open, non-invasive ventilatory support counterbalances this positive recoil pressure, effectively decreasing the work of breathing

as well as neural respiratory drive and presumably also central corollary discharge (316-321). Indeed, numerous studies have reported enhanced inspiratory and expiratory muscle activity during exercise with non-invasive ventilatory support (i.e., a decrease in Pes and EMGdi measured transcutaneously) (317, 319, 320). Therefore, relief of exertional breathlessness with non-invasive ventilatory support is likely the result of a decrease in the overall work of breathing, neural respiratory drive and central corollary discharge. In support of this hypothesis, some studies have reported a correlation between relief of exertional breathlessness and measures of enhanced respiratory muscle function (i.e., a decrease in EMGdi and Pdi) during exercise with non-invasive ventilatory support (319, 321). Furthermore, improvements in exercise tolerance during non-invasive ventilatory support therapy have been correlated with relief of isotime breathlessness (318).

GOLD guidelines

GOLD recommends that non-invasive ventilatory support may be considered on an individual basis in adults with COPD with pronounced daytime hypercapnia and recent hospitalization (23).

Summary

Acute administration of non-invasive ventilatory support decreases the work of breathing with concomitant positive effects on exertional breathlessness and exercise capacity in adults with COPD. Despite these promising effects of non-invasive ventilatory support, there is limited evidence to support the long-term use of non-invasive ventilatory support in adults with COPD (322). As such, alternative therapies for the management of exertional breathlessness and exercise intolerance in adults with COPD should be investigated.

2.9.5. Lung volume reduction surgery (LVRS)

Lung-volume reduction surgery is an invasive procedure designed to decrease lung hyperinflation in adults with COPD. In surgical lung-volume reduction, areas of the lung with high ventilation/perfusion ratios are excised (323). Conversely, endoscopic lung-volume reduction surgery decreases or obliterates ventilation to areas with high $\dot{V}/Q$ ratios (323).

Effect on exertional breathlessness and exercise performance

Numerous studies have investigated the efficacy of lung-volume reduction surgery in reducing exertional breathlessness (measured at isotime using the Borg's 0-10 scale or visual analogue scale) and improving exercise tolerance (typically evaluated with the 6MWT) at a variety of follow-up times (i.e., 3 months to 2 years) in adults with COPD (324-331). In a recent systematic review, van Agteren*, et al.* (330) reported that, compared to control medical therapy (e.g., PR), surgical lung-volume reduction significantly improved walking distance (standardized mean difference +0.70 m; 95% CI 0.42-0.98) in adults with COPD. Similarly, van Agteren*, et al.* (331) reported significant improvement in the 6-min walking distance (6MWD) at various time points following endoscopic lung-volume reduction. The effect of surgical lung-volume reduction and endoscopic lung-volume reduction on exertional breathlessness was not reported in these systematic reviews (330, 331).

By decreasing lung hyperinflation in adults with COPD (i.e., a decrease in total lung capacity and end-expiratory lung volume), lung-volume reduction effectively [1] increases the zone of apposition and length of the diaphragm (325), contributing to [2] enhanced diaphragmatic strength (e.g., maximal Pdi) (12, 13, 324, 326, 328) with attendant [3] decreases in neural respiratory drive

and presumably central corollary discharge (12, 13, 325). Collectively, these physiological changes enable greater V_T expansion for a given degree of diaphragmatic pressure development (i.e., enhanced neuromuscular efficiency of the diaphragm) (12, 13), leading to relief of exertional breathlessness.

Improvements in exercise tolerance following lung-volume reduction are positively correlated with improvements in: ventilatory capacity and neuromuscular coupling of the respiratory system at rest; and breathlessness intensity ratings at isotime (12, 13, 325, 326, 328). Therefore, by decreasing lung hyperinflation and enhancing exercise ventilatory efficiency (332), lung-volume reduction surgery enables adults with COPD to sustain exercise for a longer period of time in the setting of improved ventilatory mechanics, respiratory muscle function, and exertional breathlessness.

GOLD guidelines

LVRS is recommended for patients with COPD who meet carefully designed and highly selected criteria (see (23) for details).

Summary

Lung-volume reduction surgery enhances diaphragmatic functioning and neuromuscular efficiency, thus contributing to improvements in exertional breathlessness and exercise capacity in adults with COPD. Nevertheless, lung-volume reduction surgery procedures are expensive, limited to a highly selected group of patients with very severe disease, and may be associated with

a high potential of complications (330, 331). As such, additional therapies for the management of breathlessness and exercise intolerance in adults with COPD should be investigated.

2.10 SECTION SUMMARY

Available pharmacological and non-pharmacological therapies produce modest improvements in exertional breathlessness and exercise tolerance by enhancing the neurophysiological underpinning of breathlessness in adults with COPD (e.g., enhancing respiratory mechanics, diaphragm functional capacity, neural respiratory drive, neuromechanical coupling of the respiratory system, and central neural processing of breathlessness). Nevertheless, many adults living with COPD continue to experience severe and disabling breathlessness and exercise intolerance (1, 3). To this end, adjunct therapies targeted to relief exertional breathlessness and improved exercise tolerance in adults with COPD are needed.

2.11 PREVELANCE OF CHRONIC REFRACTORY BREATHLESSNESS

Chronic breathlessness syndrome has recently been defined as *"the experience of breathlessness that persists despite optimal treatment of the underlying pathophysiology and results in disability for the patient"* (4). Although poorly understood, studies by Mullerova*, et al.* (1) and Sundh and Ekstrom (3) have provided some insights into the prevalence and burden of both breathlessness and chronic breathlessness syndrome in COPD.

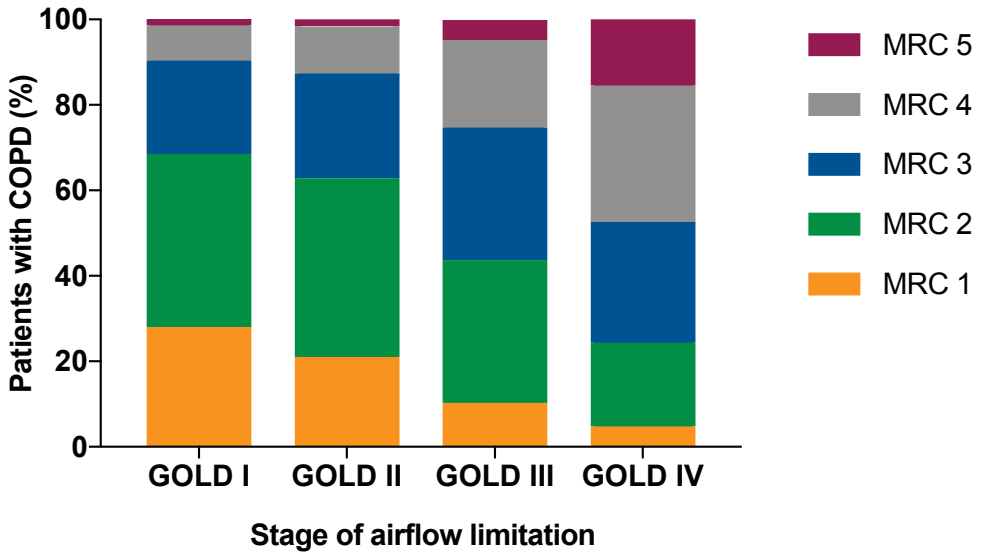

Figure 2.7. MRC dyspnoea grade distribution by stage of airflow limitation. COPD, chronic obstructive pulmonary disease; MRC, Medical Research Council. MRC of 1 = no breathlessness. MRC of 2 = mild breathlessness. MRC of ≥ 3 = moderate-to-severe or clinically significant breathlessness. MRC scoring 1-5 equals to mMRC grades 0-4. Adapted from (1).

Mullerova*, et al.* (1) evaluated factors associated with breathlessness in a cohort of over 40,000 patients with spirometrically-defined COPD and breathlessness measured using the Medical Research Council (MRC) dyspnoea scale across both England and Wales. Mullerova*, et al.* (1) demonstrated that breathlessness severity increased with increasing airflow limitation, with almost 56% of GOLD III and 76% of GOLD IV patients suffering from clinically significant breathlessness despite intensive treatment with bronchodilators, inhaled corticosteroids, theophylline and/or oral corticosteroids for management of their COPD (**Figure 2.7**).

In a longitudinal analysis of the Swedish National Registry of COPD, Sundh and Ekstrom (3) evaluated the prevalence of disabling breathlessness and persistent disabling breathlessness in relation to pharmacological and non-pharmacological treatments in a cohort of 1,689 adults with spirometrically-defined COPD. Breathlessness was evaluated using the modified Medical

Research Council (mMRC) dyspnoea scale. Disabling breathlessness was defined as a mMRC ≥2 (equivalent to MRC ≥3), and persistent disabling breathlessness was defined as disabling breathlessness at baseline and at follow-up (≥2 months). In keeping with the results of Mullerova*, et al.* (1), Sundh and Ekstrom (3) also demonstrated that disabling breathlessness (or clinically meaningful breathlessness) increased with COPD disease severity (i.e., decreasing FEV_1% predicted). Furthermore, Sundh and Ekstrom (3) demonstrated that more intense COPD treatment was associated with persistent disabling breathlessness (**Figure 2.8**). For example, the prevalence of persistent disabling breathlessness increased from ~26% in patients receiving monotherapy to ~60% in patients receiving triple inhalation therapy (**Figure 2.8**). Of note was the high prevalence (74%) of patients with persistent disabling breathlessness despite triple inhalation therapy and physiotherapy, i.e., chronic breathlessness syndrome (**Figure 2.8**). Collectively, the results of Mullerova*, et al.* (1) and Sundh and Ekstrom (3) clearly demonstrate [1] a high prevalence of breathlessness and chronic breathlessness syndrome in patients with severe to very-severe COPD (i.e., GOLD III and IV), and [2] an unmet need for the symptomatic management of chronic breathlessness in COPD. Therefore, adjunct therapies to compliment traditional pharmacological and non-pharmacological therapies are urgently needed to better optimize management of breathlessness and chronic breathlessness syndrome in COPD.

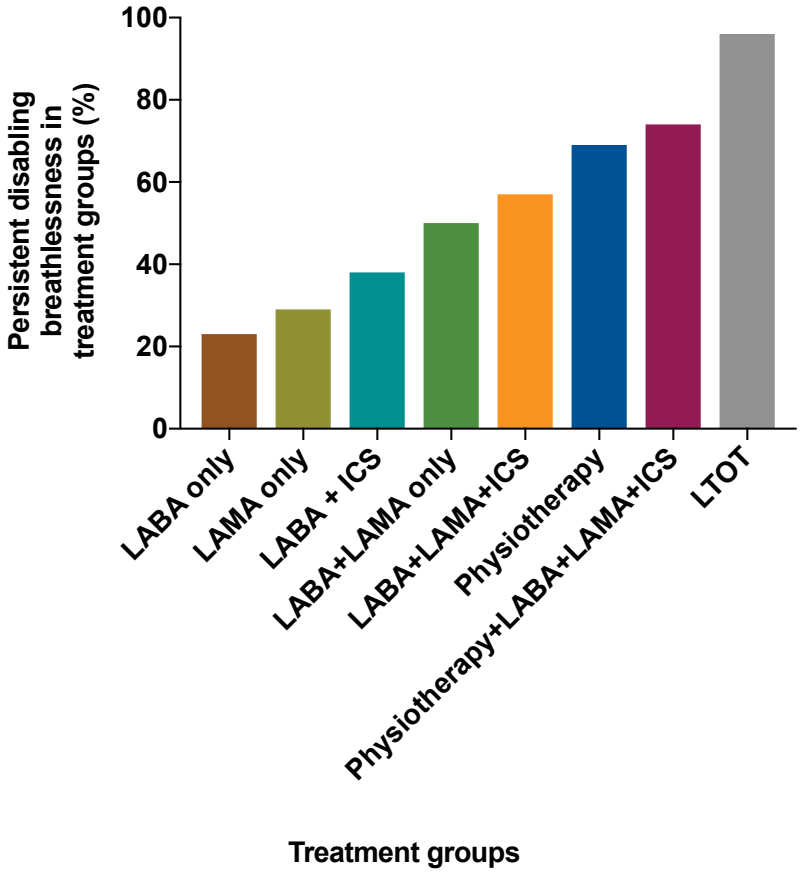

Figure 2.8. Persistent disabling breathlessness in treatment groups. Data presented as percentages of persistent disabling breathlessness in different treatment groups. Persistent disabling breathlessness is defined as an mMRC grade of ≥ 2 at both baseline and follow-up (duration ≥ 2 month). LABA, long-acting beta-2-agonists; LAMA, long-acting muscarinic antagonists; LTOT, long-term oxygen therapy; ICS, inhaled corticosteroids. Adapted from (3).

CHAPTER 3: RATIONALE, OBJECTIVES AND HYPOTHESIS

3.1 RATIONALE

Breathlessness on exertion and exercise intolerance are among the most debilitating symptoms experienced by adults with COPD (23, 55). Available evidence-based therapies (both pharmacologic and non-pharmacologic) have limited efficacy in improving exertional breathlessness and exercise tolerance, particularly in adults with GOLD stage III and IV COPD (1, 3). Therefore, the overall aim of this thesis was to evaluate novel and/or poorly studied adjunct therapies for the management of exertional breathlessness and exercise intolerance in adults with COPD.

As discussed in **Chapter 2**, the neurophysiological underpinnings of exertional breathlessness in COPD are multifactorial and include: [1] respiratory mechanical abnormalities; [2] dynamic diaphragmatic neuromuscular inefficiency; [3] altered central neural processing of breathlessness; [4] exaggerated neural respiratory drive and central corollary discharge; and [5] neuromechanical uncoupling of the respiratory system (**Figure 3.1**). Similarly, the mechanisms mediating exercise intolerance in adults with COPD are complex but are often (although not always) attributable to intolerable breathlessness and its pathophysiological underpinnings. Therefore, it may be possible to improve exercise tolerance by alleviating exertional breathlessness during submaximal exercise in adults with COPD. For this reason, the pharmacological and non-pharmacological therapies selected in this thesis were intended to alter the neurophysiological processes contributing to the symptom of breathlessness, and not exercise intolerance, per se (i.e., an intervention targeted to improve cardiac function or peripheral muscle function were not selected). Specifically, the pharmacological and non-pharmacological therapies assessed in this thesis were designed to: [1]

enhance neuromuscular efficiency of the diaphragm; [2] alter central neural processing of breathlessness; [3] decrease neural respiratory drive and corollary discharge; and [4] improve neuromechanical uncoupling of the respiratory system via improved respiratory mechanics (**Figure 3.1**). It was postulated that exertional breathlessness, and by extension, exercise endurance would improve if any one, or combination, of these aforementioned neurophysiological mediators of breathlessness were enhanced following administration of the selected pharmacological or non-pharmacological therapy.

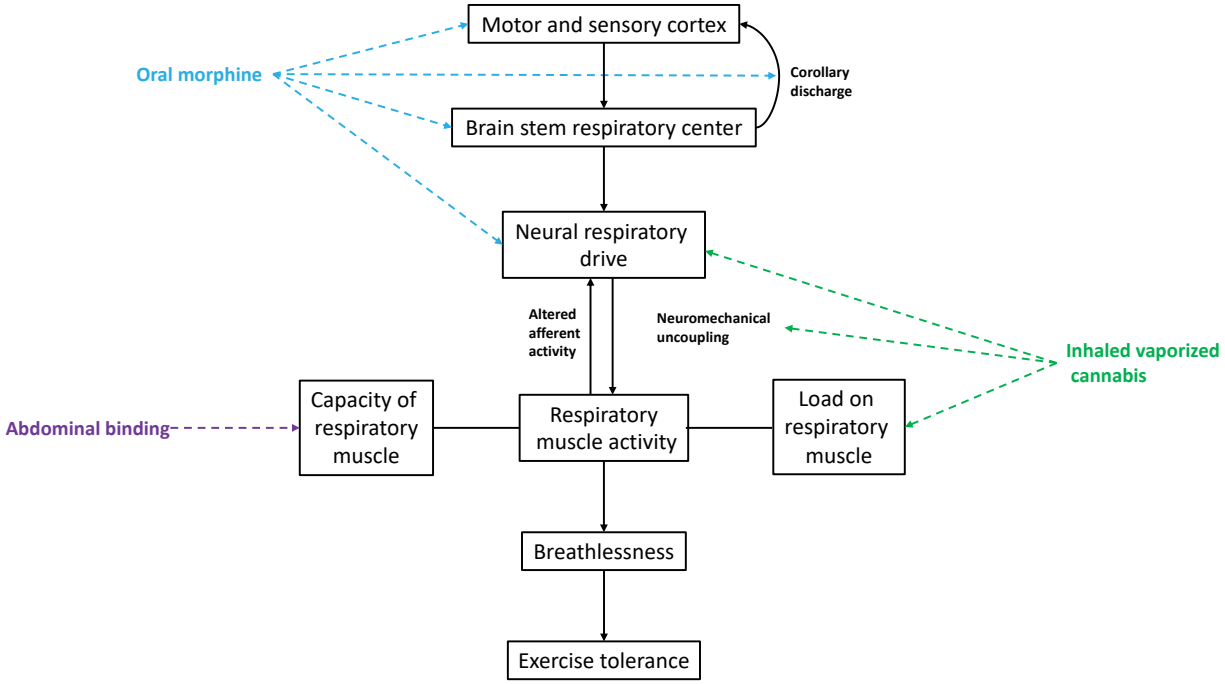

Figure 3.1. Simplified neurophysiological construct of breathlessness. An increased load on the respiratory muscle (i.e., hyperinflation) and/or a decreased capacity of the respiratory muscle increases the level of neural respiratory drive required to activate the respiratory muscle. **Abdominal binding** may decrease breathlessness and improve exercise tolerance by augmenting the capacity of the respiratory muscle (i.e., increasing transdiaphragmatic pressure for a given neural respiratory drive). **Oral morphine** may decrease breathlessness and improve exercise tolerance by altering: [1] central neural processing (i.e., motor and sensory cortex); [2] decreasing brain stem respiratory control center activity and therefore [3] neural respiratory drive and [4] central corollary discharge. **Inhaled vaporized cannabis** may decrease breathlessness and improve exercise capacity by: [1] decreasing the load on the respiratory system, therefore [2] reducing neural respiratory drive, [3] decreasing central corollary discharge and [4] improving neuromechanical coupling of the respiratory system. Modified from (37) with permission.

3.2 OBJECTIVES AND HYPOTHESES

The overall objective of this thesis was to evaluate the efficacy and physiological mechanisms of action of various pharmacological and non-pharmacological therapies in alleviating exertional breathlessness and improving exercise tolerance in adults with COPD. Specifically, this thesis had four research objectives, each of which are highlighted below and are thoroughly addressed in manuscripts 1-4.

Research objective 1 (manuscript 1): Targeting neuromuscular efficiency of the diaphragm – Abdominal binding for the management of exertional breathlessness and exercise intolerance in healthy adults.

The **overall objective** of this randomized crossover trial was to evaluate the acute effect of abdominal binding (AB) on neuromuscular coupling of the diaphragm, exertional breathlessness and exercise capacity in healthy men. This trial served as a physiological "proof of principle" study to ascertain the effects of AB in healthy subjects before examining the effect of AB on exertional breathlessness and exercise endurance in adults with COPD. The **specific objectives** were to evaluate the effect of AB vs. control on: [1] neuromuscular efficiency of the diaphragm and [2] cardiac, metabolic, ventilatory, breathing pattern, dynamic operating lung volume, inspiratory neural drive (EMGdi), respiratory pressure and breathlessness responses during high-intensity constant-load cycle exercise testing in healthy men. We **hypothesized** that AB would reduce exertional breathlessness and improve exercise endurance by improving neuromuscular efficiency of the diaphragm in healthy men (**Figure 3.1**).

Research objective 2 (manuscript 2): Targeting neuromuscular efficiency of the diaphragm – Abdominal binding for the management of exertional breathlessness and exercise intolerance in adults with COPD.

The **overall objective** of this randomized crossover trial was to evaluate the acute effect of AB on neuromuscular efficiency of the diaphragm during exercise, exertional breathlessness and exercise capacity in adults with COPD. The **specific objective** was to evaluate the effect of AB vs. control on cardiac, metabolic, ventilatory, breathing pattern, dynamic operating lung volume, inspiratory neural drive (EMGdi), respiratory pressure and breathlessness responses during high-intensity constant-load cycle exercise testing. We **hypothesized** that AB would reduce exertional breathlessness and improve exercise tolerance by enhancing neuromuscular efficiency of the diaphragm in adults with COPD (**Figure 3.1**).

Research objective 3 (manuscript 3): Targeting central neural processing of breathlessness, neural respiratory drive and central corollary discharge – Oral morphine for the relief of exertional breathlessness and improved exercise endurance in adults with COPD and chronic breathlessness syndrome.

The **overall objective** of this randomized crossover trial was to evaluate the acute effects of immediate-release oral morphine on exertional breathlessness and exercise endurance in adults with advanced COPD and chronic breathlessness syndrome. The **specific objectives** were to evaluate the effect of single-dose administration of immediate-release oral morphine vs. placebo on: [1] CO2 retention; [2] opioid-related side effects; [3] pharmacokinetics of morphine; and [4] cardiac, metabolic, ventilatory, breathing pattern, dynamic operating lung volume, inspiratory neural drive (EMGdi), respiratory pressure and breathlessness responses during high-intensity constant-load cycle exercise testing. We **hypothesized** that oral morphine vs. placebo would be associated with clinically meaningful improvements in exertional breathlessness and exercise

endurance, independent of opioid-related side effects, CO2 retention, and concurrent improvements in the physiological response to exercise. Specifically, we **hypothesized** that, in the absence of changes in the physiological response to exercise, oral morphine would improve exertional breathlessness and exercise endurance by modulating central neural processing of breathlessness, neural respiratory drive and central corollary discharge in adults with advanced COPD and chronic breathlessness syndrome (**Figure 3.1**).

Research objective 4 (manuscript 4): Targeting neuromechanical coupling of the respiratory system – Inhaled vaporized cannabis for relief of exertional breathlessness and improved exercise endurance in adults with COPD.

The **overall objective** of this randomized crossover trial was to evaluate the acute effect of inhaled vaporized cannabis on exertional breathlessness and exercise endurance in symptomatic adults with advanced COPD. The **specific objectives** were to evaluate the effect of single-dose administration of inhaled vaporized cannabis vs. control on: [1] static and dynamic lung function; [2] cannabis-related side effects; [3] pharmacokinetics of inhaled vaporized cannabis; and [4] cardiac, metabolic, ventilatory, breathing pattern, dynamic operating lung volume and breathlessness responses during high-intensity constant-load cycle exercise testing. We **hypothesized** that inhaled vaporized cannabis vs. control would alleviate exertional breathlessness and improve exercise endurance by enhancing static and dynamic airway function (**Figure 3.1**).

CHAPTER 4: MANUSCRIPT 1 "ABDOMINAL BINDING IMPROVES NEUROMUSCULAR EFFICIENCY OF THE HUMAN DIAPHRAGM DURING EXERCISE"

PREFACE TO MANUSCRIPT 1: Targeting neuromuscular efficiency of the Diaphragm – Abdominal binding for the management of exertional breathlessness and exercise endurance in healthy adults.

As discussed in **Chapter 2**, dynamic lung hyperinflation reduces functional capacity of the diaphragm with a concurrent increase in neural respiratory drive (i.e., neuromuscular inefficiency) during exercise in adults with COPD. To this end, any intervention capable of improving functional capacity of the diaphragm (i.e., increasing Pdi) may enhance diaphragmatic neuromuscular efficiency (i.e., increase the Pdi-to-EMGdi ratio), with concomitant improvements in exertional breathlessness and exercise endurance.

Previous studies have demonstrated that AB, by increasing Pdi, may enhance neuromuscular efficiency of the diaphragm at rest and during exercise in health (333), spinal cord injury (334-336) and pulmonary disease (11). However, the effects of AB on exertional breathlessness remain equivocal. Therefore, we sought to evaluate the effect of AB on diaphragmatic neuromuscular efficiency, exertional breathlessness and exercise endurance in healthy young men. This physiological "proof of principle" study served to [1] determine the viability of increasing diaphragmatic neuromuscular efficiency with AB and [2] evaluate the effect of AB on exertional breathlessness and exercise endurance in healthy young men. If successful in achieving one or

more of our outcomes, then a follow-up study would be carried out to evaluate the physiological and perceptual effects of AB in adults with COPD.

Manuscript

A published version of this manuscript has been included in **Appendix I**. For this thesis, [1] acronyms have been redefined to meet the *Frontiers in Physiology* guidelines and [2] references have been renumbered and are included in the combined bibliography at the end of the thesis.

TITLE PAGE

Abdominal binding improves neuromuscular efficiency of the human diaphragm during exercise

Running title: Abdominal Binding and Human Diaphragmatic Function

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Keywords. Breathlessness, exercise, abdominal binding, neuromuscular efficiency, diaphragm

ABSTRACT

We tested the hypothesis that elastic binding of the abdomen (AB) would enhance neuromuscular efficiency of the human diaphragm during exercise. Twelve healthy non-obese men aged 24.8 ± 1.7 yrs (mean ± SE) completed a symptom-limited constant-load cycle endurance exercise test at 85% of their peak incremental power output with diaphragmatic electromyography (EMGdi) and respiratory pressure measurements under two randomly assigned conditions: unbound control (CTRL) and AB sufficient to increase end-expiratory gastric pressure (Pga,ee) by 5-8 cmH_2O at rest. By design, AB increased Pga,ee by 6.6 ± 0.6 cmH_2O at rest. Compared to CTRL, AB significantly increased the transdiaphragmatic pressure swing-to-EMGdi ratio by 85-95% during exercise, reflecting enhanced neuromuscular efficiency of the diaphragm. By contrast, AB had no effect on spirometric parameters at rest, exercise endurance time or an effect on cardiac, metabolic, ventilatory, breathing pattern, dynamic operating lung volume and perceptual responses during exercise. In conclusion, AB was associated with isolated and acute improvements in neuromuscular efficiency of the diaphragm during exercise in healthy men. The implications of our results are that AB may be an effective means of enhancing neuromuscular efficiency of the diaphragm in clinical populations with diaphragmatic weakness/dysfunction.

INTRODUCTION

Diaphragm muscle weakness/dysfunction is pervasive in many clinical populations, including chronic obstructive pulmonary disease (COPD), interstitial lung disease, heart failure, neuromuscular disease, critical illness and mechanical ventilation, and spinal cord injury (SCI) (177, 178, 337-346). In these patient populations, diaphragm muscle weakness/dysfunction has been linked to increased breathlessness, impaired exercise tolerance, prolonged and difficult weaning from mechanical ventilation, and adverse health outcomes, including quality of life and death (178). It follows that non-disease specific interventions capable of increasing the pressure generating capacity of the diaphragm may have important clinical and pathophysiological implications. With the exception of inspiratory muscle training (347-352) and the Ca^{2+} sensitizing agent, Levosimendan (353, 354), few generalized interventions exist to improve the force generating capacity of the human diaphragm.

Accumulating evidence from studies in health (335, 355) and SCI (335, 356, 357) suggest that elastic binding of the abdomen (AB) significantly increases maximal voluntary (e.g., sniff) and involuntary (e.g., twitch) pressure generating capacity of the diaphragm, presumably by reducing abdominal wall compliance, improving the operating length of the diaphragm due to its ascent to a more mechanically advantageous (cephalad) end-expiratory position, increasing intra-abdominal pressure, increasing the area of diaphragmatic apposition to the rib cage and/or increasing diaphragm-rib cage insertional forces (358, 359). A series of studies by West, *et al.* (334-336) recently reported that AB sufficient to increase end-expiratory gastric pressure (Pga,ee) by an average of ~8 cmH_2O at rest in athletes with cervical SCI increased transdiaphragmatic twitch pressures by ~40% relative to the unbound control condition. In those studies, AB-induced

improvements in diaphragmatic function were associated with concurrent improvements in static lung volumes and capacities; cardiac output at rest; the behavior of dynamic operating lung volumes during exercise; and selected measures of field-based exercise performance.

To our knowledge, only two studies have examined the impact of AB on exercise physiological responses in healthy adults (333, 360). Vanmeenen*, et al.* (360) examined the effects of decreasing vital capacity by ~30% through the application of an inelastic canvas corset around the abdomen (extending from the xyphoid process to the hips, thus encompassing the lower 5 ribs) on exercise physiological responses in 11 healthy men. In that study, AB impaired ventilatory and cardiovascular responses to exercise with attendant reductions in exercise performance, consistent with the established effects of external thoracic restriction on exercise physiological responses in healthy men (46, 79, 361, 362). A similar study by Hussain*, et al.* (333) found that applying an inelastic corset around the abdomen of 5 healthy men as tightly as possible while interfering minimally with ribcage movements, caused a 'mild' restrictive lung deficit; significantly increased transdiaphragmatic pressure (Pdi) swings during exercise; and had no effect on exercise tolerance or an effect on ventilation ($\dot{V}_E$), breathing pattern and diaphragmatic electromyography (EMGdi) responses to exercise. While the study by Hussain*, et al.* (333) suggested that AB has the potential to enhance neuromuscular efficiency of the human diaphragm during exercise (i.e., increase ratio of Pdi-to-EMGdi), the authors did not [1] control for the degree of abdominal compression applied; [2] account for the possibility that the 'mild' restrictive lung deficit imposed by AB may have offset the potential benefits of enhanced neuromuscular efficiency of the diaphragm on exercise tolerance; and/or [3] examine the simultaneous effect of AB on cardiac, metabolic, dynamic operating lung volume and breathlessness responses to exercise.

The purpose of this study was to examine the effect of AB sufficient to increase Pga,ee by 5-8 cmH_2O at rest on cardiac, metabolic, ventilatory, breathing pattern, dynamic operating lung volume, EMGdi, respiratory pressure and breathlessness responses during high-intensity constant-load cycle endurance exercise testing in healthy men.

MATERIALS AND METHODS

Study design. This was a single-center, controlled, randomized, crossover study wherein eligible men participated in three testing visits over a period ≤2 weeks. Visit 1 included screening of medical history, spirometry and a symptom-limited incremental cycle exercise test to determine peak power output (PPO). Visits 2 and 3 included spirometry and a symptom-limited constant-load cycle endurance exercise test at 85% of PPO with added measurements of EMGdi and respiratory pressures under two randomly assigned conditions: unbound control (CTRL) and AB. Although the conditions could not be blinded to the participants and investigators, the participants were naïve to the expected outcomes of the study. Visits 1-3 were separated by ≥24 hrs and conducted at the same time of day (±1 hr) for each participant. Participants were instructed to avoid alcohol, caffeine, heavy meals and strenuous exercise on each test day. The study was approved by the Institutional Review Board of the Faculty of Medicine at McGill University (A04-M42-12B) in accordance with the *Declaration of Helsinski*. Written informed consent was obtained from all participants prior to study initiation.

Participants. Participants included 12 non-smoking, non-obese men aged 18-40 yrs with normal spirometry (forced expiratory volume in 1-sec (FEV_1) ≥80% predicted (51) and FEV_1-to-forced

vital capacity ratio ≥70%) and no known or suspected cardiovascular, respiratory, metabolic, musculoskeletal, endocrine and/or neuromuscular disorder(s).

Abdominal binding. As described in detail elsewhere (335), a binder made primarily of flexible neoprene (493R Universal Back Support; McDavid Inc., Woodridge, IL, USA) was individually sized and fitted with participants in the upright position and with the binder's upper edge below the costal margin so that it interfered minimally with rib-cage movement. The desired degree of abdominal compression – defined as an increase in Pga,ee of 5-8 cmH_2O during steady-state breathing while seated on a chair at rest prior to exercise – was achieved by tightening Velcro fasteners at the front of the binder. An earlier study by West*, et al.* (335) found that this level of abdominal compression optimized pulmonary function and twitch Pdi responses at rest in healthy adults and among individuals with cervical SCI.

Spirometry. Spirometry was performed using automated equipment (Vmax Encore™, CareFusion, Yorba Linda, CA, USA) according to recommended techniques (363).

Cardiopulmonary exercise testing. Symptom-limited exercise tests were performed on an electronically braked cycle ergometer (VIAsprint 150P; Ergoline, Bitz, Germany) using a cardiopulmonary exercise testing system (Vmax Encore™, CareFusion). Incremental exercise tests consisted of a steady-state resting period of ≥6-min, followed by 25 W increases in power output (starting at 25 W) every 2-min: PPO was defined as the highest power output that the participant was able to sustain for ≥30-sec. Constant-load exercise endurance tests consisted of a steady-state resting period of ≥6 min followed by a step increase in power output to 85% PPO.

Standard cardiopulmonary exercise test parameters were collected breath-by-breath (79, 172), while heart rate (HR), stroke volume (SV) and cardiac output (CO) were assessed using an impedance cardiograph (PhysioFlow®; NewMeDx, Bristol, PA, USA) that provides an acceptable and non-invasive evaluation of CO during symptom-limited cycle exercise testing in both health and disease (364, 365). Inspiratory capacity (IC) maneuvers were performed at rest, within the last 30-sec of every 2-min interval during exercise and at end-exercise (79, 172). Assuming that total lung capacity does not change during exercise with and without AB in normal males (366), changes in IC and inspiratory reserve volume (IRV = IC – tidal volume (V_T)) reflect changes in dynamic end-expiratory and end-inspiratory lung volume, respectively.

Breath-by-breath measures of the root mean square of EMGdi (EMGdi,rms) and of esophageal (Pes), gastric (Pga) and transdiaphragmatic pressure (Pdi = Pga – Pes) were recorded from a gastro-esophageal electrode-balloon catheter (Guangzhou Yinghui Medical Equipment Ltd., Guangzhou, China) and analyzed using published methods (79, 172). Maximum voluntary EMGdi,rms was identified as the largest of all EMGdi,rms values obtained from IC maneuvers performed either at rest or during exercise. Tidal swings in Pes (Pes,tidal), Pga (Pga,tidal) and Pdi (Pdi,tidal) were calculated as the difference between peak tidal inspiratory and peak tidal expiratory Pes, Pga and Pdi, respectively. The ratio of Pdi,tidal-to-EMGdi,rms was used as an index of neuromuscular efficiency of the diaphragm.

Using Borg's 0-10 category ratio scale (367), participants rated the intensity of their breathing overall and the intensity of their leg discomfort at rest, within the last 30-sec of every 2-min interval during exercise and at end-exercise. Breathing overall (hereafter referred to as breathlessness) was

defined as "the global awareness of your breathing," which is consistent with the American Thoracic Society's recommendation that the definition of breathlessness should be neutral with respect to any particular quality of breathing (55). Leg discomfort was defined as the "difficulty associated with pedaling". Participants were asked to verbalize their main reason(s) for stopping exercise; quantify the percentage contribution of breathlessness and leg discomfort to exercise cessation; and identify qualitative phrases that best described their breathlessness at end-exercise (46).

Analysis of exercise end-points. All physiological parameters were averaged in 30-sec intervals at rest and during exercise. These parameters, averaged over the first 30-sec of every 2-min interval during exercise, were linked with IC and symptom measurements collected during the last 30-sec of the same minute. Three main time points were used for the evaluation of measured parameters: [1] *pre-exercise rest*, defined as the average of the last 60-sec of the steady-state period after ≥3-min of breathing on the mouthpiece while seated on the cycle ergometer before the start of exercise; [2] *isotime,* defined as the average of the first 30-sec of the 2nd min of the highest equivalent 2-min interval of constant-load cycle exercise completed by a given participant with and without AB; and [3] *peak exercise*, defined as the average of the last 30-sec of loaded pedaling. Exercise endurance time (EET) was the duration of loaded pedaling.

Statistical analysis. Two-tailed paired *t*-tests were used to examine the effects of AB *vs.* CTRL on spirometric parameters, maximal voluntary EMGdi,rms, and the percentage contribution of breathlessness and leg discomfort to exercise cessation. A two-way repeated measures analysis of variance with Tukey's HSD post-hoc test was used to examine the effect of AB *vs.* CTRL on

physiological and perceptual parameters measured at rest, at standardized submaximal time points during exercise (including isotime) and at peak exercise. All analyses were performed using SigmaStat®, version 3.5 (Systat® Software, San Jose, CA, USA) and statistical significance was set at $p<0.05$. Data are presented as means ± SEM.

RESULTS

Participants, abdominal binding and spirometry. Participants were healthy, young (24.8 ± 1.7 yrs), non-obese (body mass index = 23.1 ± 0.6 $kg \cdot m^{-2}$) and non-smoking men with normal cardiorespiratory fitness: symptom-limited peak rate of O_2 consumption ($\dot{V}O_2$) of 55.1 ± 2.2 $ml \cdot kg \cdot min^{-1}$ or 121 ± 6 % predicted (368); and PPO of 267 ± 18 W or 109 ± 5 % predicted (368). By design, AB increased Pga,ee by 6.6 ± 0.6 cmH_2O above its baseline value during the AB visit, but had no effect on spirometric parameters compared with CTRL (**Table 4.1**).

Physiological and perceptual responses to exercise. The order of experimental conditions was balanced such that 7 of the 12 participants were randomized to exercise with AB at Visit 2. To rule out a potentially confounding order effect on exercise performance, we compared EET between Visits 2 and Visits 3, irrespective of experimental condition and found no statistically significant difference: 9.7 ± 1.0 min *vs.* 9.0 ± 1.2 min, respectively (p=0.290).

Compared to CTRL, AB had no effect on EET or an effect on cardiac, metabolic, perceptual, ventilatory, breathing pattern and/or operating lung volume parameters at rest or during exercise (**Table 4.2**, **Figure 4.1** and **Figure 4.2**).

The relative contributions of breathlessness (AB, 46 ± 8% *vs.* CTRL, 40 ± 7%; p=0.592) and leg discomfort (AB, 54 ± 8% *vs.* CTRL, 60 ± 7%; p=0.592) to exercise cessation were not different under AB *vs.* CTRL conditions. The distribution of reasons for stopping exercise were also similar between-tests: Breathlessness: AB, n=1 *vs.* CTRL, n=1; Leg discomfort: AB, n=0 *vs.* CTRL, n=1; Combination of breathlessness and leg discomfort: AB, n=10 *vs.* CTRL, n=9. The majority of participants self-selected phrases alluding to a heightened sense of *'work/effort of breathing'* to describe their breathlessness at end-exercise under both AB and CTRL conditions; for example, *'My breathing is heavy'* (AB, 100% *vs.* CTRL, 92%) and *'My breathing requires more work'* (AB, 92% *vs.* CTRL, 100%).

Diaphragmatic EMG and respiratory pressures. Maximal voluntary EMGdi,rms was not significantly different under AB *vs.* CTRL conditions: 227 ± 19 μV *vs.* 234 ± 25 μV, respectively (p=0.727). Peak inspiratory Pes values recorded during serial IC maneuvers did not change significantly from rest (AB, -34.2 ± 3.4 cmH_2O; CTRL, -34.5 ± 2.2 cmH_2O) and throughout exercise (e.g., AB at end-exercise, -34.8 ± 2.9 cmH_2O; CTRL at end-exercise, -36.1 ± 2.6 cmH_2O) both within and between conditions. Peak inspiratory Pdi values recorded during serial IC maneuvers (Pdi,IC) performed at rest and throughout exercise were significantly increased by 18.5-22.2 cmH_2O (or 43-53%) under AB *vs.* CTRL conditions; for example, AB, 70.3 ± 6.0 cmH_2O *vs.* CTRL, 48.5 ± 4.9 cmH_2O at rest ($p<0.001$); and AB, 59.2 ± 4.0 cmH_2O *vs.* CTRL, 40.2 ± 2.8 cmH_2O at end-exercise ($p<0.001$).

Compared with CTRL, AB had no effect on either EMGdi,rms (**Figure 4.3A**) or Pes (**Figure 4.3C**) responses during exercise (**Table 4.2**). As expected, peak tidal inspiratory Pga (Pga,inspir) and

peak tidal expiratory Pga (Pga,expir) were consistently higher at rest and during exercise with *vs.* without AB (**Table 4.2**, **Figure 4.3E**). Compared with CTRL, AB increased Pdi,tidal and peak tidal inspiratory Pdi (Pdi,inspir) at rest and during exercise; for example, by +16.5 cmH_2O at rest and by +28.2 cmH_2O during exercise at isotime with *vs.* without AB (**Table 4.2**, **Figure 4.3B**). Furthermore, AB was associated with a marked increase in the magnitude of the exercise-induced rise in Pdi,tidal and Pdi,inspir (**Table 4.2**, **Figure 4.3B**): the respective increases in Pdi,tidal and Pdi,inspir from rest to isotime during exercise were ~315% and ~223% greater with *vs.* without AB. As illustrated in **Figure 4.3D**, Pdi,tidal and Pdi,inspir were much higher at any given EMGdi,rms during exercise with *vs.* without AB, indicating enhanced neuromuscular efficiency of the diaphragm. Indeed, AB increased the Pdi,tidal:EMGdi,rms ratio by an average of 85-95% at each measurement time during exercise (**Table 4.2**, **Figure 4.3F**).

DISCUSSION

The main finding of this study was that AB sufficient to increase intra-abdominal pressure by an average of 6.6 cmH_2O at rest enhanced neuromuscular efficiency of the diaphragm during exercise but had no effect on exercise endurance nor an effect on cardiac, metabolic, ventilatory, breathing pattern, dynamic operating lung volume and perceptual responses to exercise in healthy young men.

In keeping with the results of earlier AB studies in health (333) and SCI (334-336, 357), the increased Pdi,tidal, Pdi,inspir and Pdi,IC responses observed at rest and during exercise with *vs.* without AB were mechanistically linked to increased intra-abdominal pressures (i.e., Pga,ee and Pga,expir). The increased intra-abdominal pressures associated with AB effectively shift the

abdominal contents towards the diaphragm (cephalad), thereby increasing both insertional and appositional forces of the diaphragm on the lower rib cage (358, 369). By shifting the diaphragm cephalad, AB also lengthens diaphragm muscle fibers and optimizes its length-tension relationship (358). As a result, the diaphragm initiates its inspiratory contraction at a longer length, thus generating a greater pressure at any given level of muscle activation, reflecting enhanced diaphragmatic contractility (370). Abdominal binding may further enhance pressure-generating capacity of the diaphragm by improving (reducing) abdominal compliance, thus impeding diaphragmatic descent at the costal fibers during inspiration and minimizing muscle fiber shortening, i.e., maintaining the muscle length on a more favorable region of the length-tension curve (357, 358, 370). Finally, by increasing intra-abdominal pressures and decreasing abdominal compliance, AB may increase the inflationary action of the diaphragm on the lower rib cage by increasing the zone of apposition and improving the diaphragm's ability to lift and expand the lower rib cage (358, 370). The combination of these mechanically advantageous changes to the shape and configuration of the diaphragm are most likely responsible for the 85-95% increase in neuromuscular efficiency of the diaphragm observed during exercise with *vs.* without AB.

Although AB increased diaphragmatic contractility/pressure-generating capacity, it had no demonstrable effect on EMGdi,rms, Pes, $\dot{V}_E$, breathing pattern and dynamic operating lung volume responses to exercise. These findings are similar to those of earlier AB studies by Hussain*, et al.* (333) in health and by West*, et al.* (336) in SCI, and presumably reflect the fact that AB had no untoward effect on expiratory flow generation during exercise (as evidenced by relative preservation of the relationship between exercise-induced increases in peak tidal expiratory Pes and peak expiratory flow) or an effect on exercise-induced increases the rate of CO_2 production,

which is the proximate source of increased ventilatory requirements during exercise. It could be argued that the increased intra-abdominal pressures associated with AB may have hindered descent of the diaphragm into the abdomen at rest and particularly during exercise when ventilatory requirements were ~13-fold higher than at rest. If this was true, then maximal voluntary EMGdi,rms as well as the magnitude of exercise-induced increases in EMGdi,rms should have been consistently higher under AB *vs.* CTRL conditions. However, this is not what we observed in our study nor what Hussain*, et al.* (333) reported in their AB study of 5 healthy men.

In the setting of a relatively preserved EMGdi,rms, $\dot{V}_E$, breathing pattern and dynamic operating lung volume response to exercise with *vs.* without AB, we speculate that the disparate effect of AB on Pdi and Pes responses to exercise reflected "off-loading" of the inspiratory action(s) of the rib cage muscles. In other words, by increasing Pdi,inspir and thus Pdi,tidal responses to exercise, AB effectively decreased the rib cage muscles' relative contribution to any given level of negative intrathoracic pressure development throughout inspiration during exercise. Additional research with simultaneous measures of accessory inspiratory muscle EMG activity is needed to confirm this postulate.

In the absence of changes in EMGdi,rms, $\dot{V}_E$, breathing pattern, expiratory flow generation and dynamic operating lung volume responses to exercise, isolated and acute improvements in neuromuscular efficiency of the diaphragm during exercise with *vs.* without AB had no effect on exercise endurance and/or exertional breathlessness. These findings support the view that, in healthy young adults: [1] respiratory mechanical/muscular factors do not likely contribute to the limits of exercise tolerance; and [2] progressive neuromuscular uncoupling of the diaphragm is not

likely a proximate source of exertional breathlessness. Nevertheless, the results of our study provide a physiological rationale for future examination of AB as a potentially effective non-pharmacological means of improving exercise tolerance in pathophysiological disease states where neuromuscular uncoupling of the diaphragm has been mechanistically linked to a heightened perception of exertional breathlessness, most notably in patients with COPD (178). Interestingly, a case report by Celli*, et al.* (11) found that AB sufficient to increase Pga,ee from 4 to 12 cmH_2O was associated with objective and potentially clinically meaningful improvements in diaphragmatic function, exercise tolerance, and breathlessness in a symptomatic patient with severe COPD and a large midline hernia of the anterior abdominal wall.

The collective results of studies by Vivier*, et al.* (371), Aliverti, *et al*. (372, 373) and Uva*, et al.* (374) suggest that AB, by increasing intra-abdominal pressure and/or the abdominal circulatory pump action of the diaphragm and abdominal muscles, has the potential to improve cardiac function at rest and during exercise by increasing central venous return from the splanchnic venous circulation. In our study, however, AB had no demonstrable effect on impedance cardiograph-derived estimates of CO and SV at rest and during exercise, which is in agreement with West*, et al.* (335) who reported no effect of AB on echocardiography-derived measures of cardiac function at rest (e.g., CO, SV, end-diastolic volume, end-systolic volume, ejection fraction) in 8 healthy adults. We speculate that AB-induced increases in intra-abdominal pressure and/or the abdominal circulatory pump action of the diaphragm and abdominal muscles were of insufficient magnitude(s) to shift large enough quantities of blood from the splanchnic to central venous circulation to enhance cardiac function at rest and during exercise in our participants.

In summary, the increased intra-abdominal pressures associated with AB enhanced neuromuscular efficiency of the diaphragm by 85-95% during high-intensity constant-load cycle endurance exercise testing in healthy men. Additional research is recommended to examine potential benefits of AB on exertional symptoms in clinical populations where diaphragmatic weakness/dysfunction has been implicated as a source of physical activity-related breathlessness and exercise intolerance.

Conflict of interest

None of the authors has a real or perceived conflict of interest to disclose.

Author Contributions

S.J.A., D.S.C., R.G., and D.J. contributed to the conception of the study as well as to data collection, analysis and interpretation. C.T.M. contributed to data collection and analysis. S.J.A. and D.J. wrote the manuscript, with critical input from all other authors. All authors read and approved the final version of the manuscript.

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Table 4.1. Effect of abdominal binding (AB) on spirometric pulmonary function test parameters at rest in healthy men.

Parameter	Control	AB
FVC, L	5.48 ± 0.22	5.35 ± 0.25
FEV_1, L (% predicted)	4.41 ± 0.19 (95 ± 3)	4.27 ± 0.21 (92 ± 4)
FEV_1/FVC, %	81 ± 2	80 ± 2
PEF, $L \cdot sec^{-1}$	10.4 ± 0.5	9.8 ± 0.6
$FEF_{25-75\%}$, $L \cdot sec^{-1}$	4.22 ± 0.33	4.04 ± 0.34

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

Table 4.2. Effect of abdominal binding (AB) on physiological and perceptual responses to constant-load cycle endurance exercise testing at 85% of symptom-limited peak incremental power output (equivalent to 227 ± 17 W) in healthy men.

	REST		ISO-TIME		PEAK	
Parameter	**Control**	**AB**	**Control**	**AB**	**Control**	**AB**
Exercise time, min	0 ± 0	0 ± 0	7.5 ± 0.8	7.5 ± 0.8	9.9 ± 1.0	8.9 ± 1.1
Breathlessness, Borg 0-10 units	0.2 ± 0.2	0.3 ± 0.2	6.1 ± 0.6	6.8 ± 0.6	8.1 ± 0.7	8.3 ± 0.7
Leg Discomfort, Borg 0-10 units	0.0 ± 0.0	0.0 ± 0.0	6.9 ± 0.7	7.3 ± 0.6	8.1 ± 0.7	8.3 ± 0.5
$\dot{V}O_2$, ml·kg·min^{-1}	5.6 ± 0.2	5.1 ± 0.5	51.6 ± 2.3	51.5 ± 2.5	53.6 ± 2.1	52.4 ± 2.5
$\dot{V}CO_2$, ml·kg·min^{-1}	4.4 ± 0.2	4.7 ± 1.0	53.3 ± 1.9	53.8 ± 2.2	53.9 ± 1.7	53.9 ± 2.1
CO, L·min^{-1}	5.9 ± 0.3	5.5 ± 0.4	20.3 ± 1.0	20.6 ± 1.0	21.3 ± 1.1	21.3 ± 1.0
HR, beats·min^{-1}	78.4 ± 3.2	76.3 ± 3.2	172.7 ± 3.4	174.1 ± 3.3	182.6 ± 2.3	178.7 ± 2.9
SV, ml	75.6 ± 4.3	70.9 ± 3.8	117.9 ± 6.4	118.5 ± 6.1	116.6 ± 6.5	119.8 ± 6.0
$\dot{V}_E$, L·min^{-1}	12.5 ± 0.9	11.2 ± 0.9	116.4 ± 7.3	120.7 ± 7.7	133.4 ± 8.0	130.6 ± 8.6
V_T, L	0.90 ± 0.09	0.74 ± 0.06	2.96 ± 0.17	2.90 ± 0.21	2.72 ± 0.17	2.73 ± 0.19
f_R, breaths·min^{-1}	15.2 ± 1.1	15.7 ± 0.8	39.9 ± 2.0	42.8 ± 2.4	50.4 ± 3.6	49.8 ± 4.0
IC, L	3.38 ± 0.17	3.62 ± 0.16	3.84 ± 0.22	3.82 ± 0.20	3.68 ± 0.19	3.81 ± 0.23
IRV, L	2.48 ± 0.19	2.87 ± 0.15	0.89 ± 0.14	0.92 ± 0.18	0.96 ± 0.14	1.08 ± 0.17
EMGdi,rms, μV	22.5 ± 1.9	27.4 ± 3.7	129.2 ± 13.3	120.0 ± 11.8	150.7 ± 28.1	123.2 ± 14.7
EMGdi%max	10.4 ± 1.1	13.1 ± 2.1	56.0 ± 2.8	53.0 ± 2.9	61.9 ± 5.2	53.3 ± 3.4
End-expiratory Pes, cmH_2O	-7.3 ± 0.7	-5.0 ± 0.7	-5.4 ± 1.1	-4.3 ± 1.2	-6.0 ± 1.1	-5.3 ± 0.9
Pes,tidal, cmH_2O	6.1 ± 0.9	4.8 ± 0.6	31.6 ± 2.9	31.2 ± 2.6	35.0 ± 3.0	34.5 ± 2.8
Peak inspiratory Pes, cmH_2O	-11.7 ± 1.8	-8.4 ± 0.8	-23.6 ± 1.9	-21.5 ± 1.7	-24.3 ± 1.9	-22.0 ± 1.9
Peak expiratory Pes, cmH_2O	-5.6 ± 0.7	-3.5 ± 0.8	8.1 ± 2.3	9.8 ± 1.5	10.8 ± 2.2	12.5 ± 1.7
End-expiratory Pga, cmH_2O	7.7 ± 1.2	12.3 ± 1.2*	14.3 ± 1.4	17.8 ± 0.9	14.4 ± 1.3	19.1 ± 1.1*
Pga,tidal, cmH_2O	5.2 ± 0.5	9.0 ± 0.8	17.3 ± 1.7	18.0 ± 1.3	19.2 ± 1.6	17.1 ± 1.1
Peak inspiratory Pga, cmH_2O	6.5 ± 1.3	11.7 ± 1.2†	4.3 ± 1.2	14.0 ± 1.0†	4.3 ± 1.1	14.8 ± 0.9†
Peak expiratory Pga, cmH_2O	11.7 ± 1.6	20.7 ± 1.5*	21.6 ± 1.9	32.0 ± 1.7†	23.5 ± 1.9	31.9 ± 1.6*
End-expiratory Pdi, cmH_2O	15.1 ± 0.9	17.3 ± 0.9	19.6 ± 1.3	22.0 ± 1.2	20.4 ± 1.2	24.5 ± 1.3
Pdi,tidal, cmH_2O	9.6 ± 0.9	12.8 ± 1.0	20.4 ± 1.5	36.9 ± 2.3†	21.4 ± 1.5	35.8 ± 2.1†
Peak inspiratory Pdi, cmH_2O	22.9 ± 1.2	28.8 ± 1.6	32.0 ± 1.8	50.2 ± 2.6†	32.4 ± 1.6	49.5 ± 2.5†
Peak expiratory Pdi, cmH_2O	13.4 ± 1.2	16.0 ± 1.1	11.5 ± 1.1	13.4 ± 1.1	11.0 ± 1.2	13.7 ± 1.1
Pdi,tidal:EMGdi,rms, cmH_2O·μV^{-1}	0.44 ± 0.04	0.54 ± 0.06	0.17 ± 0.0.01	0.33 ± 0.03†	0.17 ± 0.01	0.33 ± 0.03†

Values are means ± SEM. $\dot{V}O_2$ and $\dot{V}CO_2$, rate of oxygen consumption and carbon dioxide output, respectively; CO, cardiac output; HR, heart rate; SV, stroke volume; $\dot{V}_E$, minute ventilation, V_T, tidal volume; f_R, respiratory frequency; IC, inspiratory capacity; IRV, inspiratory reserve volume; EMGdi,rms, root mean square of the crural diaphragm electromyogram; EMGdi%max, root mean square of the crural diaphragm electromyogram expressed as a percentage of the maximal voluntary room mean square of the crural diaphragm electromyogram; Pes, Pga and Pdi, esophageal, gastric and transdiaphragmatic pressure, respectively; Pes,tidal, tidal esophageal pressure swing; Pga,tidal, tidal gastric pressure swing; Pdi,tidal, tidal transdiaphragmatic pressure swing; Pdi,tidal:EMGdi,rms, tidal transdiaphragmatic swing-to-root mean square of the diaphragmatic electromyogram ratio – an index of neuromuscular efficiency of the diaphragm. *$p<0.05$ and $^{\dagger}p\leq0.01$ *vs.* Control.

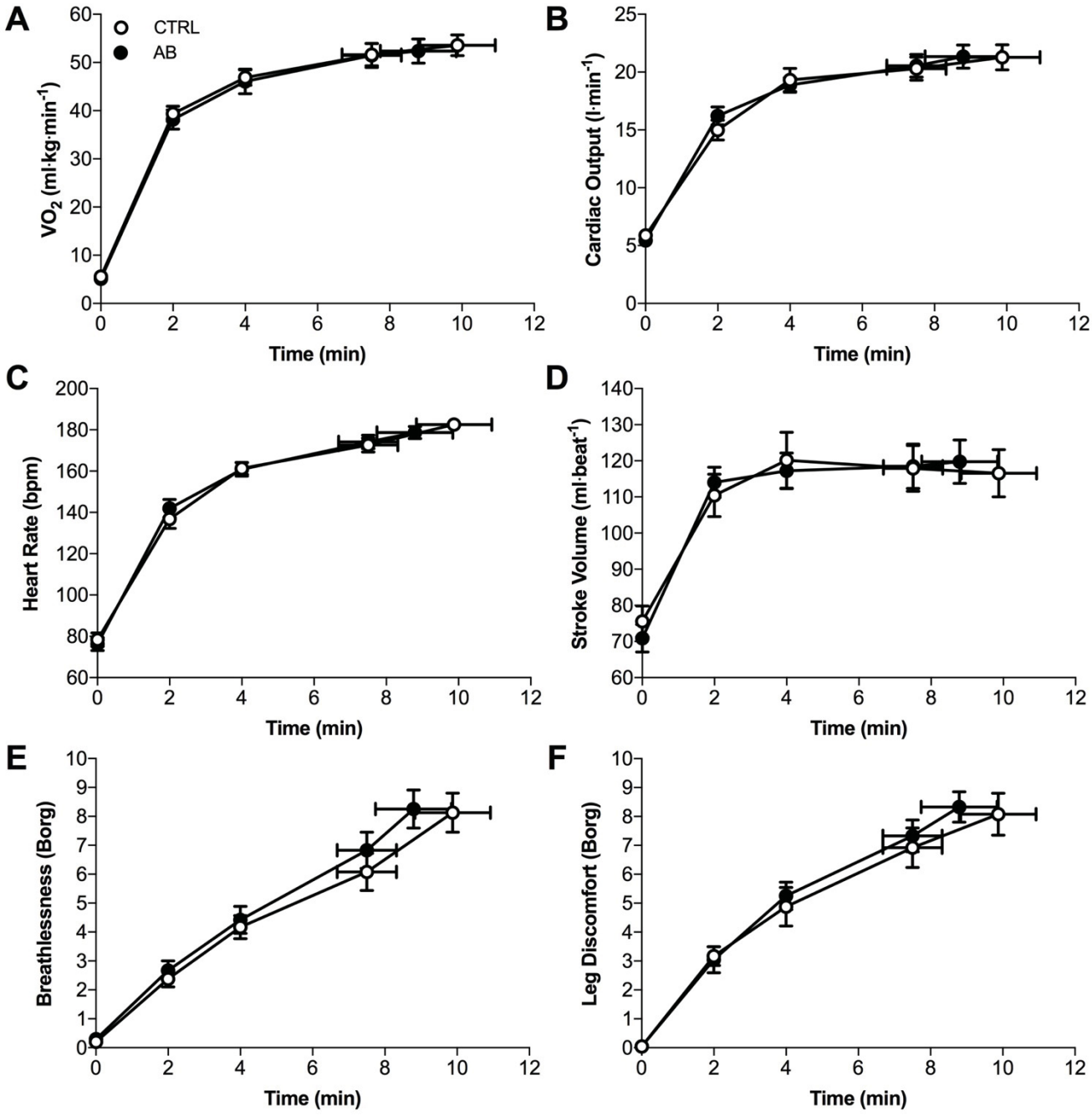

Figure 4.1. Effect of abdominal binding (AB) *vs.* control (CTRL) on (**A**) the rate of oxygen consumption (VO2), (**B**) cardiac output (**C**) heart rate, (**D**) stroke volume, (**E**) breathlessness and (**F**) leg discomfort responses during constant-load cycle endurance exercise test. Values are mean ± SEM.

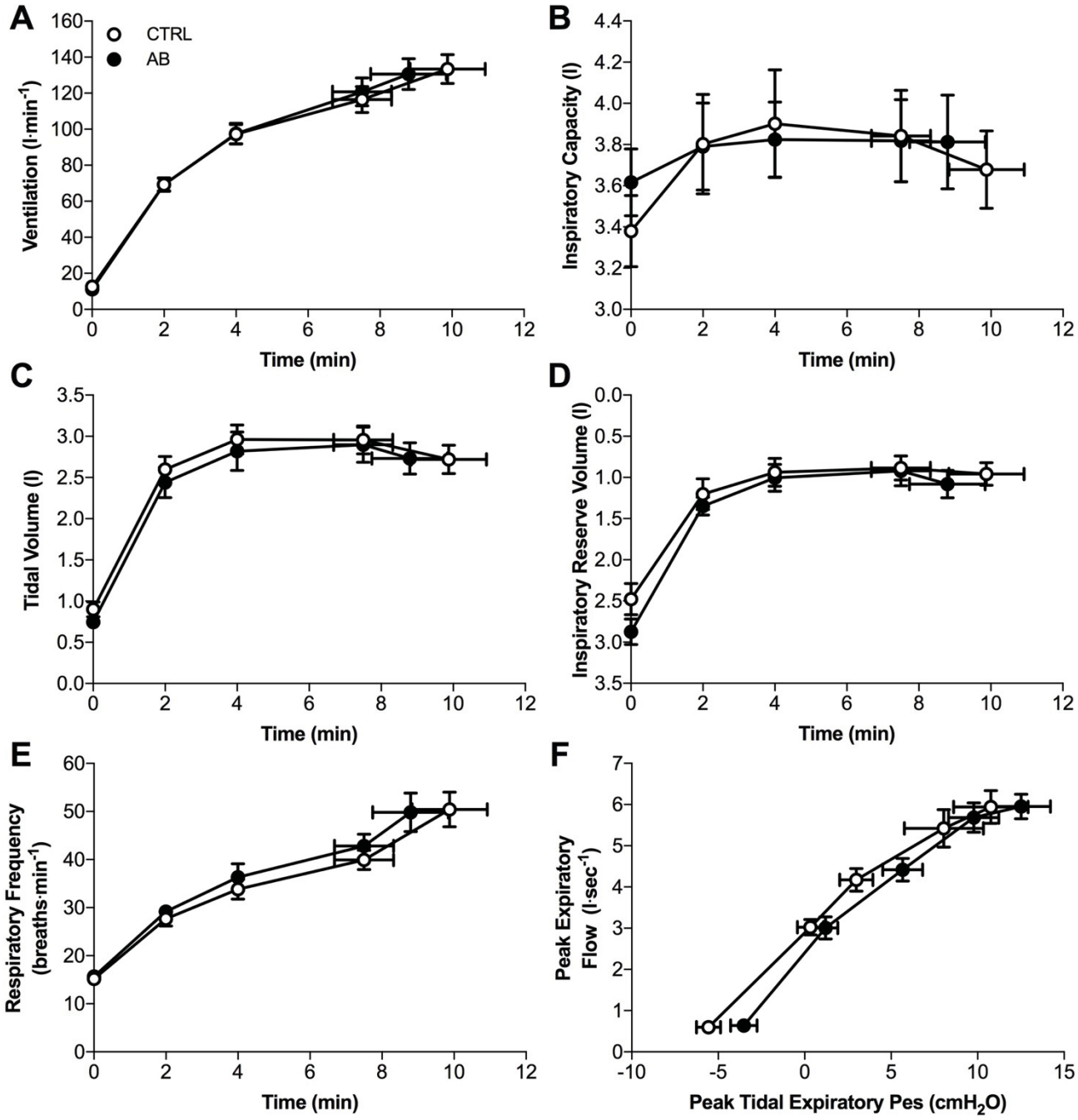

Figure 4.2. Effect of abdominal binding (AB) *vs.* control (CTRL) on (**A**) ventilation, (**B**) inspiratory capacity, (**C**) tidal volume, (**D**) inspiratory reserve volume, (**F**) respiratory frequency and (**G**) peak expiratory flow *vs.* peak tidal expiratory esophageal pressure (Pes) responses during constant-load cycle endurance exercise testing at 85% of peak incremental power output in healthy men. Values are means ± SEM.

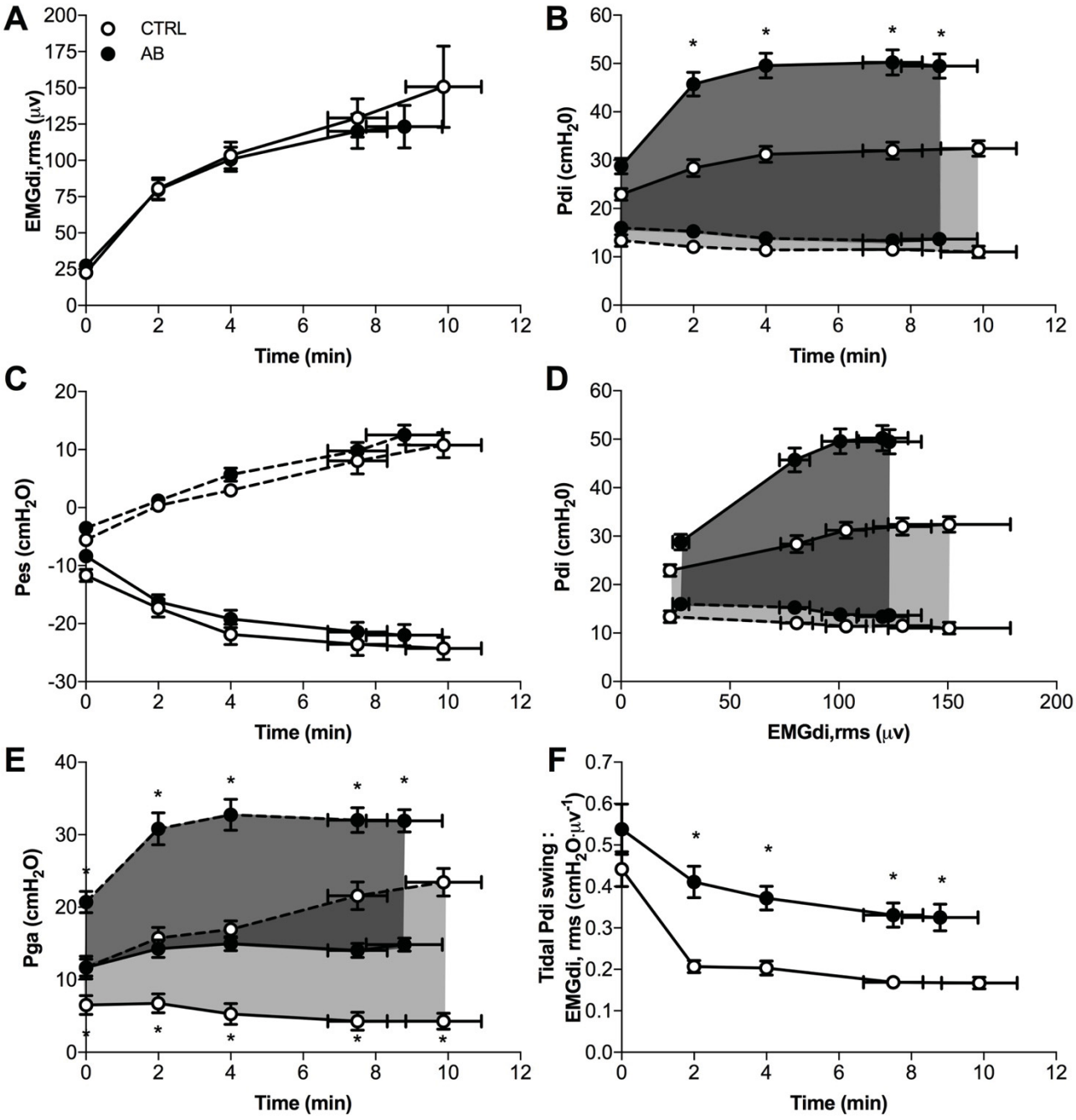

Figure 4.3. Effect of abdominal binding (AB) *vs.* control (CTRL) on (**A**) root mean square of the crural diaphragm electromyogram (EMGdi,rms), (**B**) transdiaphragmatic pressure (Pdi), (**C**) esophageal pressure (Pes), (**D**) Pdi *vs.* EMGdi,rms, (**E**) gastric pressure (Pga) and (F) tidal Pdi swing-to-EMGdi,rms ratio responses during constant-load cycle endurance exercise testing at 85% of peak incremental power output in healthy men. Dashed lines in panels B, D and E denote peak tidal expiratory Pdi, Pdi/EMGdi and Pga, respectively. Values are means ± SEM. *$p<0.05$ *vs.* CTRL.

CHAPTER 5: MANUCRIPT 2 "EFFECT OF ABDOMINAL BINDING ON DIAPHRAGMATIC NEUROMUSCULAR EFFICIENCY, EXERTIONAL BREATHLESSNESS AND EXERCISE ENDURANCE IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE"

PREFACE TO MANUSCRIPT 2: Targeting neuromuscular efficiency of the diaphragm – Abdominal binding for the management of exertional breathlessness and exercise intolerance in adults with COPD.

In **Chapter 4 (Study 1)**, we demonstrated that AB sufficient to increase Pga,ee by an average of 6.6 cmH_2O at rest significantly increased neuromuscular efficiency (defined as the Pdi,tidal-to-EMGdi,rms ratio) of the diaphragm by 85-95% during exercise in healthy young men (5). Despite enhanced neuromuscular efficiency of the diaphragm, AB had no effect on exertional breathlessness and exercise endurance in the population studied, likely because neuromuscular inefficiency of the diaphragm is not the proximate cause of breathlessness and physical activity-limitation in healthy young men. Nevertheless, the results of **Study 1** provided a physiological rationale to examine the efficacy of AB in reducing exertional breathlessness and improving exercise endurance in adults with COPD, where neuromuscular inefficiency of the diaphragm has been mechanistically linked to a heightened perception of exertional breathlessness and impaired exercise tolerance (11-13). To this end, we carried out a randomized clinical trial to evaluate the effect of AB on neuromuscular efficiency of the diaphragm, exertional breathlessness and exercise endurance in adults with COPD.

Manuscript

A published version of this manuscript has been included in **Appendix II**. For this thesis, [1] acronyms have been redefined to meet the *Frontiers in Physiology* guidelines and [2] references have been renumbered and are included in the combined bibliography at the end of the thesis.

TITLE PAGE

Effect of Abdominal Binding on Diaphragmatic Neuromuscular Efficiency, Exertional Breathlessness and Exercise Endurance in Chronic Obstructive Pulmonary Disease

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ABSTRACT

We tested the hypothesis that abdominal binding (AB) would reduce breathlessness and improve exercise tolerance by enhancing neuromuscular efficiency of the diaphragm during exercise in adults with chronic obstructive pulmonary disease (COPD). In a randomized, controlled, crossover trial, 20 adults with COPD (mean±SD FEV_1, 60±16%predicted) completed a symptom-limited constant-load cycle endurance exercise test at 75% of their peak incremental power output with concomitant measures of diaphragmatic electromyogram (EMGdi) and respiratory pressures without (CTRL) *vs.* with AB sufficient to increase end-expiratory gastric pressure (Pga,ee) by 6.7±0.3 cmH_2O at rest. Compared to CTRL, AB enhanced diaphragmatic neuromuscular efficiency during exercise ($p<0.05$), as evidenced by a 25% increase in the quotient of EMGdi to tidal transdiaphragmatic pressure swing. By contrast, AB had no demonstrable effect on exertional breathlessness and exercise tolerance; spirometry and plethysmography-derived pulmonary function test parameters at rest; and cardiac, metabolic, breathing pattern, inspiratory reserve volume and EMGdi responses during exercise (all $p>0.05$ *vs.* CTRL). In conclusion, enhanced neuromuscular efficiency of the diaphragm during exercise with AB was not associated with relief of exertional breathlessness and improved exercise tolerance in adults with COPD.

Keywords: breathlessness, abdominal binding, diaphragm, neuromuscular efficiency, exercise endurance

INTRODUCTION

In people with chronic obstructive pulmonary disease (COPD), lung hyperinflation shortens the length of the diaphragm, thereby compromising its length-tension relationship and area of apposition to the rib cage (176, 178). Collectively, these changes promote diaphragmatic neuromuscular inefficiency in COPD by decreasing diaphragm pressure-generating capacity and provoking high levels of diaphragm electrical activation (EMGdi), particularly during exercise when dynamic lung hyperinflation further shortens and weakens the diaphragm (179, 375). Diaphragmatic neuromuscular inefficiency has been mechanistically linked to abnormally high levels of exertional breathlessness and abnormally low levels of exercise tolerance in COPD (12, 13). It follows that any intervention capable of enhancing diaphragmatic neuromuscular efficiency may decrease exertional breathlessness and improve exercise tolerance in adults with COPD. Indeed, Laghi*, et al.* (12) reported that improvements in diaphragmatic neuromechanical coupling after lung volume reduction surgery (LVRS) in patients with severe emphysema correlated with relief of breathlessness at rest and improved 6-min walking distance.

In 1934, Alexander and Kontz (376) and Gordon (377) reported symptomatic improvement of breathlessness following application of a belt around the abdomen in adults with various pulmonary diseases, including bronchitis, emphysema and asthma. In keeping with these observations, Celli*, et al.* (11) reported that abdominal binding (AB) sufficient to increase end-expiratory gastric pressure (Pga,ee) by 8 cmH_2O increased maximal voluntary transdiaphragmatic pressure-generating capacity by 13 cmH_2O (93%), decreased the perception of breathlessness at rest, and increased exercise tolerance in a symptomatic patient with severe COPD and a large midline hernia of the anterior abdominal wall. Presumably, this improvement in diaphragm

pressure-generating capacity *via* AB reflected the combination of reduced abdominal wall compliance, increased intra-abdominal pressure, improved operating length of the diaphragm due to its ascent to a more mechanically advantageous (cephalad) position, increased area of diaphragm apposition to the rib cage, and increased diaphragm-rib cage insertional forces (358).

Recently, West*, et al.* (335) reported improvements in static lung volumes and capacities following AB in people with cervical spinal cord injury (SCI) as well as in healthy adults. For example, AB decreased functional residual capacity by 0.75 l (23%) in SCI and 0.46 l (14%) in health; increased inspiratory capacity (IC) by 0.47 l in SCI (20%) and 0.33 l (11%) in health; and increased inspiratory reserve volume (IRV) by 0.49 l (29%) in SCI and 0.38 l (16%) in health. A subsequent study by West*, et al.* (336) demonstrated that, compared to the unbound condition, AB shifted tidal breathing to lower and more mechanically advantageous end-expiratory and end-inspiratory lung volumes during submaximal exercise in athletes with cervical SCI.

We recently demonstrated that increasing Pga,ee by 6.6±0.6 cmH_2O (mean±SE) at rest *via* AB markedly improved diaphragmatic neuromuscular efficiency - quantified as the quotient of tidal transdiaphragmatic pressure swing (Pdi,tidal) to the root mean square of the crural diaphragm electromyogram (EMGdi,rms) - by 85-90% during cycle endurance exercise testing in healthy young men (5). Despite this improvement, AB had no effect on exertional breathlessness and exercise endurance, likely because diaphragmatic neuromuscular inefficiency is not the proximate source of exertional breathlessness and exercise limitation in healthy young adults (5). Nevertheless, the collective results of Alexander and Kontz (376), Gordon (377), Celli*, et al.* (11), West, *et al*. (335, 336) and ourselves (5) provide a physiological rationale for the use of AB as a

potentially effective non-pharmacological means of alleviating exertional breathlessness and improving exercise tolerance in adults with COPD by improving dynamic operating lung volumes and/or enhancing diaphragmatic neuromuscular efficiency.

Therefore, the primary aim of this randomized controlled trial was to evaluate the effect of AB on the inter-relationships between diaphragmatic neuromuscular efficiency, exertional breathlessness and exercise endurance in adults with COPD.

MATERIALS AND METHODS

Study design. This single-center, randomized, controlled, crossover trial was conducted at the McGill University Health Centre in Montréal, QC, Canada (Clinicaltrials.gov identifier: NCT01852006). The study protocol and informed consent form received ethics approval from the Research Institute of the McGill University Health Centre (13-075-BMA) in accordance with the *Declaration of Helsinki*.

After providing written and informed consent, participants completed a screening/familiarization visit followed by two intervention visits randomized to order. All visits were separated by ≥48-hrs. *Visit 1* included: medical history and clinical assessment; evaluation of activity-related breathlessness using the modified Medical Research Council dyspnoea scale (378), the Baseline Dyspnea Index (379) and the Oxygen Cost Diagram (380); evaluation of health status using the COPD Assessment Test (381); evaluation of anxiety and depression using the Hospital Anxiety and Depression scale (382); post-bronchodilator (400 μg salbutamol) spirometry; and a symptom-limited incremental cardiopulmonary cycle exercise test (CPET) to determine peak power output

(PPO), defined as the highest power output that the participant was able to sustain for ≥30-sec. During *Visits 2 and 3,* participants first inhaled 400 μg of salbutamol. The gastro-esophageal electrode-balloon catheter used to record EMGdi,rms and respiratory pressures (*see below*) was then inserted and positioned in accordance with established techniques (383). During the AB visit, the abdominal binder was applied and optimally fitted (*see below*). Once the AB was optimally fitted, the gastro-esophageal electrode-balloon catheter was re-positioned to achieve optimal recordings of EMGdi during resting breathing (i.e., positioned such that the amplitude of EMGdi during inspiration was greatest in electrode pairs 1 and 5, and lowest in electrode pair 3) (383). In this way, the recording electrodes were similarly positioned at the diaphragm's electrically active centre under both CTRL and AB conditions. Thereafter, participants completed spirometry and plethysmography followed by a symptom-limited constant-load cycle CPET at 75% PPO. Participants were permitted to use their respiratory medications according to their regular schedule. Participants were randomized in a 1:1 ratio according to a computer-generated block randomization schedule (Block size = 4) prepared by a third-party statistician not involved in the trial.

Participants. Participants were recruited from the Montreal Chest Institute of the McGill University Health Centre, and included men and women aged ≥40 yrs with Global Initiative for Obstructive Lung Disease (GOLD) stage II or III COPD (384), cigarette smoking history ≥15 pack-years, and no change in medication dosage or frequency of administration with no exacerbation(s) and/or hospitalization(s) in the preceding 6-weeks. Exclusion criteria were: presence of medical conditions other than COPD that could contribute to breathlessness and/or

exercise intolerance; use of domiciliary oxygen; exercise-induced oxyhemoglobin desaturation to <80% on room air; and body mass index <18.5 or ≥35.0 kg/m^2.

Intervention. A commercially available binder (493R Universal Back Support; McDavid Inc., Woodridge, IL, USA) that has been described in detail elsewhere (335) was used to bind the abdomen. The binder was fitted with the upper edge below the costal margin so that it interfered minimally with rib-cage movement. The desired degree of abdominal compression – defined as a 5-8 cmH_2O increase in Pga,ee – was achieved by tightening Velcro fasteners at the front of the binder with participants breathing normally while seated at rest. We recently demonstrated that this level of abdominal compression enhanced diaphragmatic neuromuscular efficiency during exercise in healthy young men, as evidenced by an 85-90% increase in the quotient of Pdi,tidal to EMGdi,rms (5). Furthermore, West, *et al.* (335) demonstrated that this level of abdominal compression was associated with significantly greater improvements in diaphragm function than increasing Pga,ee by 1.0-3.5 cmH_2O in healthy adults and people with cervical SCI.

Procedures.

Pulmonary function testing. Spirometry and plethysmography were performed with participants seated using automated equipment (Vmax Encore™ 29C, CareFusion, Yorba Linda, CA, USA; Medisoft body box 5500®, Medisoft Belgium, Sorinnes, Belgium) and according to recommended techniques (363, 385-387). Measurements were referenced to predicted normal values (388-391).

Cardiopulmonary Exercise Testing. Exercise tests were conducted on an electronically braked cycle ergometer (Lode Corival, Lode B.V. Medical Tech., Groningen, The Netherlands) using a

computerized CPET system (Vmax Encore^{TM} 29C). Incremental CPETs consisted of a baseline rest period of ≥6-min, followed by 10 W/min increases in power output to symptom-limitation. Constant-load CPETs consisted of a baseline rest period of ≥6-min, followed by 1-min of unloaded pedaling and then a step increase in power output to 75% PPO maintained to symptom-limitation. Cardiac, metabolic, gas exchange and breathing pattern parameters were collected breath-by-breath and analyzed as previously described (5). Inspiratory capacity maneuvers were performed at rest, every 2-min during CPET, and at end-exercise (392). Measurements of PPO, peak oxygen uptake and peak heart rate were referenced to the predicted normal values of Jones*, et al.* (368).

Published methods were used to analyze breath-by-breath measures of EMGdi,rms and of esophageal (Pes), gastric (Pga) and transdiaphragmatic pressure (Pdi=Pga-Pes) recorded from a gastro-esophageal electrode-balloon catheter (Guangzhou Yinghui Medical Equipment Ltd., Guangzhou, China) (5, 79, 383). Maximum voluntary EMGdi,rms was identified as the largest of all EMGdi,rms values obtained from IC maneuvers performed either at rest or during exercise. Tidal swings in Pes (Pes,tidal), Pga (Pga,tidal) and Pdi (Pdi,tidal) were calculated as the difference between peak tidal inspiratory and peak tidal expiratory Pes, Pga and Pdi, respectively. The quotient of Pdi,tidal to EMGdi,rms was used as an index of diaphragmatic neuromuscular efficiency (5).

Using Borg's modified 0-10 category ratio scale (367), participants rated the intensity and unpleasantness of their breathlessness, as well as the intensity of their leg discomfort at rest, every 2-min during CPET, and at end-exercise. At end-exercise, participants were asked to identify their locus of symptom limitation (breathlessness, leg discomfort, combination of breathlessness and

leg discomfort, other); to quantify the percentage contribution of their selection to exercise cessation; and identify qualitative phrases that best described their breathlessness at end-exercise (46).

Outcomes.

Primary outcomes. The primary outcome was the difference in breathlessness intensity ratings during exercise at isotime under AB *vs.* CTRL conditions, where isotime was defined as the highest equivalent 2-min interval of exercise completed by a given participant during each of the constant-load CPETs. The co-primary outcome was the difference in exercise endurance time (EET) under AB *vs.* CTRL conditions, where EET was defined as the duration of loaded pedaling during constant-load CPET.

Secondary outcomes. Spirometry and plethysmography-derived pulmonary function test parameters; physiological and perceptual parameters measured at rest, at standardized submaximal times during constant-load CPETs, and at peak-exercise (defined as the average of the last 30-sec of loaded pedaling); reasons for stopping exercise; percentage contribution of breathlessness and leg discomfort to exercise cessation; and qualitative descriptors of breathlessness at end-exercise.

Statistical methods. Using a two-tailed paired subject formula with α=0.05, β=0.90 and an expected effect size of 0.80 (393), we estimated that at least 19 participants were needed to detect a minimal clinically important difference of ±1 Borg unit in breathlessness intensity ratings (204) at isotime and of ±101-sec in EET (205) under AB *vs.* CTRL conditions.

Participants who completed both AB and CTRL arms of the trial were included in the analysis. Linear mixed-model regression with random intercepts was used to analyze differences in EET as well as in all physiological and perceptual responses to constant-load CPET under AB and CTRL conditions. Two-tailed paired t-tests were used to compare the effects of AB *vs.* CTRL on: spirometry and plethysmography-derived pulmonary function test parameters; maximal voluntary EMGdi,rms; and the percentage contribution of breathlessness and leg discomfort to exercise cessation. Fisher's exact test was used to compare the effect of AB *vs.* CTRL on the selection frequencies of reasons for stopping exercise as well as the descriptors of breathlessness at end-exercise. Data were analyzed using SAS statistical package, version 9.4 (SAS Institute Inc., Cary, NC, USA) and SigmaStat, version 3.5 (Systat Software Inc., San Jose, CA, USA). Statistical significance was set at $p<0.05$ and values are reported as mean±SEM unless stated otherwise.

RESULTS

Twenty-four participants were randomized into the trial. Four of these participants dropped out during follow-up for non-study related reasons (**Figure 5.1**). Baseline characteristics of the 20 participants (13 men) who completed the trial are presented in **Table 5.1**. By design, AB increased Pga,ee by 6.7±0.3 cmH_2O above its baseline value during the AB visit.

Primary outcomes. Compared to CTRL, AB had no effect on breathlessness intensity ratings at isotime (AB, 3.2±0.4 Borg units *vs.* CTRL, 3.0±0.4 Borg units; p=0.454) or on EET (AB, 6.7±1.1 min *vs.* CTRL, 6.9±1.1 min; p=0.853) (**Figure 5.2**). To assess for a possible confounding order effect on our primary outcomes, breathlessness intensity ratings at isotime and EET were compared between *Visits 2 and 3.* There was no statistically significant effect of visit order on

breathlessness intensity ratings at isotime (*Visit 2*, 3.2±0.5 Borg units *vs. Visit 3*, 3.1±0.4 Borg units; p=0.873) or on EET (*Visit 2*, 7.3±1.3 min *vs. Visit 3*, 6.4±1.0 min; p=0.079).

Secondary outcomes.

Pulmonary function. Compared to CTRL, AB had no effect on spirometry and plethysmography-derived pulmonary function test parameters at rest (**Table 5.2**).

Physiological and perceptual responses to exercise. Except for small and isolated decreases in IC at isotime (AB, 1.96±0.12 l *vs.* CTRL, 2.07±0.13 l; p=0.043) and at peak exercise (AB, 1.86±0.14 l *vs.* CTRL, 1.98±0.14 l; p=0.024), AB had no demonstrable effect on cardiac, metabolic, ventilatory, breathing pattern and IRV parameters at rest or during exercise (**Figure 5.3** and **Figure 5.4**).

Compared to CTRL, AB had no significant effect on maximal voluntary EMGdi,rms (AB, 162±10 μV *vs.* CTRL, 160±10 μV; p=0.737). Peak inspiratory Pes values recorded during serial IC maneuvers did not change significantly from rest (AB, -24.6±2.1 cmH_2O *vs.* CTRL, -24.9±1.5 cmH_2O; p=0.847) and throughout exercise (e.g., AB, -23.1±1.4 cmH_2O *vs.* CTRL, -22.8±1.8 cmH_2O at end-exercise; p=0.816). Peak inspiratory Pdi values recorded during serial IC maneuvers performed at rest and throughout exercise were significantly increased by 4.4-8.3 cmH_2O (10-22%) under AB *vs.* CTRL conditions (e.g., AB, 50.9±2.2 cmH_2O *vs.* CTRL, 46.4±2.5 cmH_2O at rest (p=0.014); and AB, 46.5±2.2 cmH_2O *vs.* CTRL, 38.3±2.2 cmH_2O at end-exercise (p=0.001)).

EMGdi,rms (**Figure 5.5A**) and Pes (**Figure 5.5C**) responses during exercise were not significantly

different under AB *vs.* CTRL conditions. Peak tidal inspiratory Pga and peak tidal expiratory Pga were consistently higher at rest and during exercise with *vs.* without AB (**Figure 5.5E**). Similarly, peak tidal inspiratory Pdi and Pdi,tidal were significantly higher at rest and during exercise under AB *vs.* CTRL conditions (**Figure 5.5B**). Finally, enhanced neuromuscular efficiency of the diaphragm with *vs.* without AB was evidenced by the consistently higher Pdi,tidal at any given EMGdi,rms during exercise (**Figure 5.5D**). Indeed, the quotient of Pdi,tidal to EMGdi,rms increased by an average of ~25% at each measurement time during exercise under AB *vs.* CTRL conditions (**Figure 5.5F**).

Compared to CTRL, AB had no effect on the locus of symptom-limitation (breathlessness: AB, n=7 *vs.* CTR, n=6; Leg discomfort: AB, n=6 *vs.* CTRL, n=6; and combination of breathlessness and leg discomfort: AB, n=7 *vs.* CTRL, n=7). The relative contributions of breathlessness (AB, 44±7% *vs.* CTRL, 47±8%; p=0.731) and leg discomfort (AB, 48±7% *vs.* CTRL, 52±8%; p=0.531) to exercise cessation were not different under AB *vs.* CTRL conditions. Similarly, the selection frequency of breathlessness descriptors at end-exercise was not significantly different in AB *vs.* CTRL (data not shown).

DISCUSSION

The main finding of this randomized controlled trial was that abdominal binding enhanced neuromuscular efficiency of the diaphragm during exercise but had no effect on exertional breathlessness and exercise endurance in adults with COPD.

In keeping with the results of earlier studies in health (5, 333), SCI (335, 336) and COPD (11, 376, 377, 394), AB significantly enhanced pressure-generating capacity of the diaphragm at rest and throughout exercise. Presumably, by increasing intra-abdominal pressure, AB functionally "strengthened" the diaphragm and enhanced its pressure-generating capacity by improving its length-tension relationship, thus enabling the diaphragm to initiate its inspiratory contraction at a more favourable length (358). Furthermore, cephalad displacement of the diaphragm with AB likely increased the area of diaphragmatic apposition to the rib cage with attendant increases in the inflationary action of the diaphragm on the lower rib cage (358). Abdominal binding presumably also minimized caudal shift of the diaphragm by reducing abdominal wall compliance, thus decreasing the velocity of diaphragm shortening (358). Collectively, these mechanically advantageous adaptations are likely responsible for the ~25% improvement in diaphragmatic neuromuscular efficiency during exercise with *vs.* without AB in adults with COPD.

Despite enhanced diaphragmatic neuromuscular efficiency, AB had no effect on exertional breathlessness and EET. This is in contrast to the results of LVRS studies in COPD, wherein enhanced diaphragmatic neuromuscular efficiency correlated with relief of exertional breathlessness and increased exercise capacity (12, 13, 325, 395). Enhanced diaphragmatic neuromuscular efficiency following LVRS is secondary to enhanced respiratory mechanics, as evidenced by reduced static and dynamic lung hyperinflation and improved breathing pattern (12, 13, 325, 395). By increasing the area of diaphragmatic apposition to the rib cage and improving the operating length of the diaphragm due to its cephalad displacement, these improvements in breathing mechanics following LVRS effectively decrease the load on the diaphragm, increase diaphragm pressure-generating capacity, and reduce the level of diaphragm activation needed to

support a given level of ventilation (12, 178, 325, 395). Therefore, in contrast to AB, enhanced diaphragmatic neuromuscular efficiency following LVRS is due to the combination of increased diaphragm pressure-generating capacity and reduced inspiratory neural respiratory drive. Consequently, in the absence of improvements in expiratory flow-generating capacity, static and dynamic breathing mechanics, breathing pattern and EMGdi,rms, isolated and acute improvements in diaphragmatic neuromuscular efficiency during exercise with *vs.* without AB did not translate into relief of exertional breathlessness and/or improved exercise tolerance in our participants with COPD.

Our findings substantiate the mechanistic role of pathophysiological abnormalities in breathing mechanics and inspiratory neural drive (and deemphasize the mechanistic role of diaphragmatic neuromechanical inefficiency) to the aetiology of exertional breathlessness and exercise intolerance in COPD; that is, despite improving pressure-generating capacity and neuromuscular efficiency of the diaphragm, AB had no effect on the inter-relationships between exercise-induced changes in ratings of perceived breathlessness, IRV, breathing pattern and EMGdi,rms. Our findings are consistent with those of Ciavaglia, *et al.* (396) and Faisal, *et al.* (182) who respectively reported that differences in the activity and recruitment of the diaphragm, accessory inspiratory muscles, and expiratory muscles during walking *vs.* cycling in obese adults with COPD and during symptom-limited incremental cycle CPET in adults with COPD *vs.* interstitial lung disease did not influence the relationship between exercise-induced changes in ratings of perceived breathlessness and each of the tidal volume-to-IC ratio (the inverse of IRV), breathing pattern and EMGdi,rms. Collectively, the results add to a growing body of evidence emphasizing the importance of increased inspiratory neural drive in the pathogenesis of exertional breathlessness in COPD (156,

181, 397-399), while simultaneously questioning the role of alterations in the activity of mechanosensitive afferents (i.e., Golgi tendon organs, muscle spindles) emanating from the diaphragm as well as from the chest wall and abdominal muscles in the perception of activity-related breathlessness in COPD.

Compared to CTRL, AB was associated with modest but significant decreases in IC at isotime and peak exercise by ~110 mL, which may have offset the potentially beneficial effects of enhanced diaphragmatic neuromuscular efficiency on exertional breathlessness and EET. However, this is unlikely, particularly in view of the results of Guenette*, et al.* (400) who demonstrated that the perception of breathlessness during symptom-limited constant-load CPET in adults with COPD is associated with progressive mechanical constraints on tidal volume expansion as IRV approaches its minimal value, independent of the behaviour of dynamic IC. In as much as AB did not affect the behaviour of dynamic IRV during exercise, we contend that the small and isolated decreases in IC during exercise with *vs.* without AB were unlikely to offset the potentially beneficial effects of enhanced diaphragmatic neuromuscular efficiency on exertional breathlessness and EET.

Methodological considerations. We evaluated the effects of AB sufficient to increase intra-abdominal pressures by 6.7±0.3 cmH_2O on the inter-relationships between diaphragmatic neuromuscular efficiency, exertional breathlessness and exercise endurance in adults with COPD. While this level of abdominal compression effectively enhanced diaphragmatic neuromuscular efficiency in the present study as well as in our earlier AB study of healthy younger men (5), we cannot rule out the possibility that different degrees of abdominal compression may yield different results on diaphragmatic neuromuscular efficiency, exertional breathlessness and exercise

capacity. While the observed changes in diaphragm pressure-generating capacity for a given level of diaphragm electrical activation with AB are consistent with improved length-tension relationship of the diaphragm due to its ascent to a more mechanically advantageous position, we cannot rule out the possibility that cephalad displacement of the diaphragm with AB increased pressure-generating capacity of the diaphragm by decreasing its radius of curvature, even without a change in force generation. Without radiographic evidence of cephalad displacement of the diaphragm with *vs.* without AB, we can only speculate on the determinants of improved diaphragm pressure-generating capacity and enhanced diaphragmatic neuromuscular efficiency with AB in our participants with COPD. We cannot comment on the effects of AB on cardiac function since measurements of stroke volume and cardiac output were not obtained; however, we have previously demonstrated that AB sufficient to increase intra-abdominal pressures by 6.6±0.6 cmH_2O had no demonstrable effect on stroke volume and cardiac output responses during constant-load CPET in healthy younger men (5). As the experimental conditions of this study could not be blinded to the participants and investigators, we cannot rule out the possibility that participant and/or investigator bias may have influenced our results.

Conclusions. In the absence of improved static and dynamic lung function, expiratory flow-generating capacity, ventilation, breathing pattern, and inspiratory reserve volume, isolated and acute improvements in diaphragmatic neuromuscular efficiency during exercise with abdominal binding were not associated with relief of exertional breathlessness and/or improved exercise endurance in adults with COPD.

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Author contributions

S.A., B.M.S., J.B. and D.J. contributed to the conception of the study and data collection, analysis and interpretation. C.M. contributed to data collection and analysis. P.L. contributed to data analysis. S.A. and D.J. wrote the manuscript with critical input from all authors. All authors read and approved the final version of the manuscript.

Conflict of interest

The authors have no conflicts of interest to report.

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Table 5.1. Baseline participant characteristics (n=20).

Parameter	Value
Male:Female, n	13:7
Age, yrs	69.8 ± 8.7
Height, cm	170.1 ± 9.9
Body mass, kg	78.4 ± 15.5
Body mass index, kg・m^{-2}	27.1 ± 1.1
Smoking history, pack-years	56.1 ± 30.1
Post-bronchodilator spirometry	
FEV_1, L (% predicted)	1.56 ± 0.57 (60±16)
FEV_1/FVC, %	46.3 ± 12.3
$FEF_{25-75\%}$, L・s^{-1} (% predicted)	0.57 ± 0.31(23±11)
PEF, L・s^{-1} (% predicted)	4.55 ± 1.98 (59±18)
Breathlessness and health status	
mMRC score, 0-4	1.8 ± 0.9
BDI focal score, out of 12	6.0 ± 2.0
Oxygen cost diagram, % full scale	51 ± 12
CAT score, out of 40	17.0 ± 7.8
HADS score, out of 42	9.8 ± 4.9

Values are mean±SD. FEV_1, forced expiratory volume in 1-sec; FEV_1/FVC, FEV_1 to forced vital capacity ratio; $FEF_{25-75\%}$, forced expiratory flow between 25 and 75% of the FVC maneuver; PEF, peak expiratory flow; mMRC, modified Medical Research Council dyspnoea scale; BDI, Baseline Dyspnoea Index; CAT, COPD Assessment Test; HADS, Hospital Anxiety and Depression Scale.

Table 5.2. Effect of abdominal binding (AB) on spirometry and plethysmography-derived pulmonary function test parameters in adults with chronic obstructive pulmonary disease (n=20).

Parameter	Control	AB
FEV_1, L	1.53 ± 0.56	1.54 ± 0.64
FEV_1/FVC, %	45.9 ± 12.7	45.1 ± 13.1
$FEF_{25-75\%}$, L・s^{-1}	0.56 ± 0.29	0.52 ± 0.24
PEF, L・s^{-1}	4.23 ± 1.63	4.16 ± 1.57
TLC, L (% predicted)	7.14 ± 1.39 (117±19)	6.89 ± 1.45
RV, L (% predicted)	3.37 ± 0.88 (150±45)	3.35 ± 0.99
FRC, L (% predicted)	4.61 ± 1.12 (140±32)	4.28 ± 1.12
IC, L (% predicted)	2.55 ± 0.70 (89±15)	2.67 ± 0.75
sRaw, cmH_2O・L・s^{-1} (% predicted)	17.1 ± 11.2 (406±261)	20.6 ± 15.5

Values are mean±SD. FEV_1, forced expiratory volume in 1-sec; FEV_1/FVC, FEV_1 to forced vital capacity ratio; $FEF_{25-75\%}$, forced expiratory flow between 25 and 75% of the FVC maneuver; PEF, peak expiratory flow; TLC, total lung capacity; RV, residual volume; FRC, functional residual capacity; IC, inspiratory capacity; sRaw, specific airway resistance.

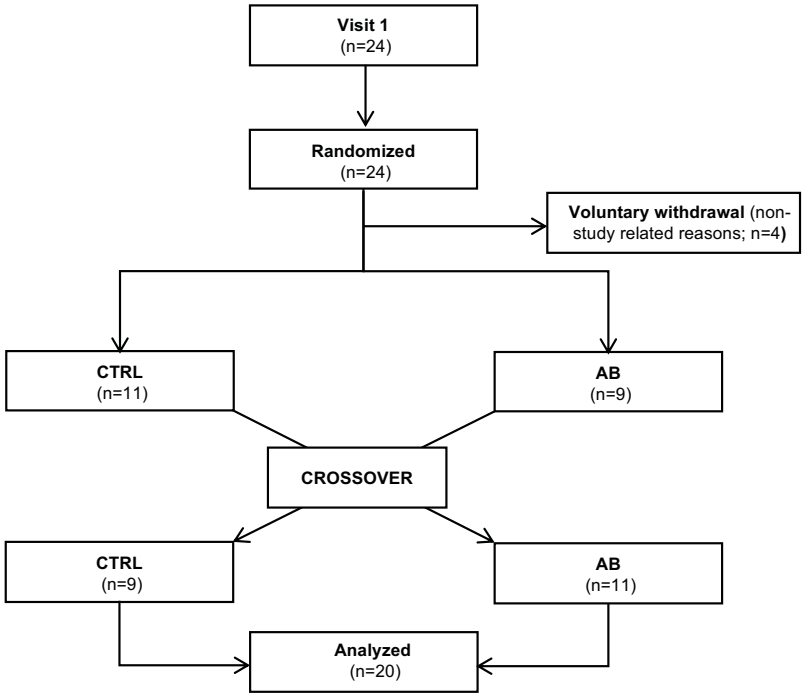

Figure 5.1. Consort diagram of the study population

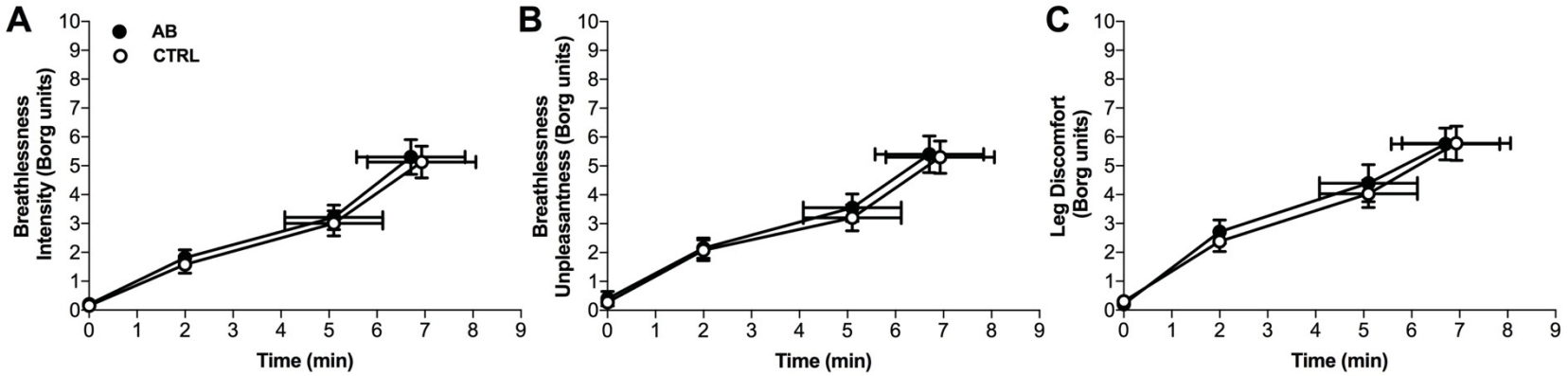

Figure 5.2. Effects of abdominal binding (AB) *vs.* control (CTRL) on (**A**) breathlessness intensity, (**B**) breathlessness unpleasantness and (**C**) leg discomfort during constant-load cycle endurance exercise testing at 75% of peak incremental power output in adults with chronic obstructive pulmonary disease (n=20). Data points are mean±SEM values at rest, at standardized submaximal times during exercise (including isotime of 5.1±1.0 min), and at peak exercise.

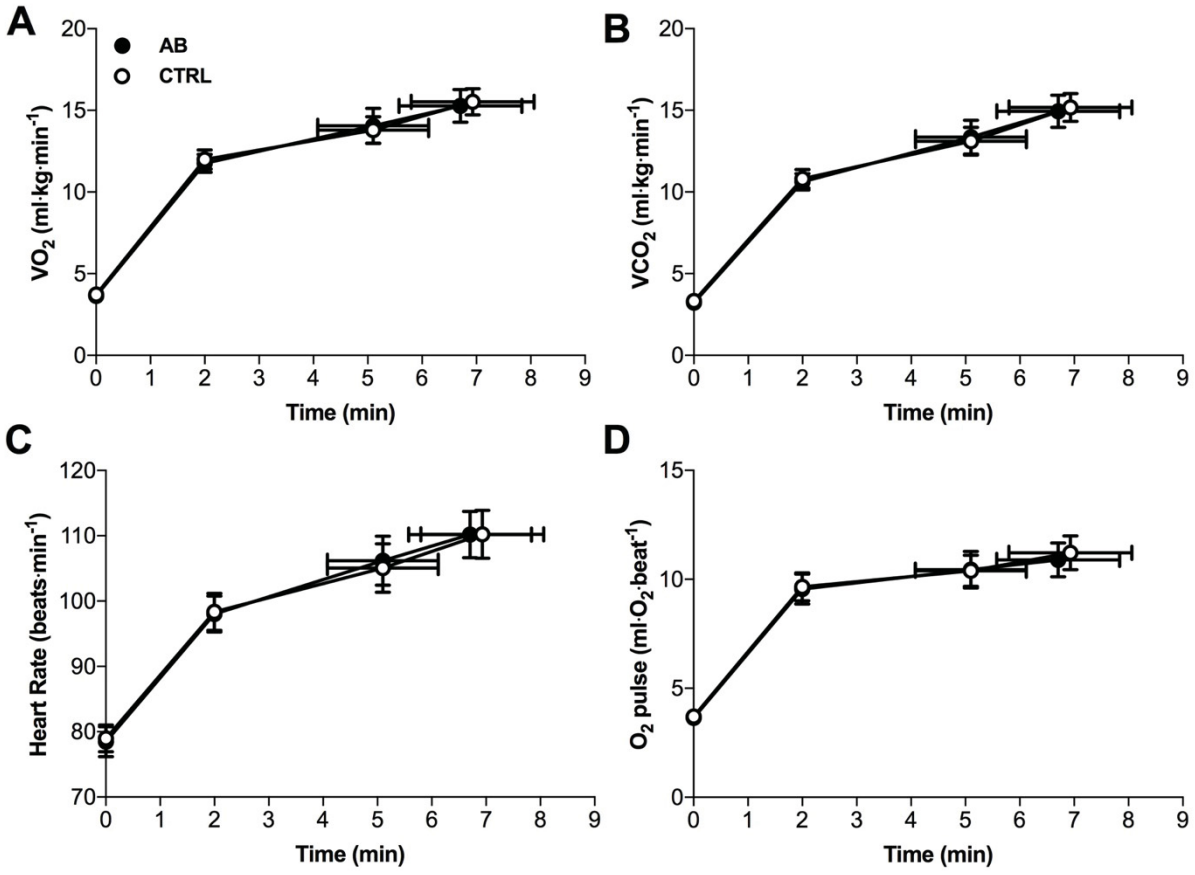

Figure 5.3. Effects of abdominal binding (AB) *vs.* control (CTRL) on (**A**) the rate of oxygen consumption (VO_2), (**B**) the rate of carbon dioxide production (VCO_2), (**C**) heart rate and (**D**) oxygen pulse (O_2pulse) during constant-load cycle endurance exercise testing at 75% of peak incremental power output in adults with chronic obstructive pulmonary disease (n=20). Data points are mean±SEM values at rest, at standardized submaximal times during exercise (including isotime of 5.1±1.0 min), and at peak exercise.

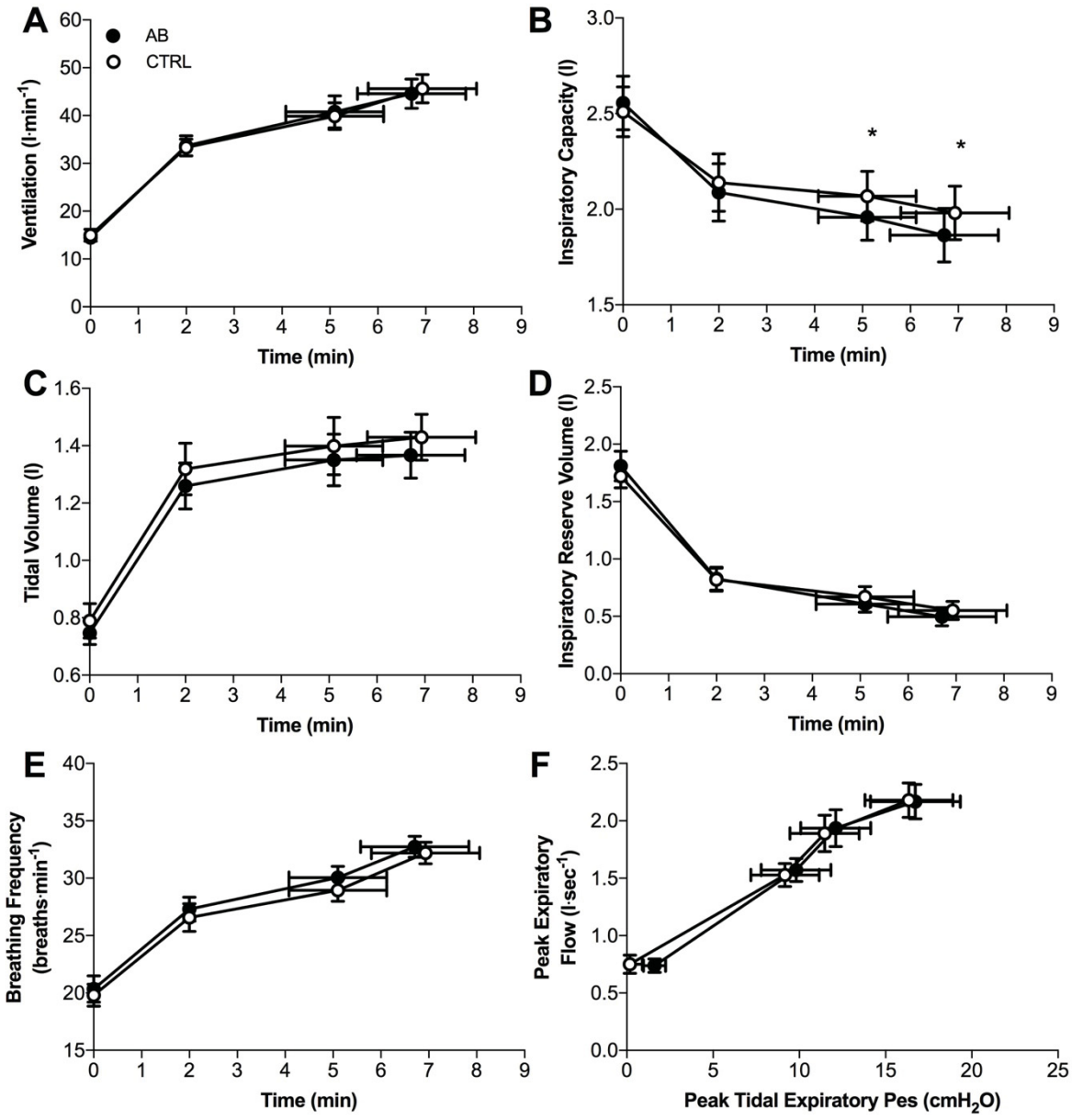

Figure 5.4. Effects of abdominal binding (AB) *vs.* control (CTRL) on (**A**) ventilation, (**B**) inspiratory capacity, (**C**) tidal volume, (**D**) inspiratory reserve volume, (**E**) breathing frequency and (**F**) peak expiratory flow during constant-load cycle endurance exercise testing at 75% of peak incremental power output in adults with chronic obstructive pulmonary disease (n=20). Data points are mean±SEM values at rest, at standardized submaximal times during exercise (including isotime of 5.1±1.0 min), and at peak exercise. *$p<0.05$ *vs.* CTRL.

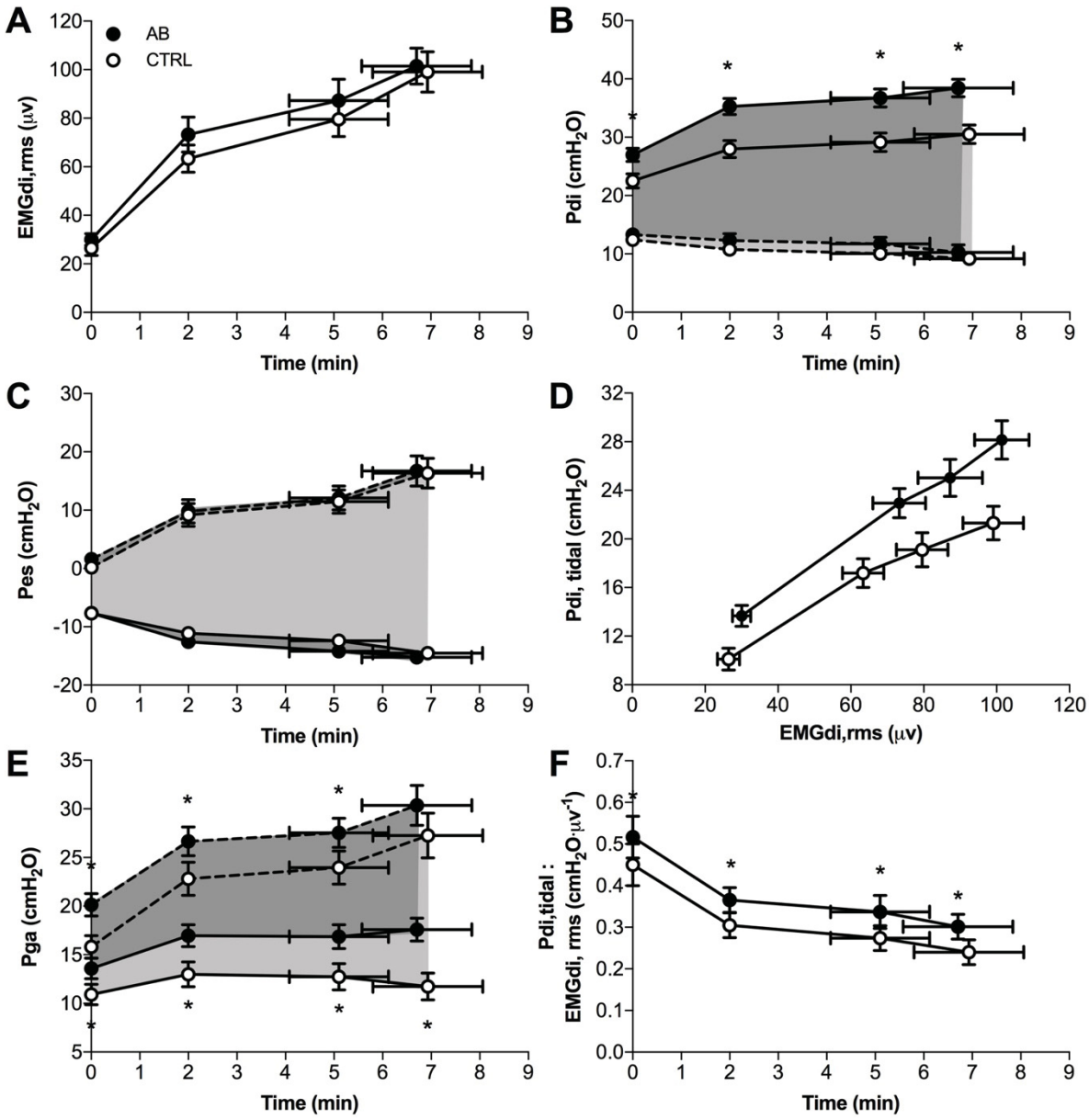

Figure 5.5. Effects of abdominal binding (AB) *vs.* control (CTRL) on (**A**) the root mean square of the crural diaphragm electromyogram (EMGdi,rms), (**B**) transdiaphragmatic pressure (Pdi), (**C**) esophageal pressure (Pes), (**D**) tidal Pdi swing (Pdi,tidal) *vs.* EMGdi,rms, (**E**) gastric pressure (Pga) and (**F**) the quotient of Pdi,tidal to EMGdi,rms (an index of diaphragmatic neuromuscular efficiency) responses during constant-load cycle endurance exercise testing at 75% of peak incremental power output in adults with chronic obstructive pulmonary disease (n=20). Dashed lines in panels B, C and E denote peak tidal expiratory Pdi, Pes and Pga, respectively. Data points are mean±SEM values at rest, at standardized submaximal times during exercise (including isotime of 5.1±1.0 min), and at peak exercise. *p<0.05 *vs.* CTRL.

CHAPTER 6: MANUSCRIPT 3 "EFFECT OF MORPHINE ON BREATHLESSNESS AND EXERCISE ENDURANCE IN ADVANCED COPD: A RANDOMIZED CROSSOVER TRIAL"

PREFACE TO MANUSCRIPT 3: Targeting central neural processing of breathlessness, neural respiratory drive and central corollary discharge – Oral morphine for the relief of exertional breathlessness and improved exercise endurance in adults with COPD and chronic breathlessness syndrome.

The results of **Chapter 5 (Study 2)** demonstrated that, in the absence of changes in ventilation, breathing pattern, neural respiratory drive and dynamic airway function, improved diaphragm function with AB had no effect on breathlessness and exercise endurance in adults with COPD. Therefore, in keeping with the neurophysiological construct of breathlessness presented earlier (see **Figure 2.2** and **Figure 3.1**), other "targets" for breathlessness relief include central neural substrates implicated in the perception of breathlessness. For this reason, as described in **Chapter 3**, immediate-release oral morphine was selected as the pharmacological therapy with the potential to decrease exertional breathlessness and improve exercise endurance by modulating central neural processing of breathlessness and by decreasing neural respiratory drive and corollary discharge.

Opioids

Opioids are considered an appropriate therapy for the management of refractory pain in adults with advanced disease irrespective of the underlying pathophysiology (e.g., cancer and non-cancer pain; neuropathic pain, etc.) (401). With the new classification of chronic refractory breathlessness as a

syndrome (4), there has been a growing interest in understanding the therapeutic potential of opioids, especially morphine, in the symptomatic management of breathlessness in adults with various diagnoses, including COPD.

Pharmacological mechanisms

Morphine and its metabolites, namely morphine-6-glucuronide (M6G), mediate their pharmacodynamic effects by binding to μ-opioid receptors, which are expressed in the central and peripheral nervous system, and some peripheral organs, including the lungs and airways (402, 403). Centrally, μ-opioid receptors are highly expressed in various brain regions implicated in the neural processing of breathlessness, including the: respiratory neural network (i.e., medulla and pons); insula; anterior cingulate cortex; and AMGY (404). Peripherally, μ-opioid receptors are expressed within the tracheobronchial tree and alveoli, where they relay information to the central nervous system *via* vagal afferent bronchopulmonary C-fibers (403). Therefore, both systemic and inhaled opioids have the potential to alleviate breathlessness and improve exercise endurance in COPD (14).

Effect on exertional breathlessness and exercise performance in adults with COPD

The effect of inhaled and systemic opioids (14) on exertional breathlessness and exercise capacity has been evaluated in a small number of clinical trials using various measures of breathlessness and exercise modalities (see **Table 6.1** for an overview of studies evaluating systemic opioids). In a recent systematic review and meta-analysis, Ekstrom*, et al.* (14) reported that, on average, inhaled opioids (low evidence; standardized mean difference of -0.39; 95% CI -0.71 to -0.07) and systemic opioids (moderate evidence; standardized mean difference of -0.34; 95% CI -0.58 to -

0.10) reduce breathlessness but have no effect on exercise capacity (measured as distance on 6MWT) in adults with COPD. Evidence-based clinical practice guidelines support use of systemic opioids to manage refractory breathlessness in selected patients with advanced COPD (23, 54). Nevertheless, systemic opioids are rarely prescribed for relief of breathlessness in COPD (405) largely owing to [1] longstanding and unwarranted fears of respiratory depression and [2] a poor understanding of the mechanisms of action mediating opioid-induced relief of breathlessness.

Mechanistically, relief of breathlessness following administration of systemic opioids has been associated with [1] a reduction in $\dot{V}_E$ during exercise (**Table 6.1**) and [2] a decrease in the activity of the brainstem respiratory neural network and various brain centers implicated in the central neural processing of breathlessness, most notably the anterior cingulate cortex and amygdala (406). Therefore, it is reasonable to postulate that in symptomatic adults with advanced COPD, opioids may reduce exertional breathlessness by [1] decreasing neural respiratory drive and central corollary discharge and/or [2] altering central neural processing of breathlessness (i.e., blunting the activity of brain centers implicated in breathlessness). Furthermore, it is reasonable to suggest, that by reducing breathlessness during sub-maximal exercise and delaying the onset of intolerable breathlessness, opioids may enable adults with COPD to improve their exercise endurance.

Summary

In adults with COPD, there is limited evidence to support the use of opioids for the management of breathlessness, but not exercise intolerance (14). The physiological mechanisms of action mediating opioid-induced relief of activity-related breathlessness in adults with COPD remain poorly understood and largely understudied. To this end, the following study represents the most

comprehensive randomized controlled clinical study to explore the physiological mechanisms of exertional breathlessness relief following single-dose administration of immediate-release oral morphine in adults with advanced COPD and chronic breathlessness syndrome.

Manuscript

A published version of this manuscript has been included in **Appendix III**. For this thesis: [1] the information presented in the online supplement of the published manuscript has been included as part of this chapter so that experimental methods were described in sufficient detail; [2] acronyms have been redefined to meet the *European Respiratory Journal* guidelines; and [3] references have been renumbered and are included in the combined bibliography at the end of the thesis.

Table 6.1. Summary of studies examining the effect of opioid therapy on breathlessness and exercise tolerance in adults with COPD

Study	N (% women)	Population	COPD severity mean (SD)	Design	Intervention	Outcomes	Reported side effects	Main outcome/findings
Woodcock, *et al.* (407)	12 (17)	COPD (n=12)	FEV_1 = 26% predicted; MRC 4 (0.85)	Randomized, double-blind, placebo-controlled CO. Single dose treatment.	Caffeine anhydrous (5 mg/kg BW), alcohol (vodka 1ml/kg BW), DHC (1mg/kg BW) vs. placebo.	Breathlessness (VAS during exercise) and EC (incremental treadmill).	n= 2 nausea, n=2 dizziness, n=5 constipation.	Breathlessness was reduced by 20 % with DHC treatment. Distance walked to exhaustion on the treadmill increased by 18% after DHC treatment. $\dot{V}_E$ at rest was reduced after DHC in 10 patients compared with placebo.
Woodcock (408)	16 (no data)	COPD	FEV_1 = 0.75 L (0.27); MRC >3	Randomized, double-blind, placebo-controlled. Treatment period: two weeks.	DHC, dose = 30 or 60 mg three times/day or placebo.	Breathlessness (Oxygen Cost Diagram) and EC (6MWT); exercise physiology (cycle ergometer at 50% of max).	n=5 withdrew because of nausea and vomiting after taking DHC. Of the 11 who completed the study, 2 were constipated and drowsy, and 2 had symptoms of opiate withdrawal after 60 mg dose was stopped.	Breathlessness was significant improved during 30 mg but not 60 mg DHC treatment. There was no significant difference in the distance walked during the 6 MWT. Arterial partial pressure of CO_2 was significantly increased by either dose of DHC when compared with placebo, but it did not rise above 40 mmHg. $\dot{V}_E$ and $\dot{V}O_2$ were reduced at rest and on exercise during DHC.
Johnson, *et al.* (409)	19 (17)	COPD (n=18)	FEV_1 = 0.83 L (0.26); MRC >3	Randomized, double-blind, placebo-controlled. Treatment period: one week.	DHC, dose = 15 mg or placebo 30 min before exercise as required up to three times daily. At the end of the study period, the average DHC tablets per day were 2.8 (i.e. 42 mg).	Breathlessness (VAS at rest and during exercise) and EC (incremental treadmill), PA (pedometer).	No significant difference in drowsiness, nausea, constipation or anxiety between placebo and DHC.	Breathlessness was significantly reduced by DHC (-17.8%) at rest (i.e. evaluated every day at home). Patients walked significantly further on the treadmill (+16.5%) during DHC when compared to placebo. Breathlessness ratings during exercise were significantly decreased at 75% of the distance walked on the placebo day (-11.8%). Patients walked significantly further (16.8%) during the week of DHC treatment compared to placebo as assessed by a pedometer.
Rice, *et al.* (410)	11 (0)	COPD (n=11)	FEV_1 = 0.70 L (0.11); Oxygen Cost Diagram 4.9 cm (0.5)	Randomized, double-blind, placebo-controlled. Treatment period: one month.	Codeine (30 mg 4 times a day) or promethazine (25 mg 4 times a day).	Breathlessness (VAS), EC (12-minute walk distance).	4 patients withdrew from the study (n=3 due to worsening of COPD). Drowsiness reported by 4 of the 7 patients that completed the study while on codeine, and 3 of the 7 while on promethazine.	Neither codeine nor promethazine produces substantial improvement in breathlessness or exercise tolerance.

Light, *et al.* (411)	13 (0)	COPD (n=13)	FEV_1 = 0.98 L (0.41)	Randomized, double-blind, placebo-controlled CO. Single dose treatment.	IR morphine. Dose: 0.8 mg/kg BW.	Breathlessness (Borg score during exercise) and EC (cycle ergometer incremental exercise test).	No formal evaluation of mental function, but the authors reported dizziness and nausea. Some patients intermittently dozed between the administration of morphine and the exercise test.	At HEWR, breathlessness was significantly lower after morphine treatment. $\dot{V}_E$ was significantly reduced after morphine at rest; respiratory rate and V_T were lower and the $PaCO_2$ was slightly higher. Mean maximal work rate increased by 18.6% on morphine treatment. EET increased from 6.5 to 7.5 min. VO_2 increased by 19.3% and VCO_2 increased by 13%. At peak, patients had higher $\dot{V}_E$ after morphine ingestion.
Munck, *et al.* (412)	21	COPD (n=21)	FEV_1 = median 0.94 L (range 0.60-1.09)	Part I: Dose titration. Part II: randomized, double-blind CO. Treatment period: 7 days; washout period of 3 days.	Codeine and paracetamol. Part 1 of the study: 60 mg codeine and 1 g paracetamol on day 1, on day 2: 120 mg codeine and 2 g of paracetamol. Part II of study: 1 g of paracetamol t.i.d or a combination of 1 g of paracetamol and 60 mg of codeine t.i.d.	Respiration.	Two patients withdrew due to worsening of their COPD. n=11 gastrointestinal complaints. There was no correlation between the plasma concentration of codeine or morphine and changes in respiratory parameters or adverse effects.	Authors concluded that 60 mg of codeine and 1 g of paracetamol is a safe short-time analgesic in COPD patients as it does not cause a deterioration of pulmonary function.
Eiser, *et al.* (413)	Part I: 18 (39) Part II: 10 (40)	COPD (n=18)	FEV_1 = 32% predicted	Randomized, double-blind, placebo-controlled. Study 1 treatment period: two weeks. No wash-out period. Study 2: single dose administration.	Study 1: dihydromorphine; dose: 2.5 or 5 mg every 6 hours vs. placebo. Study 2: dihydromorphine; dose: 7.5 mg vs. placebo (single administration).	Breathlessness (VAS at rest) and EC (6MWT, and time walked on treadmill).	Four patients withdrew from the study (n= 1 itching, n= 1 constipation, n=1 headache and n=1 unknown). Two other patients developed constipation and vomiting but continued the study.	Study 1: Repeated doses of oral dihydromorphine failed to ameliorate breathlessness or to increase EC. No significant difference between study periods on the degree of perceived breathlessness, arterial blood gases, nor distance walked on treadmill. Study 2: no effect of dihydromorphine treatment on breathlessness or walking distance during 6MWT.
Light, *et al.* (414)	7 (0)	COPD (n=7)	FEV_1 = 0.6 L (0.16)	Randomized, double-blind, placebo-controlled CO. Single dose treatment	Morphine. Dose: 30 mg vs. placebo.	Breathlessness (Borg score during exercise) and EC (cycle ergometer).	None.	At rest, there were no significant differences in spirometry, $\dot{V}_E$, V_T , $\dot{V}O_2$, $\dot{V}CO_2$, and $PETCO_2$. There was an increase, although insignificant, in exercise tolerance in patients after the administration of morphine.
Poole, *et al.* (415)	16 (31)	COPD (n=16)	FEV_1 = 0.6 L (0.16)	Randomized, double-blind, placebo-controlled. Treatment period: 6 weeks. Washout period: 2 weeks.	SR morphine. Starting dose: 10 mg/day, titrated to 20 mg twice per day as tolerated over the first 2 weeks of the study period. At the end of the dose titration period, the mean daily total dose was 25 mg of SR morphine.	Breathlessness (Likert scale, at rest) and EC (6MWT).	Twelve of the 16 patients reported nausea or anorexia, 14 reported constipation and 10 reported drowsiness.	Morphine improved breathlessness, but it was not significantly different from placebo. There was no significant change in O_2 saturation at rest or during treatment with morphine. Morphine decrease distance covered during 6MWT.
Abernethy, *et al.* (416)	48 (27)	COPD (n=42), Cancer (n=3), Motor	No data.	Randomized, double-blind, placebo-controlled. Treatment period:	SR morphine (Kapanol). Dose: 20 mg vs. Placebo.	Breathlessness (VAS, at rest).	Respiratory rate was similar for patients receiving morphine and placebo. No episodes of severe sedation were recorded. Morphine	Morphine significantly reduced VAS ratings of breathlessness at rest.

		neuron (n=1)		4 days, no washout period.			caused constipation, but neither treatment caused significantly more distressing vomiting, confusion, sedation or suppression of appetite.	
Currow, *et al.* (417)	Phase I = 83 (64) Phase II= 52	COPD (n=45), Cancer (n=24), ILD (n=10)	mMRC 3.8 (0.4)	Phase I: dose titration; Phase II: follow-up study to define safety and long-term effectiveness of daily SR morphine. Treatment period of 3 months.	SR morphine. Part I of the study: dose titration starting at 10 mg/ 24 h titrated weekly by 10 mg/24 h in non-responders to a max of 30 mg/24 hr. Part II of study: long-term study with the dose reached during part I (dose titration, if necessary was allowed). At the end of Part I, the mean dose of SR morphine was 16.5 mg (median 10 mg). At the end of Part II, the mean dose was 14.0 mg.	Breathlessness (VAS, at rest)	In Part I of the study, 15 patients reported side effects (n =4 drowsiness, n= 3 confusion, n= 2 constipation, n=2 vomiting, n=1 dizziness, n=1 hallucination). In Part II of the study, 6 people withdrew due to side effects (n= 6 constipation, n= 4 drowsiness, n=4 nausea and vomiting). All side effects settled with the cessation of opioids and no hospitalization were required.	Part I conclusion: 70% of individuals that derived >10% improvement in breathlessness were at a dosage of 10 mg/24 h. Part II conclusion: patients with chronic refractory breathlessness derive a benefit from opioid therapy without compromising respiratory function. 10 mg of SR oral morphine once daily is a safe and effective dosage for patients with refractory breathlessness.
Rocker, *et al.* (418)	44 (58)	COPD (n=44)	FEV_1 =26.8 % predicted	Prospective, longitudinal, observational, interventional study. Treatment period: 4-6 months after initiation of therapy.	IR and SR morphine. Part 1, dose titration phase: started at 0.5 mg IR morphine twice daily, and slowly titrated upward based on weekly assessments of symptoms. Part 2: switched to SR morphine at the dose that achieved symptom relief during part 1 with IR morphine for episodic dyspnea. At the end of the study period, 38% of patients were on 10 mg/d, 38% were on 12 mg/d, 22% on 15 mg/d and 3% on 30 mg/d. 28% remained on IR morphine, with an average daily dose of 5.4 mg.	Breathlessness (Likert scale, at rest; and CRQ)	Dry mouth increased from baseline, but all other side effects (e.g., constipation, nausea, dizziness) did not change.	Significant improvements were observed in the CRQ-breathlessness domain. Patients reported tangible improvements in breathing, activity/mobility and independence. Health-related quality of life significantly improved post-opioid therapy (defined by CRQ).

BW = body weight; COPD = chronic obstructive pulmonary disease; CO = crossover; CRQ = Chronic Respiratory Disease Questionnaire; DHC= dihydrocodeine; EC= exercise capacity; EET = exercise endurance time; HEWR = highest equivalent work rate; HR = heart rate; ILD = interstitial lung disease; IR = immediate release; PA= physical activity; $PaCO_2$ = arterial carbon dioxide; $PETCO_2$ = end-expiratory partial pressure for carbon dioxide; SR= sustained release; VAS = Visual Analogue Scale; $\dot{V}_E$ = minute ventilation; V_T = tidal volume; $\dot{V}O_2$ = rate of oxygen consumption; $\dot{V}CO_2$ = rate of carbon dioxide output; 6MWT = 6-minute walk test.

TITLE PAGE

Effect of Morphine on Breathlessness and Exercise Endurance in Advanced COPD: A Randomized Crossover Trial

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"Take home" message: Immediate-release oral morphine decreased exertional breathlessness and improved exercise endurance in advanced COPD

ABSTRACT

Objective: Evaluate the effect of morphine on exertional breathlessness and exercise endurance in advanced chronic obstructive pulmonary disease (COPD). **Methods:** In a randomized crossover trial, we compared the acute effect of immediate-release oral morphine *vs.* placebo on physiological and perceptual responses during constant-load cardiopulmonary cycle exercise testing (CPET) in 20 adults with advanced COPD and chronic breathlessness syndrome. **Results:** Compared with placebo, morphine reduced exertional breathlessness at isotime by 1.2±0.4 Borg units and increased exercise endurance time by 2.5±0.9 min (both $p \leq 0.014$). During exercise at isotime, morphine decreased ventilation by 1.3±0.5 $l \cdot min^{-1}$ and breathing frequency by 2.0±0.9 $breaths \cdot min^{-1}$ (both $p=0.041$). Compared with placebo, morphine decreased exertional breathlessness at isotime by ≥1 Borg unit in 11 participants (responders [R]) and by <1 Borg unit in 9 participants (non-responders [NR]). Baseline participant characteristics, including pulmonary function and cardiorespiratory fitness, were similar between R and NR. A higher percentage of R *vs.* NR stopped incremental CPET due to intolerable breathlessness: 82 *vs.* 33% ($p=0.028$). **Conclusions:** Immediate-release oral morphine improved exertional breathlessness and exercise endurance in some, but not all, adults with advanced COPD. The locus of symptom-limitation on laboratory-based CPET may help to identify patients most likely to benefit from morphine.

INTRODUCTION

Breathlessness and exercise intolerance are independently associated with increased morbidity and mortality in chronic obstructive pulmonary disease (COPD) (419). Despite optimal treatment of their underlying disease with bronchodilators, corticosteroids and/or phosphodiesterase inhibitors, 46-91% of patients with severe-to-very-severe COPD suffer from chronic and disabling breathlessness at rest and on minimal exertion (1, 3, 420), i.e., chronic breathlessness syndrome (4). Therefore, symptom-specific therapies that alleviate refractory breathlessness and improve exercise capacity are needed to enhance health outcomes in advanced COPD.

A systematic review and meta-analysis by Ekstrom*, et al.* (14) recently concluded that systemic low-dose opioids are safe and effective for decreasing refractory breathlessness but do not improve exercise capacity in advanced COPD. Importantly, published studies have provided little insight into the mechanism(s) mediating opioid-induced relief of breathlessness in COPD, although reductions in ventilation ($\dot{V}_E$) *via* reduced central neural respiratory drive and/or a blunted central perception of breathlessness have been proposed (98, 407, 411, 421). A better understanding of the physiological mechanism(s) of action of systemic opioids on breathlessness is essential to optimizing symptom control in advanced COPD.

Although Canadian, American, European and International clinical practice guidelines support the use of systemic low-dose opioids for decreasing refractory breathlessness in advanced COPD (54, 422-424) ,many physicians do not prescribe opioids for breathlessness (15) due to fear of adverse side-effects (e.g., respiratory depression), insufficient scientific evidence supporting a benefit of

opioids on refractory breathlessness and an inability to predict which patient(s) will respond to opioids (16, 17).

The primary objective of this randomized crossover trial was to evaluate the acute effect of oral morphine on exertional breathlessness and exercise endurance in advanced COPD. Our secondary objective was to elucidate the physiological mechanism(s) of action of oral morphine on exertional breathlessness and exercise endurance in advanced COPD. We compared detailed physiological and perceptual responses to cycle endurance exercise testing after single-dose administration of immediate-release oral morphine and placebo in participants with advanced COPD and chronic breathlessness syndrome. We hypothesized that oral morphine *vs.* placebo would be associated with clinically meaningful improvements in exertional breathlessness and exercise endurance, independent of opioid-related side effects, CO_2 retention, and concurrent improvements in the physiological response to exercise.

MATERIALS AND METHODS

Participants. Participants included men and women aged ≥40 yrs with clinically stable Global Initiative for Obstructive Lung Disease stage 3 or 4 COPD (424) and chronic breathlessness syndrome (4), defined as a modified Medical Research Council (mMRC) dyspnoea score of ≥3 (378), a Baseline Dyspnoea Index (BDI) focal score of ≤6 (379) and/or an Oxygen Cost Diagram rating of ≤50% full scale (380) despite optimal treatment with bronchodilators, corticosteroids and/or phosphodiesterase inhibitors (424). Exclusion criteria included: smoking history <20 pack-years; change in medication dosage and/or frequency of administration in preceding 2-weeks; exacerbation and/or hospitalization in preceding 6-weeks; arterialized capillary PCO_2 ($P_{ac}CO_2$)

>50 mmHg at rest; presence of other medical condition(s) that could contribute to breathlessness and/or exercise intolerance; important contraindications to cardiopulmonary exercise testing (CPET); self-reported history of addiction and/or substance abuse; use of anti-seizure drugs or opioids; use of daytime oxygen; and exercise-induced oxyhemoglobin desaturation to <80% on room air.

Study design. This single-center, randomized, double-blind, placebo-controlled, crossover trial (ClinicalTrials.gov identifier NCT01718496) consisted of two intervention periods separated by a washout period of ≥48 hrs. Participants were randomized in a 1:1 ratio to receive immediate-release oral morphine sulphate (0.1 mg/kg body mass to a maximum dose of 10 mg; Statex™, Paladin Labs Inc., Montreal, QC, Canada) or diluted simple syrup (placebo) prepared in 250 ml of orange juice. A computer-generated block randomization schedule was prepared by a third-party not involved in the trial. The study protocol and informed consent form received ethical approval from Health Canada (File No. 9427-M1647-48C) and the Research Ethics Board of the Research Institute of the McGill University Health Centre (MP-CUSM-12-325-T).

After providing written informed consent, participants completed a screening/familiarization visit followed by two randomly assigned treatment visits. *Visit 1* included: medical history; clinical assessment; evaluation of participant-reported breathlessness (378-380), health status (381) and anxiety/depression (382); measurement of $P_{ac}CO_2$ at rest; post-bronchodilator (400 μg salbutamol) pulmonary function testing; and a symptom-limited incremental CPET to determine peak power output (PPO), defined as the highest power output that the participant was able to sustain for ≥30-sec. At the start of *Visits 2 and 3*, participants inhaled 400 μg of salbutamol to standardize the time

since last bronchodilator administration. Fifteen minutes thereafter, participants completed the opioid-related symptom distress scale (ORSDS) (425, 426) followed by blood sampling for measurement of $P_{ac}CO_2$ and of plasma concentrations of morphine ([MOR]) and its two metabolites, morphine-3-glucuronide ([M3G]) and morphine-6-glucuronide ([M6G]). Participants were then administered oral morphine or placebo. Thirty-minutes thereafter, participants completed the ORSDS and blood for measurement of $P_{ac}CO_2$, [MOR], [M3G] and [M6G] was collected. Participants then completed a symptom-limited constant-load cycle CPET at 75% PPO.

Procedures. Spirometry, plethysmography and single-breath diffusion capacity of the lung for carbon monoxide were performed using automated equipment and recommended techniques (363, 385-387). Measurements were referenced to predicted normal values (388-391):predicted normal inspiratory capacity (IC) was calculated as the difference between predicted normal total lung capacity and predicted normal functional residual capacity.

Symptom-limited exercise tests were conducted on an electronically braked cycle ergometer (Lode Corival, Lode B.V. Medical Tech., Groningen, The Netherlands) using a computerized CPET system (Vmax Encore^{TM} 29C). Incremental CPETs consisted of a steady-state rest period of at least 6-min, followed by 1-min of unloaded pedalling and then 5 W/min increases in power output. Constant-load CPETs consisted of a steady-state rest period of at least 6-min, followed by 1-min of unloaded pedaling and then a step increase in power output to 75% PPO. Cardiac, metabolic, breathing pattern and gas exchange parameters were collected and analyzed as previously described (427). Inspiratory capacity (IC) maneuvers were performed at rest, every 2-min during

CPET, and at end-exercise (368, 392). Measurements of PPO and of peak oxygen uptake and peak heart rate were referenced to the predicted normal values of Jones and colleagues (368). Using Borg's modified 0-10 category ratio scale (367), participants rated the intensity and unpleasantness of their breathlessness, as well as the intensity of their leg discomfort at rest, every 2-min during CPET, and at end-exercise. In a subgroup of 7 consenting adults, breath-by-breath measures of the crural diaphragm electromyogram (EMGdi) were recorded and analyzed using published methods (79, 172). Participants verbalized their main reason(s) for stopping exercise; quantified the percentage contribution of breathlessness and leg discomfort to exercise cessation; and identified qualitative phrases that best described their breathlessness at end-exercise (46). Each participant's blinded treatment preference was assessed at the end of *Visit 3* by having them identify the visit wherein "exercise felt easier" and providing a reason for their selection.

Blood for measurement of $P_{ac}CO_2$ was drawn from a warmed earlobe (Finalgon® Cream, Boehringer Ingelheim GmbH) into a pre-heparinized capillary tube (safe CLINITUBES, D957P-70-125; Radiometer Copenhagen, Denmark) and analyzed immediately using an OPTI^{TM} CCA-TS2 blood gas analyzer (OPTI Medical Systems Inc., Roswell, GA, USA).

Plasma [MOR], [M3G] and [M6G] were analyzed by high-performance liquid chromatography mass spectrometry (EliaPharma Services Inc., Montreal, QC, Canada). Proteins from 20 µl of plasma were precipitated with the addition of 3 volumes of acetonitrile containing 2 ng of morphine D6 (internal standard). The molecules of interest were isolated by mixed mode strong cation exchange solid phase extraction (Strata-X-C, Phenomenex, Torrance, USA). A 9 point standard curve ranging from 0 to 500 ng/ml of each of the molecules was prepared in plasma

alongside the samples (r^2 = 0.99 for morphine, 0.97 for M3G and 0.98 for M6G). Acquisition was performed in positive mode with a Sciex TripleTOF 5600 (Sciex, Concord, Canada) equipped with an electrospray interface with 50 µm iD capillary and coupled to an Eksigent µUHPLC (Sciex,Concord, Canada). Analyst TF 1.7 software was used to control the instrument and for data processing and acquisition. Acquisition was performed in MRM mode with the following transitions, declustering potential and collision energy: 286.1 → 165.06, DP: 100V, CE: 49V for morphine; and 462.18 → 207.03, DP: 100V, CE: 50V for both M3G and M6G. Separation was performed on a reversed phase HALO PFP column 0.5 mm i.d., 2.7 µm particles, 50mm long (Advance Materials Technology, Wilmington, USA). For the 3-min liquid chromatography gradient, the mobile phase consisted of solvent A (0.2% v/v formic acid in water) and solvent B (0.2% v/v formic acid + 50% methanol + 49.8% acetonitrile). Molecule quantification was done using peak area with a 0.05 Da extraction window with the MultiQuant software (Sciex, Concord, Canada).

Primary outcome variables. The primary outcome was the post-dose difference in breathlessness intensity ratings during exercise at isotime, defined as the highest equivalent 2-min interval of exercise completed by a given participant during each of the constant-load CPETs. The co-primary outcome was the post-dose difference in exercise endurance time (EET), defined as the duration of loaded pedaling during constant-load CPET.

Secondary outcome variables. $P_{ac}CO_2$ at rest; plasma [MOR], [M3G] and [M6G]; OSRDS-derived measures of opioid-related side effects; physiological and perceptual parameters measured at rest, at standardized submaximal times during constant-load CPETs and at end-exercise; reasons

for stopping exercise; percentage contribution of breathlessness and leg discomfort to exercise cessation; qualitative descriptors of breathlessness at end-exercise; and participant's blinded treatment preference

Statistical analyses. Using a two-tailed paired subject formula with α=0.05, β=0.90, and an expected effect size of 0.80 (393), we estimated that 20 participants were needed to detect a minimal clinically important difference (MCID) of 1 Borg unit in breathlessness intensity during exercise at isotime (204) and of 101-sec or 1.68-min in EET (205) after taking morphine *vs.* placebo.

All participants who completed both morphine and placebo arms of the trial were included in the analysis. Linear mixed-models regression with random intercepts was used to analyze post-dose differences in EET as well as in all physiological and perceptual responses to constant-load CPET, accounting for period and sequence effects.

Post-dose differences in the percentage contribution of breathlessness and leg discomfort to exercise cessation, and in the intensity and bothersomeness of each symptom assessed on the ORSDS were analyzed using two-tailed paired t-tests.

Post-dose differences in the selection frequencies of each symptom on the ORSDS, the individual reasons for stopping exercise, and the individual descriptors of breathlessness at end-exercise were analyzed using the chi-squared test.

Linear mixed-model regression with random intercepts was used to examine treatment, time and treatment*time interaction effects on $P_{ac}CO_2$. Multiple imputations (n=30) were performed to impute missing $P_{ac}CO_2$ values.

A secondary analysis was conducted after examination of the data showed that 11 participants reported a morphine-induced decrease in breathlessness intensity at isotime by the MCID of ≥1 Borg unit (responders [R]) compared with the remaining 9 participants who did not (non-responders [NR]). Baseline characteristics were compared between-groups using two-tailed unpaired t-tests. The effect of oral morphine *vs.* placebo on EET was analyzed using two-tailed paired t-tests within each group. A two-tailed two-way repeated measures analysis of variance with Tukey's HSD post-hoc test was used to examine treatment, time and treatment*time interaction effects on physiological and perceptual parameters measured at rest and during exercise within each group.

Data were analyzed using SAS statistical package, version 9.1.3 (SAS Institute Inc., Cary, NC, USA) and SigmaStat, version 3.5 (Systat Software Inc., San Jose, CA, USA). Statistical significance was set at $p<0.05$ and values are reported as mean±SEM unless stated otherwise.

RESULTS

Twenty-three of 128 participants assessed for eligibility were randomized (**Figure 6.1**). Two of these 23 participants were lost during follow-up for non-study related reasons, while one was lost following a serious adverse event (**Figure 6.1**). Baseline characteristics of the 20 participants who completed the trial are presented in **Table 6.2** and **Table 6.3**.

Primary outcomes. There was no statistically significant sequence or period effect of treatment. Compared with placebo, morphine (mean±SEM dose, 7.2±3.2 mg [range: 4.4-9.2 mg]) decreased breathlessness intensity ratings at isotime by 1.2±0.4 Borg units (p=0.011) and increased EET by 2.5±0.9 min (148±52 sec) or 41±13% (p=0.014) (**Figure 6.2**, **Table 6.4**).

Secondary outcomes.

Perceptual responses. Compared with placebo, morphine decreased breathlessness unpleasantness ratings by 1.4±0.4 Borg units at isotime (p=0.003) (**Figure 6.2B**, **Table 6.4**), but had no effect on intensity ratings of leg discomfort at rest or during exercise (**Figure 6.2C**, **Table 6.4**). Despite differences in EET, breathlessness intensity and unpleasantness ratings were similar between treatments at end-exercise (**Figure 6.2A** and **B**, **Table 6.4**).

Compared with placebo, morphine had no effect on the locus of symptom limitation (**Table 6.4**), the selection frequency of breathlessness descriptors at end-exercise (**Figure 6.3**), and the relative contributions of breathlessness (morphine, 61±8% *vs.* placebo, 66±8%, p=0.260) and leg discomfort (morphine, 19±6% *vs.* placebo, 23±6%, p=0.305) to exercise cessation.

Blood biochemistry. Oral morphine increased plasma [MOR], [M3G] and [M6G] (**Figure 6.4A**). Morphine-induced changes in breathlessness intensity ratings at isotime and in EET were unrelated to plasma [MOR], [M3G] and [M6G] (Pearson $r \leq 0.43$, $p \geq 0.09$ for all). There was no treatment, time or treatment*time interaction effect for $P_{ac}CO_2$ (**Figure 6.4B**). There was also no evidence of CO_2 retention at rest: all pre- and post-dose measurements of $P_{ac}CO_2$ were <50 mmHg (**Figure 6.4B**).

Physiological responses. With the exception of a small but significant increase in the end-tidal partial pressure of CO_2 ($P_{ET}CO_2$) by just 0.9±0.3 mmHg (p=0.002), morphine had no effect on physiological variables at rest (**Figure 6.5**, **Table 6.4**). During exercise at isotime after taking morphine *vs.* placebo, there were small but significant decreases in $\dot{V}_E$ (p=0.031) and breathing frequency (f_R; p=0.041) (**Figure 6.5**, **Table 6.4**). At peak exercise, f_R decreased (p=0.041) after taking morphine *vs.* placebo (**Table 6.4**). Compared with placebo, morphine had no statistically significant effect on EMGdi or the EMGdi-$\dot{V}_E$ ratio at any measurement time (**Table 6.4**), although EMGdi was reduced by ~13% during exercise at isotime after taking morphine *vs.* placebo (p=0.061).

Opioid-related side effects and adverse events. Eighteen of 20 participants reported no pre- to post-dose change in ORSDS ratings of headache, nausea, difficulty concentrating, drowsiness, lightheadedness/dizziness, confusion, and fatigue after taking either morphine or placebo. One participant with no pre-dose symptoms reported lightheadedness/dizziness and difficulty concentrating 30-min after taking morphine, although the severity and bothersomeness of these symptoms were mild with ratings of <20-mm on a 100-mm visual analog scale. Another participant with no pre-dose symptoms reported nausea and drowsiness 30-min after taking morphine and placebo, respectively. In both cases, the severity and bothersomeness of these symptoms were moderate, with visual analog scale ratings of <50-mm. One serious adverse event occurred in a woman with an unreported intolerance to opioids. This participant experienced severe abdominal pain 20-min after taking morphine, was admitted to the emergency department, treated with epinephrine, and discharged 4-hrs after admission.

Participant's blinded treatment preference. Fifteen of 20 participants reported a preference for morphine over placebo for exercise: 12 participants volunteered that their breathing was easier during exercise, while 3 participants volunteered that exercise was less demanding. Three of 20 participants reported a preference for placebo because they felt more prepared for the study visit (i.e., they received placebo at visit 3), while the remaining 2 participants reported no treatment preference.

Secondary analysis. Baseline characteristics were similar between R and NR (**Table 6.5**), with the exception of forced expiratory volume in 1-sec expressed as a percentage of predicted (FEV_1%pred), which tended to be lower in R *vs.* NR (p=0.050).

Intensity and unpleasantness ratings of breathlessness were higher in R *vs.* NR at the symptom-limited peak of incremental CPET (Intensity, 6.9±0.7 *vs.* 5.1±0.8 Borg units [p=0.077]; Unpleasantness, 7.0±0.7 *vs.* 5.5±0.5 Borg units [p=0.119]) and constant-load CPET during the placebo treatment period (Intensity, 6.8±0.8 *vs.* 4.7±0.6 Borg units [p=0.046]; Unpleasantness, 7.1±0.8 *vs.* 4.9±0.6 Borg units [p=0.050]), even though PPO, EET, peak $\dot{V}_E$ and peak $\dot{V}O_2$ were not significantly different between-groups. A greater percentage of participants within the R *vs.* NR subgroup identified intolerable breathlessness as their primary reason for stopping incremental CPET (82% [n=9/11] *vs.* 33% [n=3/9], p=0.028) and constant-load CPET during the placebo treatment period (73% [n=8/11] *vs.* 33% [n=3/9], p=0.078). The relative contribution of intolerable breathlessness to the cessation of incremental CPET (76±6 *vs.* 51±11%, p=0.042) and constant-load CPET during the placebo treatment period (76±9 *vs.* 52±13%, p=0.139) was also higher in R *vs.* NR.

Plasma [MOR], [M3G] and [M6G] were not different between R and NR, while post-dose measures of $P_{ac}CO_2$ were similar within- and between-groups (**Figure 6.6**). The effect of oral morphine *vs.* placebo on breathlessness and selected ventilatory responses to constant-load CPET within R and NR are shown in **Figure 6.7**. After taking morphine *vs.* placebo within the R subgroup: EET increased by 3.6±1.3 min (p=0.005), while breathlessness intensity and unpleasantness ratings during exercise at the highest equivalent isotime of 6.0±1.5 min decreased by 2.3±0.6 Borg units (p<0.001) and 2.3±0.6 Borg units (p=0.001), respectively. Although the differences were not statistically significant, f_R decreased by 3.2±1.4 breaths · min^{-1} and tidal volume (V_T) increased by 0.08±0.04 L at isotime (with no corresponding change in $\dot{V}_E$) after taking morphine *vs.* placebo within R. By contrast, morphine had no effect on EET or an effect on ventilatory and breathlessness responses to exercise within NR.

Compared with placebo, morphine had no effect on either the locus of symptom limitation, the selection frequency of breathlessness descriptors at end-exercise or the percentage contributions of breathlessness and leg discomfort to exercise cessation within either R or NR (data not shown).

DISCUSSION

The main findings of this randomized crossover trial are as follows: (i) single-dose administration of immediate-release oral morphine *vs.* placebo improved exertional breathlessness and exercise endurance among participants with advanced COPD and chronic breathlessness syndrome; (ii) morphine-induced improvements in exertional breathlessness and exercise endurance were accompanied by small but statistically significant decreases in $\dot{V}_E$ and f_R during exercise at isotime,

without significant opioid-related side effects and/or gas exchange impairment at rest and during exercise; and (iii) the locus of symptom-limitation on laboratory-based CPET may help to identify adults with advanced COPD and chronic breathlessness syndrome most likely to respond to morphine.

Compared with placebo, morphine decreased breathlessness intensity ratings during exercise at isotime by 1.2 Borg units and increased EET by 2.5-min (148-sec) or 41%. The magnitudes of these improvements exceeded their respective MCIDs (234, 292) and were thus clinically meaningful. Our results are comparable to those of Woodcock, *et al.* (407) and Light, *et al*. (411) who respectively reported on the acute effect of single-dose oral dihydrocodeine (1 mg/kg body mass) and oral morphine (0.8 mg/kg body mass) on exertional breathlessness and exercise tolerance in advanced COPD.

It is noteworthy that improvements in exertional breathlessness and EET after taking morphine *vs.* placebo occurred following apparent maximal or near maximal bronchodilatation with 400 μg of inhaled salbutamol. Indeed, morphine-induced improvements in exertional breathlessness and EET could not be easily explained by improved dynamic respiratory mechanics.

In keeping with the results of Woodcock, *et al.* (407) and Light, *et al.* (411), single-dose administration of oral morphine *vs.* placebo was associated with modest but statistically significant reductions in $\dot{V}_E$ and f_R during exercise at isotime, which were accompanied by concomitant reductions in neural inspiratory drive to the crural diaphragm (i.e., EMGdi). These results are consistent with the known effect of systemic opioids on central and peripheral chemoreflex drives

to breathe (421). Importantly, the observed changes in $\dot{V}_E$, f_R and EMGdi occurred in the absence of any untoward effect of morphine on cardiac, metabolic and/or gas exchange parameters at rest (e.g., $P_{ac}CO_2$) and during exercise (e.g., $P_{ET}CO_2$, oxygen saturation). In view of the observed reductions in $\dot{V}_E$, f_R, EMGdi and the relatively preserved EMGdi-$\dot{V}_E$ ratio during exercise at isotime after taking morphine *vs.* placebo, we speculate that morphine-induced improvements in exertional breathlessness and EET reflected, at least in part, the awareness of reduced central neural respiratory drive as sensed by reduced central corollary discharge from brainstem respiratory centers to various cortical and sub-cortical regions implicated in the neurophysiology of breathlessness (100). These regions, all of which express high densities of opioid receptors, include the prefrontal, insular and motor cortices, operculum, anterior and posterior cingulate cortices, amygdala and periaqueductal grey matter (98, 100, 406, 428).

It is unlikely that awareness of reduced central neural respiratory drive was the only mechanism responsible for relief of exertional breathlessness and improved EET after taking morphine *vs.* placebo, particularly in view of (i) the small reductions in $\dot{V}_E$, f_R and EMGdi at isotime relative to the large improvements in exertional breathlessness and EET and (ii) our finding that exertional breathlessness was reduced and EET increased after taking morphine *vs.* placebo within R despite no statistically significant decreases in $\dot{V}_E$ and f_R. It is possible that morphine relieved breathlessness and improved EET by suppressing activity of the cortico-limbic regions implicated in the perception of breathlessness, independent of, or in conjunction with, its effect on central neural respiratory drive (98, 100, 404, 406, 429).

Although one serious adverse event occurred in a participant with an unreported intolerance to opioids, no meaningful pre-to-post dose changes in any of the symptoms evaluated using the ORSDS were observed following the administration of immediate-release oral morphine in our participants with advanced COPD. These findings are consistent with the results of earlier studies that informally assessed opioid-related side effects in COPD (407, 411).

Secondary exploratory analysis

With the exception of FEV_1%pred, which tended to be lower in R *vs.* NR, baseline participant characteristics, resting $P_{ac}CO_2$, and plasma [MOR], [M3G] and [M6G] were similar between-groups (**Table 6.5**). Compared to NR, the R subgroup (i) reported higher intensity and unpleasantness ratings of breathlessness at the symptom-limited peak of CPET and (ii) were more likely to identify intolerable breathlessness as the primary reason for stopping CPET, despite exercising to a similar PPO, EET, peak $\dot{V}O_2$ and peak $\dot{V}_E$. Factors contributing to these between-group differences are unclear, particularly in the absence of notable differences in the physiological response to exercise. Collectively, these results suggest that adults with advanced COPD and chronic breathlessness syndrome who achieve relatively high ratings of breathlessness at the symptom-limited peak of exercise and/or who report intolerable breathlessness as their main exercise-limiting symptom may be the most responsive to immediate-release oral morphine.

Although we were unable to elucidate the mechanism(s) responsible for the contrasting effect of oral morphine *vs.* placebo on exertional breathlessness and EET in R *vs.* NR, we speculate that any one or combination of the following factors may be at least partly responsible: relatively greater morphine-induced suppression of central neural respiratory drive in R *vs.* NR, as evidenced by the

3.2 breath · min^{-1} decrease in f_R at isotime after taking morphine *vs.* placebo within R alone; unmeasured between-group differences in genetic variability (e.g., single nucleotide polymorphisms in opioid receptors) (430); and unmeasured between-group differences in conditioned anticipatory/associative learning responses to breathlessness (406).

Methodological considerations

The generalizability of our results may be restricted to a relatively small and homogenous group of clinically stable, normocapnic, non-oxygen dependent and opioid-naïve adults with severe-to-very severe COPD and chronic breathlessness syndrome. The 0.1 mg/kg body mass dose of immediate-release oral morphine used in this trial may be considered relatively high, particularly in comparison to current recommendations on the use of opioids for managing refractory breathlessness in advanced COPD (54). Dose-ranging studies are needed to identify the lowest effective dose of immediate-release oral morphine required to achieve clinically meaningful improvements in exertional breathlessness and EET in adults with advanced COPD and chronic breathlessness syndrome. We caution against the extrapolation of our results concerning the acute effect of single-dose immediate-release oral morphine on exertional symptoms in advanced COPD to other modes (e.g., inhaled, sublingual), types (e.g., fentanyl) and regimens of opioid administration (e.g., repeat-dose, sustained-release) in this patient population. Safety aspects of this trial should be interpreted cautiously as it was not powered to detect differences in safety outcomes. Although the results of our exploratory analysis may be limited by a small sample size (i.e., susceptible to a type 2 error), we nevertheless identified factors related to the locus of symptom-limitation on laboratory-based cycle CPET as being potentially helpful in identifying which patient(s) will likely respond to a single-dose of immediate-release oral morphine, as

previously demonstrated by Deschenes*, et al.* (431) for bronchodilator therapy. In moving forward, it will be important to prospectively validate our post hoc classification of R and NR by observing the effects of chronic dosing of morphine on breathlessness and exercise endurance in adults with advanced COPD and chronic breathlessness syndrome.

Conclusions

Single-dose administration of immediate-release oral morphine (0.1 mg/kg body mass) was associated with statistically significant and clinically meaningful improvements in exertional breathlessness and exercise endurance in adults with advanced COPD and chronic breathlessness syndrome. The observed changes in breathlessness and exercise endurance after taking oral morphine *vs.* placebo could not be explained by concurrent changes in cardiac, metabolic, gas exchange and/or dynamic operating lung volume responses to exercise, but were associated with reductions in ventilation, breathing frequency and the diaphragm electromyogram during exercise at isotime. Although additional research is necessary, the locus of symptom-limitation on laboratory-based CPET has the potential to help healthcare providers better predict which patient(s) with advanced COPD and chronic breathlessness syndrome are most likely to achieve clinically meaningful improvements in exertional breathlessness and exercise endurance in response to morphine.

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Table 6.2. Baseline participant characteristics.

Parameter	Value
Male:Female, n	15:5
Age, yrs	63.6 ± 7.1
Height, cm	169.0 ± 8.3
Body mass, kg	71.6 ± 14.4
Body mass index, kg · m^{-2}	25.3 ± 5.2
$P_{ac}CO_2$, mmHg [range]†	37.7 ± 3.4 [32 - 45]
Smoking history, pack years	59.3 ± 22.8
GOLD stage, 3:4	12:8
Post-bronchodilator pulmonary function	
FEV_1, L (% predicted)	0.93 ± 0.21 (35 ± 9)
FEV_1/FVC, %	36.3 ± 10.3
TLC, L (% predicted)	7.79 ± 1.70 (126 ± 17)
RV, L (% predicted)	4.70 ± 1.40 (217 ± 57)
FRC, L (% predicted)	5.68 ± 1.57 (174 ± 38)
IC, L (% predicted)	2.08 ± 0.61(72 ± 17)
D_LCO, ml · min · $mmHg^{-1}$ (% predicted)*	12.9 ± 6.2 (52 ± 30)
sRaw, cmH_2O · L · s^{-1} (% predicted)†	46.4 ± 35.8 (949 ± 821)
Breathlessness and health status	
mMRC score, 0-4	3.0 ± 0.6
BDI focal score, out of 12	3.9 ± 1.9
Oxygen cost diagram, % full scale	39 ± 16
CAT score, out of 40	21.3 ± 6.4
CAT breathlessness item, out of 5	4.1 ± 0.9
CAT activity limitation item, out of 5	3.3 ± 1.5
HADS score, out of 42	12.2 ± 6.1
COPD medication summary	
LABA + LAMA, n	4
LABA + LAMA + ICS, n	14
LABA + LAMA + PI, n	1
LABA + LAMA + ICS + PI, n	1

Values are mean ± SD. *n=17. †n=18. $P_{ac}CO_2$, partial pressure of carbon dioxide in arterialized capillary blood; GOLD, global initiative for obstructive lung disease; FEV_1, forced expiratory volume in 1-sec; FEV_1/FVC, forced expiratory volume in 1-sec to forced vital capacity ratio; TLC, total lung capacity; RV, residual volume; FRC, functional residual capacity; IC, inspiratory capacity; D_LCO, diffusing capacity of the lung for carbon monoxide; sRaw, specific airway resistance; mMRC, modified Medical Research Council Dyspnoea Scale; BDI, Baseline Dyspnoea Index; CAT, COPD Assessment Test; HADS, Hospital Anxiety and Depression Scale; LABA, long-acting β_2 agonist; LAMA, long-acting muscarinic antagonist; ICS, inhaled corticosteroid; PI, phosphodiesterase inhibitor.

2.9

Table 6.3. Physiological and perceptual responses at the symptom-limited peak of incremental cycle exercise testing in adults with advanced COPD and chronic breathlessness syndrome.

Parameter	Value
Cycle exercise time, min	6.5 ± 2.7
Power output, watts (% predicted)	37.8 ± 17.7 (27 ± 10)
$\dot{V}O_2$, ml · kg · min^{-1} (% predicted)	12.7 ± 2.6 (53 ± 16)
HR, beats · min^{-1} (% predicted)	113 ± 22 (67 ± 13)
Breathlessness intensity, Borg units	6.1 ± 2.3
Breathlessness unpleasantness, Borg units	6.3 ± 2.1
Leg discomfort, Borg units	5.6 ± 3.2
$\dot{V}_E$, L · min^{-1} (% estimated MVV)	31.3 ± 7.9 (97 ± 17)
V_T, L	1.11 ± 0.31
f_R, breaths · min^{-1}	29.2 ± 6.9
Δ IC from rest, L	-0.94 ± 0.61
IRV, L	0.29 ± 0.20
$\dot{V}_E/\dot{V}CO_2$	37.9 ± 1.5
$P_{ET}CO_2$, mmHg	36.4 ± 5.0
SpO_2, %	93 ± 3
Δ SpO_2 from rest, %	-2.4 ± 2.8
Reasons for stopping exercise	
Breathlessness, n	12
Leg discomfort, n	1
Breathlessness and leg discomfort, n	7

Values are mean ± SD. $\dot{V}O_2$, rate of oxygen uptake; HR, heart rate; $\dot{V}_E$, minute ventilation; MVV, maximal voluntary ventilation estimated as forced expiratory volume in 1-sec x 35; V_T, tidal volume, f_R, breathing frequency; Δ, exercise-induced change; IC, inspiratory capacity; IRV, inspiratory reserve volume; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent for carbon dioxide; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; SpO_2, oxygen saturation by pulse oximetry.

Table 6.4. Effect of immediate-release oral morphine (0.1 mg/kg body mass) *vs.* placebo on physiological and perceptual responses at rest, at a standardized submaximal time during constant-load cycle exercise testing (isotime), and at the symptom-limited peak of constant-load cycle exercise testing in adults with advanced COPD and chronic breathlessness syndrome.

	Rest		Isotime		Peak	
	Placebo	**Morphine**	**Placebo**	**Morphine**	**Placebo**	**Morphine**
Cycle exercise time, min	-	-	5.3 ± 4.5	5.3 ± 4.5	6.2 ± 4.4	8.6 ± 6.5*
Breathlessness intensity, Borg units	0.6 ± 0.9	0.4 ± 0.7	4.2 ± 2.6	3.0 ± 1.6*	5.9 ± 2.4	5.6 ± 2.2
Breathlessness unpleasantness, Borg units	0.5 ± 0.8	0.6 ± 1.2	4.5 ± 2.6	3.1 ± 1.7†	6.1 ± 2.5	5.6 ± 2.0
Leg discomfort, Borg units	0.5 ± 1.1	0.4 ± 0.7	3.9 ± 3.2	3.7 ± 3.2	4.9 ± 3.6	5.1 ± 3.4
$\dot{V}O_2$, ml · kg · min^{-1}	4.5 ± 1.1	4.4 ± 1.1	11.7 ± 2.4	11.3 ± 2.5	12.5 ± 2.3	12.5 ± 2.5
$\dot{V}CO_2$, ml · kg · min^{-1}	4.3 ± 1.1	4.1 ± 1.1	11.1 ± 2.6	10.8 ± 2.7	12.0 ± 2.5	12.1 ± 2.5
HR, beats · min^{-1}	86 ± 13	87 ± 14	110 ± 21	110 ± 21	112 ± 21	114 ± 21
$\dot{V}_E$, L · min^{-1}	14.6 ± 2.3	14.0 ± 1.9	28.9 ± 7.4	27.6 ± 7.5*	30.9 ± 7.2	30.6 ± 7.8
V_T, L	0.89 ± 0.24	0.87 ± 0.25	1.12 ± 0.28	1.16 ± 0.28	1.08 ± 0.29	1.14 ± 0.26
f_R, breaths · min^{-1}	18.0 ± 6.2	18.0 ± 6.2	26.9 ± 7.2	24.9 ± 7.3*	30.2 ± 7.7	28.1 ± 7.3*
V_T/T_i	0.74 ± 0.16	0.72 ± 0.17	1.48 ± 0.32	1.42 ± 0.31	1.60 ± 0.34	1.58 ± 0.32
T_i/T_{tot}	34.0 ± 5.5	34.3 ± 5.4	32.8 ± 5.0	32.9 ± 4.8	33.1 ± 5.2	32.6 ± 4.6
IC, L	2.03 ± 0.51	2.11 ± 0.50	1.43 ± 0.33	1.51 ± 0.31	1.34 ± 0.30	1.38 ± 0.28
Δ IC from rest, L	-	-	-0.61 ± 0.37	-0.60 ± 0.31	-0.70 ± 0.31	-0.73 ± 0.41
IRV, L	1.15 ± 0.43	1.25 ± 0.49	0.31 ± 0.21	0.35 ± 0.19	0.26 ± 0.19	0.25 ± 0.20
$\dot{V}_E/\dot{V}CO_2$	49.3 ± 5.6	49.6 ± 6.6	37.7 ± 6.7	36.9 ± 5.6	37.3 ± 6.7	38.2 ± 5.5
$P_{ET}CO_2$, mmHg	32.6 ± 2.9	33.5 ± 3.2†	37.5 ± 5.7	37.9 ± 5.2	37.8 ± 6.0	38.2 ± 5.5
SpO_2, %	96 ± 2	96 ± 2	93 ± 4	93 ± 4	93 ± 4	93 ± 4
Gastro-esophageal balloon-electrode catheter-derived parameters (n=7)						
EMGdi,rms, μV	59.5 ± 37.1	47.1 ± 26.3	134.2 ± 50.3	117.2 ± 50.9	161.2 ± 63.8	149.8 ± 65.8
EMGdi,rms/$\dot{V}_E$, μV · L · min^{-1}	4.3 ± 2.3	3.5 ± 2.0	5.2 ± 2.9	4.8 ± 2.7	5.6 ± 2.9	5.4 ± 3.2
EMGdi,rms%max	28 ± 11	23 ± 9	64 ± 12	59 ± 17	76 ± 15	74 ± 17
Reasons for stopping exercise						
Breathlessness, n	-	-	-	-	11	9
Leg discomfort, n	-	-	-	-	2	4
Breathlessness and leg discomfort, n	-	-	-	-	5	2
Other, n	-	-	-	-	2	5

Values are mean ± SD. $\dot{V}O_2$, rate of oxygen uptake; $\dot{V}CO_2$, rate of carbon dioxide production; HR, heart rate; $\dot{V}_E$, minute ventilation; V_T, tidal volume; f_R, breathing frequency; V_T/T_i, mean tidal inspiratory flow, where T_i represents inspiratory time; T_i/T_{tot}, inspiratory duty cycle, where T_{tot} represents total breath duration; IC, inspiratory capacity; Δ, exercise-induced change; IRV, inspiratory reserve volume; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent for carbon dioxide; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; SpO_2, oxygen saturation by pulse oximetry; EMGdi,rms, root mean square of the crural diaphragm electromyogram; EMGdi,rms%max, EMGdi,rms expressed as a percentage of maximum voluntary EMGdi,rms. *$p<0.05$ and †$p<0.01$ *vs.* placebo.

Table 6.5. Baseline characteristics of the participants with advanced COPD and chronic breathlessness syndrome that did (Responders) and did not (Non-Responders) report a decrease in breathlessness intensity of ≥1 Borg unit during exercise at isotime after taking oral morphine *vs.* placebo.

Parameter	Responders	Non-responders
Male:Female, n	9:2	6:3
Age, yrs	63.3 ± 6.9	64.0 ± 7.8
Height, cm	171.7 ± 8.9	165.7 ± 6.4
Body mass, kg	69.1 ± 15.7	74.6 ± 13.0
Body mass index, kg · m^{-2}	23.4 ± 4.9	27.3 ± 2.0
Smoking history, pack years	57.6 ± 18.0	61.3 ± 28.7
$P_{ac}CO_2$, mmHg [range] †	38.2 ± 3.6 [32 – 45]	37.1 ± 3.0 [35 – 44]
Incremental cycle exercise time, min	6.2 ± 2.4	6.8 ± 3.2
Peak incremental power output, watts (% predicted)	38.6 ± 20.0 (26 ± 12)	36.7 ± 15.4 (27 ± 9)
Peak incremental $\dot{V}O_2$, ml · kg · min^{-1} (% predicted)	12.8 ± 2.8 (53 ± 14)	12.5 ± 2.5 (57 ± 15)
GOLD stage, 3:4	5:6	6:3
Post-bronchodilator pulmonary function		
FEV_1, % predicted	32 ± 7	40 ± 9
FEV_1/FVC, %	33 ± 6	40 ± 14
TLC, % predicted	129 ± 17	123 ± 16
RV, % predicted	227 ± 71	206 ± 34
FRC, % predicted	180 ± 42	167 ± 35
IC, % predicted	70 ± 19	75 ± 12
D_LCO, % predicted††	59 ± 27	65 ± 15
sRaw, % predicted†††	1131 ± 687	992 ± 924
Breathlessness and health status		
mMRC score, 0-4	2.8 ± 0.75	3.1 ± 0.33
BDI focal score, out of 12	4.3 ± 1.9	3.3 ± 2.0
Oxygen cost diagram, % full scale	40 ± 18	38 ± 12
CAT score, out of 40	22.4 ± 7.7	19.9 ± 4.6
CAT breathlessness item, out of 5	4.2 ± 0.9	4.1 ± 0.9
CAT activity limitation item, out of 5	2.9 ± 1.6	3.8 ± 1.1
HADS score, out of 42	12.9 ± 6.8	11.2 ± 5.4
COPD Medication Summary		
LABA + LAMA, n	2	2
LABA + LAMA + ICS, n	8	6
LABA + LAMA + PI, n	0	1
LABA + LAMA + ICS + PI, n	1	0

Values are means ± SD. †n=10 for responder and 8 for non-responder. ††n=10 for responders and 7 for non-responders. †††n=9 for both responders and non-responders. $P_{ac}CO_2$, partial pressure of carbon dioxide in arterialized capillary blood; $\dot{V}O_2$, rate of oxygen uptake; GOLD, Global Initiative for Obstructive Lung Disease; FEV_1, forced expiratory volume in 1-sec; FEV_1/FVC, forced expiratory volume in 1-sec to forced vital capacity ratio; TLC, total lung capacity; RV, residual volume; FRC, functional residual capacity; IC, inspiratory capacity; D_LCO, diffusing capacity of the lung for carbon monoxide; sRaw, specific airway resistance; mMRC, modified Medical Research Council Dyspnoea Scale; BDI, Baseline Dyspnoea Index; CAT, COPD Assessment Test; HADS, Hospital Anxiety and Depression Scale; LABA, long-acting β_2 agonist; LAMA, long-acting muscarinic antagonist; ICS, inhaled corticosteroid; PI, phosphodiesterase inhibitor.

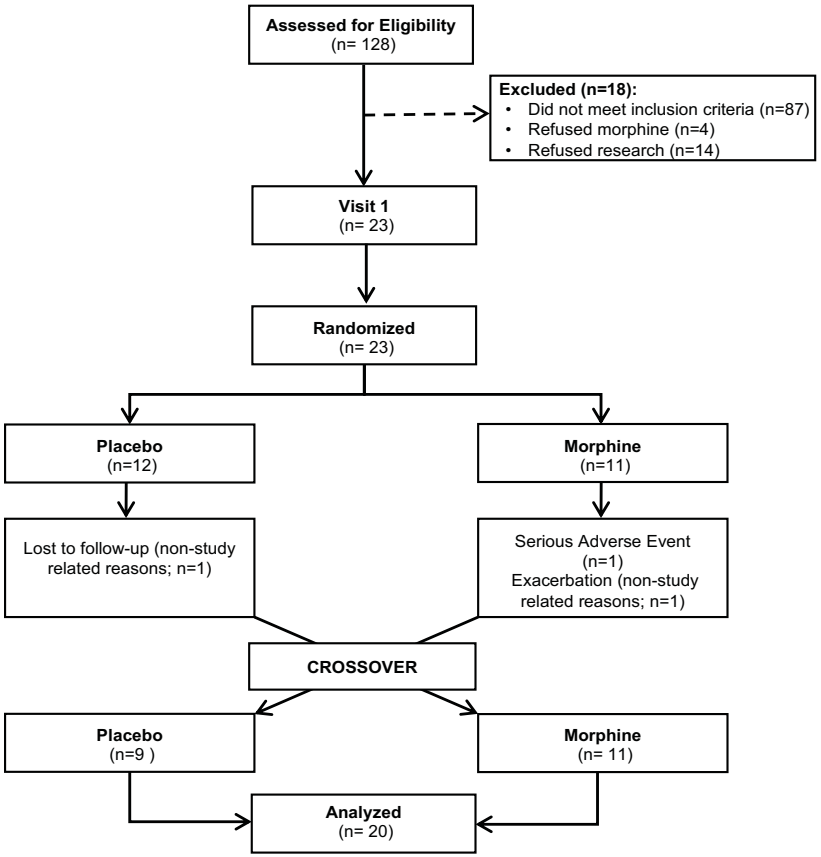

Figure 6.1. Consort diagram of the study population.

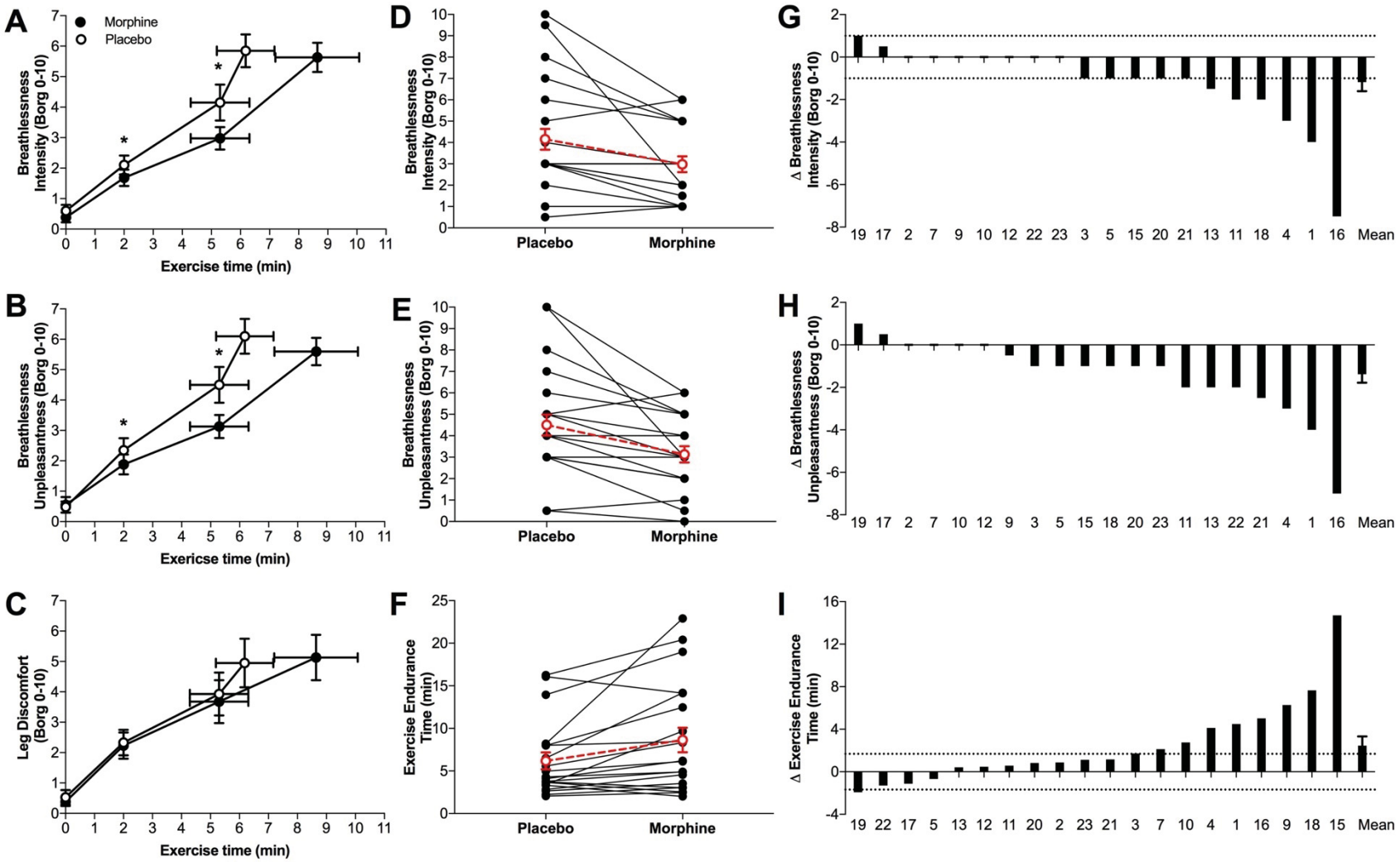

Figure 6.2. Effect of immediate-release oral morphine *vs.* placebo on exertional breathlessness and exercise endurance in adults with advanced COPD and chronic breathlessness syndrome. Mean±SEM (**A**) breathlessness intensity ratings, (**B**) breathlessness unpleasantness ratings and (**C**) leg discomfort ratings at rest and during constant-load cycle exercise testing at 75% of peak incremental power output. Individual participant post-dose values and post-dose differences in (**D,G**) breathlessness intensity ratings during exercise at isotime, (**E,H**) breathlessness unpleasantness ratings during exercise at isotime and (**F,I**) exercise endurance time, where red symbols with dashed horizontal lines in panels **D**, **E** and **F** denote mean±SEM. Dashed horizontal lines in panels **G** and **I** denote minimally clinically important difference for breathlessness intensity [Ries, 2005 #774] and exercise endurance time (205). Δ, post-dose difference (i.e., morphine minus placebo). *p<0.05 versus placebo.

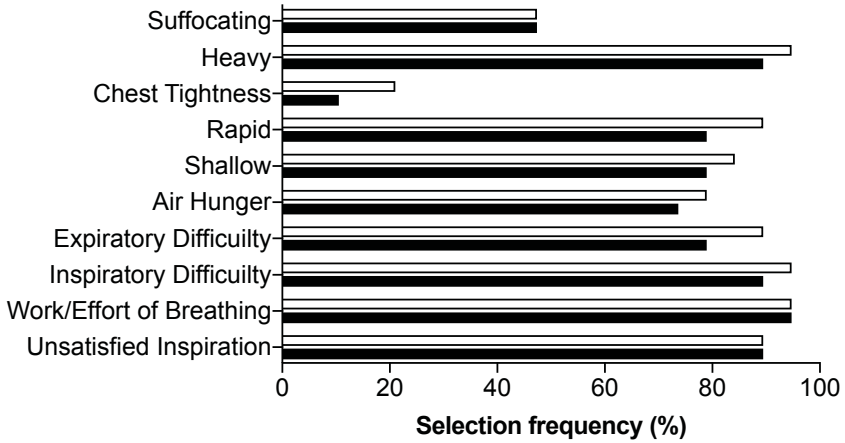

Figure 6.3. Effect of immediate-release oral morphine (closed bars) *vs.* placebo (open bars) on the selection frequency of breathlessness descriptors at the symptom-limited peak of constant-load cycle exercise testing in adults with advanced COPD and chronic breathlessness syndrome.

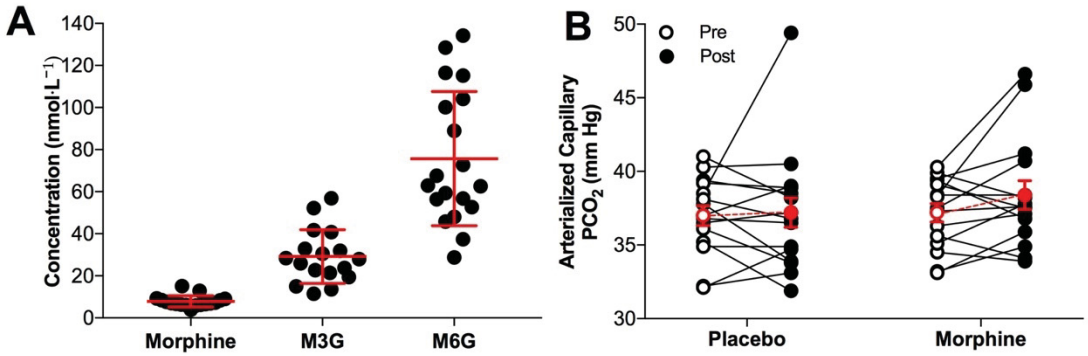

Figure 6.4. Effect of immediate-release oral morphine on blood biochemistry parameters in adults with advanced COPD and chronic breathlessness syndrome. (**A**) Individual participant (closed circles; n=19) and mean±SEM (red lines) plasma morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) concentrations measured 30-min after taking oral morphine. (**B**) Arterialized capillary PCO2 measurements made at rest before and 30-min after taking oral morphine and placebo among individual participants (n=14), where red symbols with dashed horizontal lines denote mean±SEM.

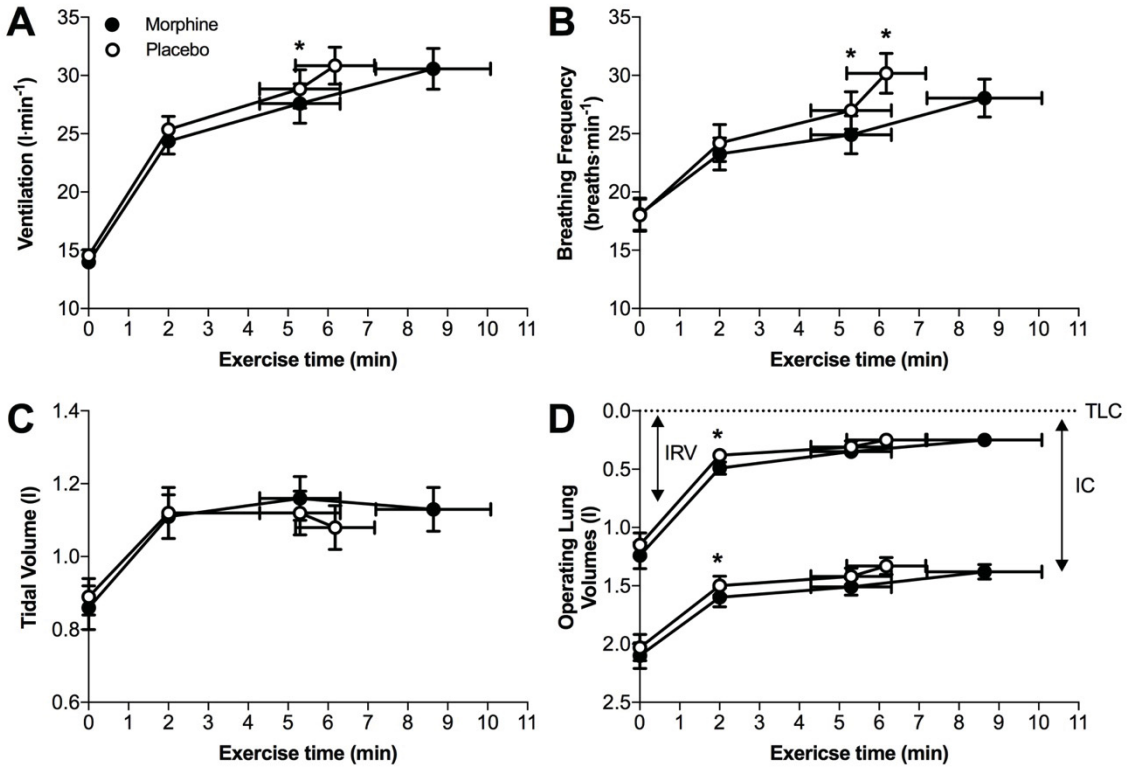

Figure 6.5. Effect of immediate-release oral morphine *vs.* placebo on (**A**) minute ventilation, (**B**) breathing frequency, (**C**) tidal volume and (**D**) dynamic operating lung volume responses during constant-load cycle exercise testing at 75% of peak incremental power output in adults with advanced chronic obstructive pulmonary disease and chronic breathlessness syndrome. IRV, inspiratory reserve volume; IC, inspiratory capacity; TLC, total lung capacity. Values are mean±SEM. *p<0.05 *vs.* placebo.

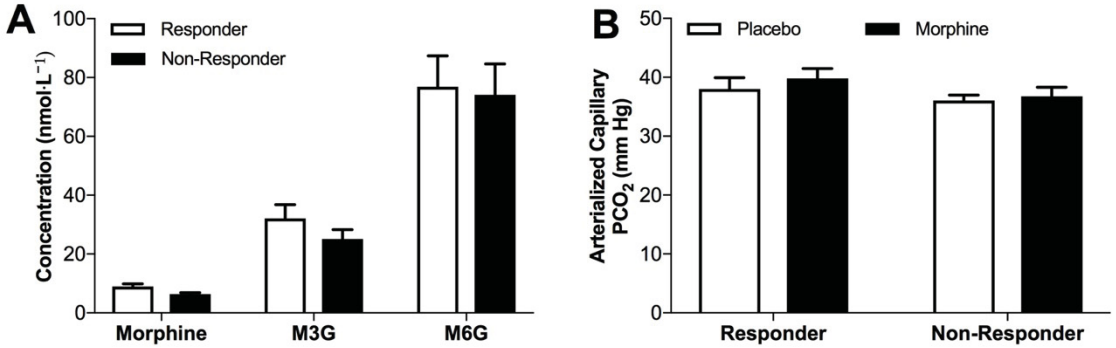

Figure 6.6. Effect of immediate-release oral morphine *vs.* placebo on blood biochemistry parameters in adults with advanced COPD and chronic breathlessness syndrome that did (Responders [n=11]) and did not (Non-Responders [n=9]) report a decrease in breathlessness intensity of ≥1 Borg unit during exercise at isotime after ingestion of morphine *vs.* placebo. (**A**) Plasma morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) concentrations measured 30-min after taking oral morphine in responders and non-responders. (**B**) Arterialized capillary PCO_2 measurements made at rest 30-min after taking oral morphine and placebo in responders (n=8) and non-responders (n=6). Values are mean±SEM.

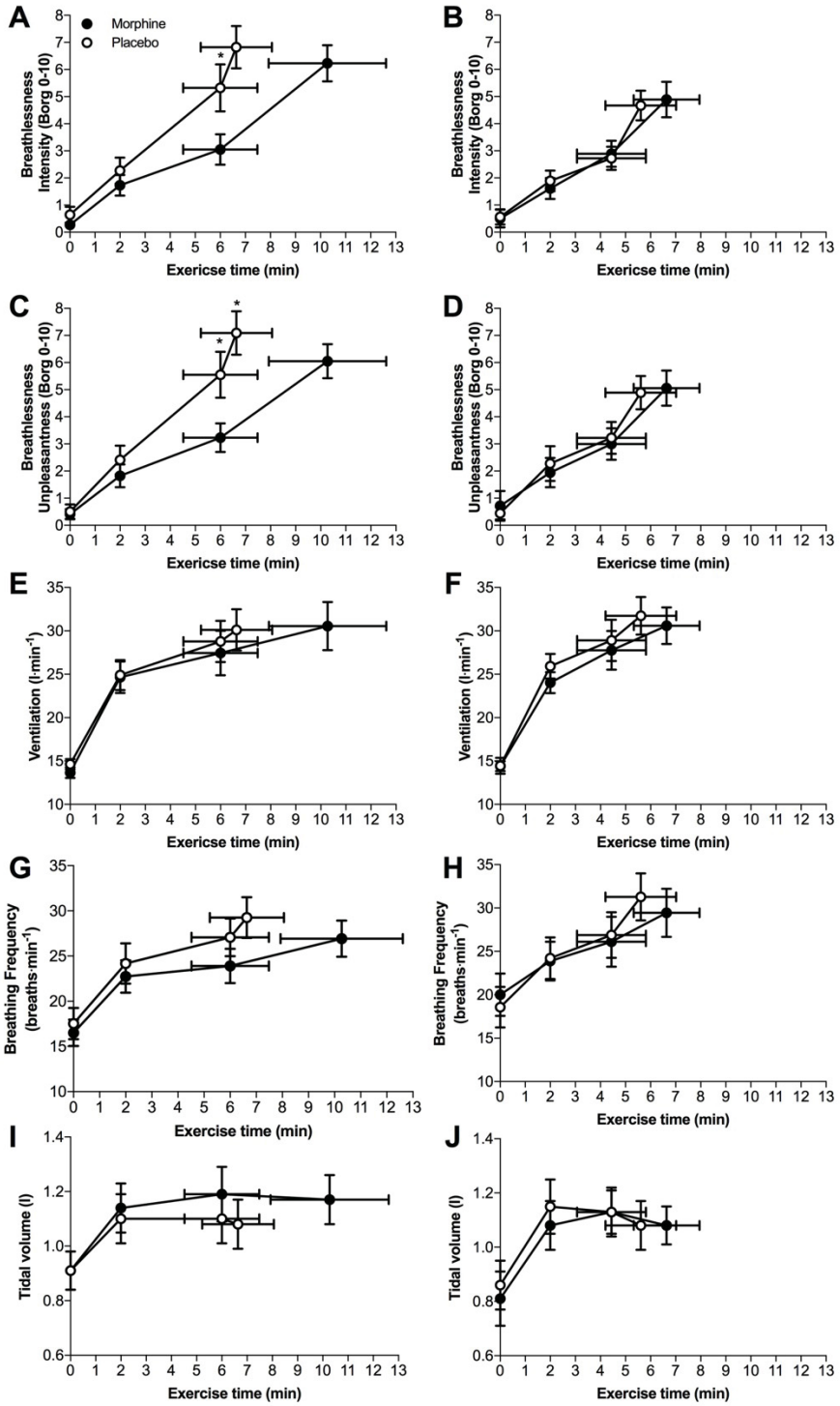

Figure 6.7. Effect of immediate-release oral morphine *vs.* placebo on (**A,B**) breathlessness intensity, (**C,D**) breathlessness unpleasantness, (**E,F**) minute ventilation, (**G,H**) breathing frequency and (**I,J**) tidal volume responses during constant-load cycle exercise testing at 75% of peak incremental power output in adults with advanced COPD and chronic breathlessness syndrome that did (Responders [n=11]; panels a,c,e,g,i) and did not (Non-Responders [n=9]; panels **B,D,F,H,J**) report a decrease in breathlessness intensity of ≥1 Borg unit during exercise at isotime after taking oral morphine *vs.* placebo. Values are mean±SEM. *p<0.05 *vs.* placebo.

CHAPTER 7: MANUSCIPRT 4 "EFFECT OF VAPORIZED CANNABIS ON EXERTIONAL BREATHLESSNESS AND EXERCISE ENDURANCE IN ADVANCED COPD: A RANDOMIZED CONTROLLED TRIALS"

PREFACE TO MANUSCRIPT 6: Targeting neuromechanical uncoupling of the respiratory system – Inhaled vaporized cannabis for the management of exertional breathlessness and exercise intolerance in adults with COPD.

The results of **Chapter 6 (Study 3)** demonstrated that single-dose administration of immediate-release oral morphine may alleviate exertional breathlessness and improve exercise endurance in adults with COPD by modulating central neural processing of breathlessness and by decreasing neural respiratory drive and central corollary discharge. Despite these promising therapeutic effects of morphine, additional therapies for the management of breathlessness and exercise intolerance in adults with COPD should be investigated, particularly in light of the relatively low responsiveness rate to opioid therapy (i.e., only 55% of adults with COPD reported clinically-meaningful relief of breathlessness during exercise after morphine *vs.* placebo in **Study 3**). Therefore, in keeping with the neurophysiological construct of breathlessness presented earlier (see **Figure 2.2** and **Figure 3.1**), enhanced airway function may be yet another potential "target" for breathlessness relief. For this reason, as described in **Chapter 3**, inhaled vaporized cannabis was selected as a pharmacological therapy with the potential to decrease exertional breathlessness and improve exercise tolerance by enhancing static and dynamic airway function (therefore decreasing neural respiratory drive and improving neuromechanical coupling of the respiratory system).

Cannabis

Historically, the cannabis plant (*Cannabis sativa L.*) has been used as a therapeutic agent for the management of pain, neurological conditions, inflammation, HIV/AIDS, cancer, anxiety, sleep, convulsions, and asthma (432). The active constituents of the cannabis plant are known as cannabinoids. Delta-9-tetrahydrocannabinol (THC) has been identified as the principle cannabinoid responsible for cannabis' psychoactive and therapeutic effects (433).

In addition to its established analgesic effects, cannabis may also enhance airway function in healthy individuals and in individuals with pulmonary disease. Indeed, Tashkin, *et al.* (18-20, 22) and others (21) have provided evidence that inhalation of smoked cannabis induces bronchodilatation in healthy and asthmatic adults. To date, however, no published study has examined the effects of inhaled cannabis on airway function, exertional breathlessness and exercise endurance in adults with COPD.

Pharmacological mechanisms

THC mediates its effects by binding to cannabinoid receptor type 1 (CB_1) and/or cannabinoid receptor type 2 (CB_2) (434, 435). CB_1 receptors are abundantly expressed in the central and peripheral nervous systems, whereas CB_2 receptors are selectively expressed in peripheral tissues (434, 435).

The mechanism(s) of action underlying THC-induced bronchodilatation have not been fully elucidated, although an anti-cholinergic mechanism has been implicated. In a recent study, Grassin-Delyle*, et al.* (436) evaluated the effects of THC and several other cannabinoids on the

contractions of the isolated human bronchi induced by electrical field stimulation. Remarkably, Grassin-Delyle*, et al.* (436) demonstrated that [1] THC inhibited electrical filed stimulation-induced contractions in a concentration-dependent manner and [2] the bronchodilator effect of THC was antagonized in the presence of a CB_1 receptor inhibitor. Based on these observations, Grassin-Delyle*, et al.* (436) concluded that THC most likely binds to presynaptic CB_1 receptors, thereby inhibiting acetylcholine release and bronchial contraction.

Effect on exertional breathlessness and exercise performance in adults with COPD

No previous study has examined the effects of inhaled vaporized cannabis on airway function, exertional breathlessness and exercise performance in adults with COPD. However, the collective results of Tashkin, *et al.* (18-20, 22) and Grassin-Delyle*, et al.* (436) provide sufficient physiological evidence to support a role of THC as a potential bronchodilator in adults with COPD. Therefore, it is reasonable to postulate that inhaled vaporized THC may reduce exertional breathlessness and improve exercise endurance in adults with COPD much in the same way that traditional bronchodilators do. That is, by inducing bronchodilatation in adults with COPD, inhaled vaporized cannabis may enhance neuromechanical coupling of the respiratory system and delay the onset of critical dynamic mechanical constraints on V_T expansion, therefore leading to relief of exertional breathlessness with concomitant improvements in exercise endurance.

Summary

There is sufficient physiological evidence to support a bronchodilator effect of cannabis in healthy and asthmatic adults. However, the therapeutic effects of cannabis in adults with COPD remain unknown. The following study represents the first randomized controlled trial to evaluate the acute

effects of inhaled vaporized cannabis on airway function, exertional breathlessness and exercise endurance in adults with advanced COPD.

Manuscript

A published version of this manuscript has been included in **Appendix IV**. For this thesis: [1] the information presented in the online supplement of the published manuscript has been included as part of this chapter so that the experimental methods were described in sufficient detail; [2] acronyms have been redefined to meet the *Annals of the American Thoracic Society* guidelines; and [3] references have been renumbered and are included in the combined bibliography at the end of the thesis.

Abdallah *et al*./2018/Effect of Vaporized Cannabis on Exertional Breathlessness and Exercise Endurance in Advanced COPD: A Randomized Controlled Trial/Annals of the ATS/15/1146-1158.

Annals of the American Thoracic Society is an official journal of the American Thoracic Society.

TITLE PAGE

Effect of vaporized cannabis on exertional breathlessness and exercise endurance in advanced COPD: A randomized controlled trial

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Running head: Cannabis, breathlessness & exercise capacity in COPD

Descriptor number: 9.14
Keywords: dyspnea, functional capacity, marijuana, chronic obstructive pulmonary disease

ABSTRACT

Rationale. A series of studies conducted ~40 years ago demonstrated an acute bronchodilator effect of smoked cannabis in healthy and asthmatic adults. However, the acute effects of vaporized cannabis on airway function in adults with advanced chronic obstructive pulmonary disease (COPD) remain unknown. **Objective.** To test the hypothesis that inhaled vaporized cannabis would alleviate exertional breathlessness and improve exercise endurance by enhancing static and dynamic airway function in COPD. **Methods.** In a randomized controlled trial of 16 adults with advanced COPD (mean±SD forced expiratory volume in 1-sec, 36±11% predicted), we compared the acute effect of 35 mg inhaled vaporized cannabis (18.2% delta-9-tetrahdyrocannabinol (THC), <0.1% cannabidiol (CBD)) *vs.* 35 mg of a placebo control cannabis (CTRL; 0.33% THC, <0.99% CBD) on physiological and perceptual responses during cardiopulmonary cycle endurance exercise testing; spirometry and impulse oscillometry (iOS) at rest; and cognitive function, psychoactivity and mood. **Results.** Compared with CTRL, cannabis had no effect on: breathlessness intensity ratings during exercise at isotime (cannabis, 2.7±1.2 Borg units *vs.* CTRL, 2.6±1.3 Borg units); exercise endurance time (cannabis, 3.8±1.9 min *vs.* CTRL, 4.2±1.9 min); cardiac, metabolic, gas exchange, ventilatory, breathing pattern and/or operating lung volume parameters at rest and during exercise; spirometry and iOS-derived pulmonary function test parameters at rest; and cognitive function, psychoactivity and mood. **Conclusion.** Single-dose inhalation of vaporized cannabis had no clinically meaningful positive or negative effect on airway function, exertional breathlessness and exercise endurance in adults with advanced COPD. Clinical trial registration: NCT03060993.

Abstract word count: 241

INTRODUCTION

In adults with chronic obstructive pulmonary disease (COPD), pathophysiological abnormalities in static and dynamic airway function (e.g., hyperinflation) are mechanistically linked to breathlessness and exercise intolerance (104, 437), which are independently associated with increased morbidity and mortality (2, 29). Despite intensive management of their underlying pulmonary pathophysiology with inhaled bronchodilators and anti-inflammatory agents, 46-91% of adults with advanced COPD suffer from persistent and disabling breathlessness at rest and on minimal exertion (3, 420, 438, 439). Therefore, it is important to identify adjunct therapies to help alleviate breathlessness and improve exercise tolerance in advanced COPD.

Amidst widespread changes in the regulatory landscape of recreational and medicinal use of cannabis, there has been a growing interest in understanding the therapeutic potential of its main cannabinoid constituent, delta-9 (Δ^9)-tetrahydrocannabinol (THC) (440), which provides symptomatic relief of acute and chronic pain across a range of malignant and non-malignant diagnoses (441).

Mechanistically, THC exerts its effects by binding to cannabinoid type 1 (CB_1) and to a lesser extent type 2 (CB_2) receptors, which are differentially expressed in the central and peripheral nervous systems as well as in some peripheral tissues, including the lungs (434, 435). Grassin-Delyle, *et al.* (436) demonstrated that THC induced a concentration-dependent inhibition of cholinergic contraction in human airway smooth cells *via* activation of prejunctional CB_1 receptors. In keeping with these observations, Vachon, *et al.* (21) and Tashkin, *et al.* (18-20, 22) demonstrated an acute bronchodilator effect of smoked cannabis (~500 mg of 1-2% THC) in

healthy and asthmatic adults that was comparable in magnitude and duration of effect to the β_2-adrenergic receptor agonist, isoproterenol. While no study has evaluated the bronchodilator and therapeutic potential of inhaled cannabis in COPD, a large cross-sectional study of adults with COPD reported a positive association between cannabis use and forced expiratory volume in 1-sec (FEV_1) and forced vital capacity (FVC), even after adjusting for cigarette smoking history (442). Together, these studies suggest that the endocannabinoid system may represent a novel therapeutic target to enhance static and dynamic airway function, with attendant improvements in exertional breathlessness and exercise tolerance in advanced COPD.

The aim of this randomized controlled trial was to evaluate the acute effect of inhaled vaporized cannabis *vs.* a placebo control (CTRL) on exertional breathlessness and exercise endurance in symptomatic adults with advanced COPD. We hypothesized that single-dose inhalation of vaporized cannabis *vs.* CTRL would alleviate exertional breathlessness and improve exercise endurance by enhancing static and dynamic airway function.

MATERIALS AND METHODS

Study design. This single-center, randomized, double-blind, crossover trial (ClinicalTrials.gov NCT03060993) consisted of two intervention periods separated by a washout period of ≥5 days. The study protocol and informed consent form received regulatory approval from Health Canada (Control number: 202091) and ethics approval from the Research Institute of the McGill University Health Centre (COPD-THC / 2017-2614). The study took place at the McConnell Centre for Innovative Medicine of the McGill University Health Centre, and participants were recruited from the Montreal Chest Institute (Montréal, QC, Canada).

After providing written and informed consent, participants completed a screening/familiarization visit followed by two randomly assigned treatment visits. Participants were permitted to take their respiratory medication(s) as prescribed prior to each study visit. *Visit 1* included: medical history and clinical assessment; urine toxicology screening for use of delta-9 (Δ^9)-tetrahdyrocannabinol (THC) in preceding 15 days; evaluation of participant-reported breathlessness using the modified Medical Research Council Dyspnoea scale (378), the Baseline Dyspnea Index (379) and the Oxygen Cost Diagram (379); evaluation of health status using the COPD Assessment Test (381); evaluation of anxiety and depression using the Hospital Anxiety and Depression Scale (382); post-bronchodilator (400 μg salbutamol) pulmonary function testing, including spirometry, impulse oscillometry (iOS), plethysmography, and single-breath diffusing capacity of the lung for carbon monoxide (D_LCO); and a symptom-limited incremental cardiopulmonary cycle exercise test (CPET) to determine peak power output (PPO), defined as the highest power output that the participant was able to sustain for ≥30-sec. Following a rest period of ≥20-min, participants completed a symptom-limited constant-load cycle CPET at 75% of PPO for familiarization purposes, and were then familiarized with the Foltin Puff Procedure (443) used to administer vaporized cannabis and control (CTRL) during subsequent treatment visits.

Upon arrival to the laboratory for *Visits 2 and 3*, participants reported the time of day that they used their short-acting inhaled β_2-agonist bronchodilator, if applicable. If a participant used his/her short-acting β_2-agonists bronchodilator prior the start of *Visit 2*, then they were required to use it again prior to the start of *Visit 3*, making sure to take the same number of puffs at the same time of day. Prior to the administration of cannabis or CTRL at *Visits 2 and 3*, a urine sample was collected for toxicology screening of THC; cognitive function was assessed using the Mini-Mental

State Exam (MMSE) (444); psychoactivity and mood were assessed using the Psychoactive State and Mood Visual Analogue Scales (VAS, 100-mm) (445), respectively; and spirometry and iOS were performed. Participants then inhaled vaporized cannabis or CTRL. Two minutes thereafter, participants completed tests of cognitive function (444), psychoactivity and mood (445) followed immediately by spirometry, iOS and a symptom-limited constant-load cycle CPET at 75% of PPO. Intravenous blood samples for measurement of plasma concentrations of THC, trans-Δ^9-tetrahydrocannabinol-9-acid A (THCA), 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC), 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) and cannabidiol (CBD) were obtained before and 2-, 30-, 75- and 180-min after inhalation of cannabis and CTRL.

Participants. Participants included men and women aged ≥40 yrs with Global Initiative for Obstructive Lung Disease stage 3 or 4 COPD (424). Exclusion criteria were as follows: cigarette smoking history <10 pack-years; change in dosage and/or frequency of administration of COPD medication(s) in preceding 4-weeks; exacerbation of COPD in preceding 6-weeks; presence of unstable medical condition(s) other than COPD that could contribute to breathlessness and/or exercise intolerance; important contraindications to CPET (e.g., abnormal 12-lead electrocardiogram); recent diagnosis of lung cancer; use of ketoconazole, levodopa, sildenafil, and/or high-dose opioids (i.e., ≥30 mg of oral morphine equivalents/day); positive urine toxicology for THC; positive urine pregnancy test for women of childbearing age; and self-reported history of (i) hepatic or renal impairment, (ii) epilepsy or convulsions, (iii) psychiatric disturbance(s) other than depression and/or anxiety, and (iv) allergy/sensitivity to cannabis.

Intervention. Participants received 35 mg of cannabis (Tilray House Blend-active, THC 18.2%, CBD <0.1%; Tilray, Nanaimo, BC, Canada) or 35 mg of a placebo control (CTRL, Tilray House Blend-control, THC 0.33%, CBD 0.99%; Tilray, Nanaimo, BC, Canada) administered using the Volcano Digit® vaporizer (Storz and Bickel America, Inc., Oakland, CA, USA). Vaporization of cannabis was preferentially selected over smoked and oral administration to limit exposure of our participants with advanced COPD to the noxious particulates in cannabis smoke, while maximizing rapid delivery of THC to the tracheobronchial tree.

Procedures.

Vaporization of cannabis and placebo. Thirty-five mg of dried plant cannabis and CTRL material were dispensed into the Volcano Digit® filling chamber (Storz and Bickel America, Inc.) by the McGill University Health Centre's research pharmacist. The filling chamber was placed in the vaporizer at a heating temperature and filling time of 190°C and 30-sec, respectively. Approximately 5.5L of the vaporized compounds were collected in a balloon fitted with a mouthpiece and a one-way valve (Storz and Bickel America, Inc.), allowing the vapor to remain in the balloon until inhalation. A filling volume of 5.5L was estimated based on the Volcano Digit's® airflow of 11 L/min and a filling time of 30-sec.

To standardize the administration of vaporized cannabis and CTRL, participants inhaled the contents of the balloon using the Foltin Puff Procedure until the balloon was completely empty (443). Briefly, participants were instructed to "hold the balloon with one hand and put the mouthpiece in your mouth", "inhale for 5-sec", "hold vapor in your lungs for 10-sec", "exhale and wait for 40-sec before repeating puff cycle" (443).

Pulmonary function testing. Spirometry, iOS, plethysmography (*Visit 1* only) and D_LCO (*Visit 1* only) were performed with participants seated using automated equipment and according to recommended techniques (363, 385-387). Measurements were referenced to predicted normal values (388, 389).

Cardiopulmonary exercise testing. Exercise tests were conducted on an electronically braked cycle ergometer (Lode Corival, Lode B.V. Medical Tech., Groningen, The Netherlands) using a computerized CPET system (Vmax Encore^{TM} 29C). Incremental CPETs consisted of a steady-state rest period of ≥6-min, followed by 1-min of unloaded pedaling, and then 10 W/min increases in power output to the point of symptom-limitation. Constant-load CPETs consisted of a steady-state rest period of ≥6-min, followed by 1-min of unloaded pedaling, and then a step increase in power output to 75% of PPO maintained to the point of symptom-limitation. Cardiac, metabolic, breathing pattern and gas exchange parameters were collected and analyzed as previously described (427). Inspiratory capacity (IC) maneuvers were performed at rest, every 2-min during CPET, and at end-exercise (392). Measurements of PPO, peak oxygen uptake ($\dot{V}O_{2peak}$) and peak heart rate were referenced to the predicted normal values of Jones and colleagues (368). Using Borg's modified 0-10 category ratio scale (367), participants rated the intensity and unpleasantness of their breathlessness, as well as the intensity of their leg discomfort at rest, every 2-min during CPET, and at end-exercise. Participants verbalized their main reason(s) for stopping exercise; quantified the percentage contribution of breathlessness and leg discomfort to exercise cessation; and identified qualitative phrases that best described their breathlessness at end-exercise (46).

Blood biochemistry. Plasma THC, THCA, TH-COOH, 11-OH-THC and CBD were analyzed by high-performance liquid chromatography (HPLC) with mass spectrometric (MS/MS API 5000) detection (Algorithme Pharma, Laval, QC, Canada). Briefly, 200 μL of human plasma was denatured with internal standards of THC, THCA, 11-OH-THC, THC-COOH, and CBD. The supernatant was transferred into a clean glass culture tube, evaporated, reconstituted in an organic solvent, transferred into a 96-well plate and stored at 4°C until injection on an HPLC. An Ascentis® Express C18 2.7 μm analytical column was used (Sigma-Aldrich, Oakville, ON).

Cannabis-related side effects and adverse events. The presence or absence of cough induced by inhalation of cannabis and/or CTRL was documented. Cognitive function was evaluated using the MMSE (444), which includes 11 questions that test five areas of cognition: orientation; attention; verbal recall; comprehension; and naming. Psychoactivity was evaluated using the Psychoactive State 100-mm visual analogue scale (VAS), with 0 defined as "nothing at all" and 100 defined as "strongest effect" (445). Using this scale, participants were asked to rate the severity of the following psychoactive symptoms: "down", "anxious", "hungry", "sedated", "impaired", "drunk", "stoned", "high", "good drug effect", "bad drug effect" and "like drug effect". Mood was similarly evaluated using a 100-mm VAS, with participants asked to rate their feelings of being: sad *vs.* happy; anxious *vs.* relaxed; jittery *vs.* calm; bad *vs.* good; paranoid *vs.* self-assured; and fearful *vs.* unafraid (445).

Participant's blinded treatment preference. Each participant's blinded treatment preference was assessed at the end of *Visit 3* by asking the following questions: [1] Did you feel that you were less breathless during exercise at one treatment visit compared to the other? If 'YES', which treatment

visit did you feel less breathless during exercise? [2] Did you feel that exercising was easier during one treatment visit compared to the other? If 'YES', which treatment visit did you feel that exercising was easier? [3] On which treatment visit do you think you received cannabis and why?

Outcomes.

Primary outcomes. The primary outcome was the post-treatment difference in breathlessness intensity ratings during exercise at isotime, defined as the highest equivalent 2-min interval of exercise completed by a given participant during each of the constant-load CPETs. The co-primary outcome was the post-treatment difference in exercise endurance time (EET), defined as the duration of loaded pedaling during constant-load CPET. The constant-load cycle CPET was selected over other exercise test modalities (e.g., endurance shuttle walking test), as it is generally regarded as the most responsive exercise testing modality in the evaluation of interventional efficacy in COPD, particularly as it relates to exertional breathlessness and EET (234).

Secondary outcomes. Plasma concentrations of THC, THCA, 11-OH-THC, THC-COOH and CBD; MMSE; psychoactive state VAS; mood effect VAS; spirometry and iOS-derived parameters; physiological and perceptual parameters measured at rest, at standardized submaximal times during constant-load CPETs, and at end-exercise; reasons for stopping exercise; percentage contribution of breathlessness and leg discomfort to exercise cessation; qualitative descriptors of breathlessness at end-exercise; and participant's blinded treatment preference.

Sample size. Using a two-tailed paired subject formula with α=0.05, β=0.80 and an expected effect size of 0.80 (393), we estimated that at least 15 participants were needed to detect a minimal

clinically important difference (MCID) of ±1 Borg unit in breathlessness intensity during exercise at isotime (204) and of ±101-sec in EET (205) after inhalation of vaporized cannabis *vs.* CTRL.

Randomisation. Participants were randomized in a 1:1 ratio according to a computer-generated block randomization schedule (Block size = 4) prepared by a third-party statistician not involved in the trial.

Blinding. Except for the research pharmacist, all investigators, staff and participants were blinded to treatment allocation/randomization schedule. During treatment visits, the dried plant cannabis or CTRL material was maintained in the Volcano Digit® filling chamber and never revealed to the investigators or participants. The vapors produced by cannabis and CTRL were colorless.

Statistical methods. An independent Data Safety Monitoring Committee reviewed un-blinded data for participant safety; no interim analysis for efficacy was done. All participants who completed both cannabis and CTRL arms of the trial were included in the analysis. Linear mixed-models regression with random intercepts was used to analyze post-treatment differences in EET as well as in all physiological and perceptual responses to constant-load CPET, accounting for period and sequence effects. Post-treatment differences in the percentage contribution of breathlessness and leg discomfort to exercise cessation were analyzed using two-tailed paired t-tests. Individual reasons for stopping exercise, and individual descriptors of breathlessness at end-exercise were analyzed using the chi-squared test. Data were analyzed using SAS statistical package, version 9.4 (SAS Institute Inc., Cary, NC, USA) and SigmaStat, version 3.5 (Systat

Software Inc., San Jose, CA, USA). Statistical significance was set at $p<0.05$ and values are reported as mean±SEM unless stated otherwise.

RESULTS

Participants were recruited from March 2018 to May 2018. Eighteen of 31 participants assessed for eligibility were randomized (**Figure 7.1**). One of these 18 participants voluntarily withdrew between *Visits 1* and *2*, while another participant was excluded following an adverse event (*see below*). Baseline characteristics of the 16 participants who completed the trial are presented in **Table 7.1** and **Table 7.2**. Twelve of the 16 participants had a self-reported cannabis smoking history of <1 joint in their lifetime. The other 4 participants had a mean±SD self-reported cannabis smoking history of 34±99 joint years (range: 1.4 – 392), where a joint year was calculated as the number joints smoked per day multiplied by the number of years of smoking. Six of the 16 participants used their short-acting β_2-agonist bronchodilator 212±101 min and 250±77 min prior to *Visits 2 and 3*, respectively.

Time course of vaporization and post-treatment PFTs and CPET. There was no statistically significant difference in the mean±SD number of puffs (cannabis, 7.3±2.1 puffs *vs.* CTRL, 7.3±2.4 puffs; p=0.774 by two-tailed, paired t-test) and the time required to inhale cannabis and CTRL (cannabis, 7.2±2.1 min *vs.* CTRL, 7.1±2.5 min; p=0.744 by two-tailed, paired t-test). The time from the end of vaporization to the start of iOS (mean±SD cannabis, 6.5±2.4 min *vs.* CTRL, 6.9±2.2 min; p=0.542 by two-tailed, paired t-test) and the start of spirometry (cannabis, 10.8±2.7 min *vs.* CTRL, 11.3±2.6 min; p=0.567 by two-tailed, paired t-test) was not significantly different between treatments. The time from the end of vaporization to the start of CPET was not

significantly different between treatments (cannabis, 32.5±5.6 min *vs.* CTRL, 31.9±6.4 min; p=0.517 by two-tailed, paired t-test).

Primary outcomes. Compared with CTRL, cannabis had no effect on breathlessness intensity ratings at isotime or on EET (**Figure 7.2**, **Table 7.3**). There was no period or sequence effect on our primary outcomes. Four participants had a cannabis-induced decrease in breathlessness intensity ratings at isotime by the MCID of ≥1 Borg unit (responders) compared with the remaining 12 participants who did not (non-responders) (**Figure 7.2D** and **G**). Two participants had a cannabis-induced increase in EET by the MCID of ≥101-sec compared with the remaining 14 participants who did not (**Figure 7.2F** and **I**). A significant negative correlation was observed between cannabis-induced changes in breathlessness intensity ratings at isotime and in EET (**Figure 7.3**).

Secondary outcomes.

Pulmonary function. Compared with CTRL, cannabis had no effect on spirometry and iOS-derived pulmonary function parameters at rest (**Figure 7.4**, **Table 7.4**).

Physiological and perceptual responses to exercise. Compared with CTRL, cannabis had no effect on cardiac, metabolic, gas exchange, ventilatory, breathing pattern, operating lung volume, breathlessness unpleasantness and leg discomfort responses at rest or during exercise (**Figure 7.2**, **Figure 7.3** and **Figure 7.6**, **Table 7.3**). The locus of symptom limitation (**Table 7.3**), the relative contributions of breathlessness and leg discomfort to exercise cessation (**Table 7.3**), and the

selection frequency of breathlessness descriptors at end-exercise (**Figure 7.5**) were not different after inhalation of cannabis *vs.* CTRL.

Blood biochemistry. Plasma THC levels were ~17 and 44 times higher after inhalation of cannabis *vs.* CTRL at the 2- and 30-min post-treatment time periods, respectively. Plasma TH-COOH levels were ~16 times higher after inhalation of cannabis *vs.* CTRL at each of the 2-, 30-, 75- and 180-min post-treatment time periods (**Table 7.5**). Peak plasma THC, THCA and 11-OH-THC levels during the cannabis condition, and of THC and CBD during the CTRL condition were achieved 2-min post-treatment. Peak plasma THC-COOH levels were achieved 30-min following cannabis and CTRL conditions (**Figure 7.8**, **Table 7.5**). Compared to the pre-treatment condition, inhaled cannabis increased plasma THCA and 11-OH-THC levels at 2-, 30- and 75-min post-treatment, whereas inhaled CTRL had no effect (**Table 7.5**). Compared to the pre-treatment condition, inhaled CTRL increased plasma CBD levels at 2-, 30- and 75-min post-treatment, whereas inhaled cannabis had no effect (**Table 7.5**).

Cannabis-related side effects and adverse events. None of the participants coughed following inhalation of CTRL. By contrast, 6 participants coughed following inhalation of cannabis, with 5 of these 6 participants reporting clinically significant worsening of exertional breathlessness at isotime by ≥1 Borg unit (**Figure 7.2G** and **Figure 7.3**).

Measures of cognitive function, psychoactivity and mood were not significantly different after inhalation of vaporized cannabis *vs.* CTRL (**Figure 7.9**, **Figure 7.10** and **Table 7.6**). Compared to the pre-treatment condition, inhalation of cannabis was associated with modest and statistically

significant: decreases in ratings of anxiety; and increases in ratings of feeling drunk, feeling stoned, feeling high, experiencing good drug effects, experiencing bad drug effects, and liking the drug effects (**Table 7.6**). In contrast, psychoactivity and mood ratings were not different before *vs.* after inhalation of CTRL.

A participant experienced vasovagal syncope during the 2-min venous blood-sampling period during the cannabis visit. After a few hours of rest while under medical observation, the participant was permitted to go home. Both the study physician and data safety committee determined that this adverse event was most likely due to the blood-sampling procedure itself and not inhalation of vaporized cannabis.

Participant's blinded treatment preference. Of the 12 participants who identified that their breathlessness during CPET was lower during one treatment period *vs.* the other, 6 volunteered a preference for cannabis and 6 volunteered a preference for CTRL. Of the 13 participants who identified that exercise felt easier during one treatment period *vs.* the other, 6 volunteered a preference for cannabis and 7 volunteered a preference for CTRL. Twelve of the 16 participants correctly identified the visit at which they received cannabis, and provided the following reason(s) for their choice: feeling high (n=4); feeling lightheaded/dizzy (n=1); noticeable smell/taste (n=4); cough/throat irritation (n=2); felt more anxious (n=1); felt more calm (n=1); felt more breathless (n=1); felt less breathless (n=2); felt that exercise capacity was better (n=1); felt better overall (n=3).

DISCUSSION

This randomized controlled trial is the first to demonstrate that single-dose inhalation of vaporized cannabis *vs.* CTRL had no effect on exertional breathlessness, exercise endurance and airway function in symptomatic adults with advanced COPD receiving dual or triple inhalation therapy for management of their underlying pulmonary pathophysiology.

We administered 35 mg of dried herbal cannabis containing 18.2% THC, a dose comparable to that used in earlier studies by Vachon, *et al.* (21) and Tashkin, *et al.* (18-20, 22) wherein smoked, aerosolized and orally administered THC induced bronchodilation in adults with and without asthma. Despite using a similar dose, inhaled vaporized cannabis did not enhance static and dynamic airway function in our participants with advanced COPD.

We offer the following explanations for the lack of effect of inhaled vaporized cannabis *vs.* CTRL on airway function and, by extension, exertional breathlessness and EET in our trial. First, previous studies reporting bronchodilation following administration of smoked cannabis used "blended natural marijuana" assayed at 1 or 2% THC (19, 20, 22). It is unclear if these cannabis preparations were devoid of other cannabinoids (e.g., CBD, cannabinol (CBN)) that may have had a direct bronchodilator effect and/or facilitated the bronchodilator effect of THC. However, this is unlikely as large doses (up to 1,200 mg) of orally administered CBD and CBN, in the absence of THC, did not induce bronchodilation in healthy men when compared to placebo (446). Second, previous studies that have demonstrated a bronchodilator effect of smoked cannabis used a uniform smoking procedure that consisted of "smoking deeply" over 2-4 seconds followed by a 15-sec breathhold (19, 20, 22). To standardize drug delivery, we utilized the Foltin puff procedure where participants

were instructed to inhale the vaporized cannabis for 5-sec, and to hold the vapor in their lungs for 10-sec. It is possible that relatively shallower inhalations and shorter breathholding times used in our trial might have diminished the potential positive effects of inhaled THC on static and dynamic airway function in our participants. Third, adults with COPD have abnormal airway geometry and fewer terminal bronchioles compared to their healthy counterparts (447-449). Therefore, limited delivery of vaporized THC into the airways and lungs of our participants may explain our null results. Structural abnormalities of the tracheobronchial tree in our participants may also account for the lower observed peak plasma THC levels of ~14 ng/mL *vs.* ~45 ng/mL reported by Ware*, et al.* (450) in adults with neuropathic pain following single-dose inhalation (smoked) of a comparatively low dose of 25 mg of dried herbal cannabis containing 9.4% THC. Our relatively low peak plasma THC levels may also reflect the vaporization temperature of 190°C used in this trial. Pomahacova*, et al.* (451) reported that vaporizing dried herbal cannabis at 230°C *vs.* 185°C produced a vapor with a 3-fold higher yield of THC. Finally, all of our participants were receiving inhaled dual or triple therapy for management of their COPD, while 6 participants used their short-acting inhaled β_2-agonist (SABA) bronchodilator 3.5±1.7 hrs and 4.2±1.3 hrs prior to *Visits 2 and* 3, respectively. It is unlikely that the SABA used by 6 of our 16 participants significantly altered the effect of inhaled vaporized cannabis airway physiology, breathlessness and EET, particularly as the duration of efficacy of the SABA is 3-4 hrs. Indeed, we found no significant effect of inhaled vaporized cannabis *vs.* CTRL on spirometry and iOS-derived pulmonary function parameters at rest in COPD participants that used their SABA *vs.* those that did not.

We observed a negative correlation between the cannabis-induced change in exertional breathlessness intensity ratings at isotime and EET. We identified 4 cannabis responders

(participants with a cannabis-induced relief of exertional breathlessness at isotime by the MCID of ≥1 Borg unit) and 12 non-responders. Importantly, 5 of the non-responders coughed following inhalation of vaporized cannabis and reported clinically significant worsening of their exertional breathlessness at isotime following inhalation of cannabis *vs.* CTRL (**Figure 7.2G**). Tashkin, *et al.* (18) similarly reported that inhalation of 5 and 10 mg of aerosolized THC provoked a cough in 4 of 5 asthmatics, two of whom exhibited THC-induced bronchospasm. Therefore, the cough induced by vaporized cannabis in 5 of the 12 non-responders could have masked a potentially positive effect of inhaled vaporized cannabis *vs.* CTRL on airway function, exertional breathlessness and EET in our participants. The mechanisms mediating the THC-induced cough-reflex are not fully understood. Previous studies have demonstrated that CB_1 receptor agonists may inhibit or induce bronchospasm; this dual effect of CB_1 receptor activation on bronchial responsiveness is dependent on cholinergic tone (452). As all of our participants were receiving at least dual inhalation therapy for management of their COPD, we cannot rule out the possibility that differences in bronchial smooth muscle tone may have contributed to the observed heterogeneity in the cough-reflex elicited by inhalation of vaporized cannabis. Future studies should evaluate the effects of inhaled vaporized cannabis on airway function, exertional breathlessness and EET in adults with COPD receiving anticholinergic bronchodilator therapy *vs.* those that are not.

Neuroimaging studies evaluating the effects of cannabis on pain have demonstrated altered activity in brain regions (453) associated with negative affect and implicated in the perception of breathlessness (454), particularly its affective (unpleasantness) dimension. To this end, cannabis could alter the central perception of breathlessness and improve EET by reducing negative affect

and/or increasing feelings of euphoria. Indeed, earlier studies demonstrating cannabis-induced bronchodilation often reported concomitant psychoactive effects, particularly a feeling of being "high" within minutes of treatment administration (18-20, 22). Importantly, these studies reported a greater degree of intoxication following administration of smoked cannabis (i.e., the degree of "high" was rated ~6 on a 7-point scale) relative to that observed in our participants following inhalation of vaporized cannabis (i.e., the degree of "high" was rated ~4.8 mm on a 100-mm VAS) (19). The low peak plasma THC levels achieved in our study likely account for the relatively modest effects of inhaled vaporized cannabis on psychoactivity. Nevertheless, we observed a modest but significant within-treatment effect (i.e. pre-to-post) of inhaled vaporized cannabis on psychoactivity, including decreased ratings of anxiety and increased ratings of feeling high, drunk and stoned. It is possible that the potentially positive effects of this altered psychoactive state on exertional breathlessness and EET may have been confounded by the cough-reflex and its effect on exertional breathlessness exhibited in some of our participants following inhalation of vaporized cannabis. Moreover, a preliminary study of 5 adults with mild-to-moderate COPD by Pickering, *et al.* (455) reported that sublingual administration of Sativex® - a cannabis-based medicinal extract containing both THC and CBD - reduced the selection frequency of respiratory descriptors associated with air hunger, an inherently unpleasant form of breathlessness (456). By contrast, we observed no effect of inhaled vaporized cannabis *vs.* CTRL on unpleasantness ratings of exertional breathlessness and the selection frequency of breathlessness descriptors at end-exercise.

Earlier studies demonstrating cannabis-induced bronchodilation in healthy and asthmatic adults often reported a concomitant increase in heart rate that was sustained for ~60 min post-inhalation

(18, 19, 22). In contrast to these findings, we did not observe a significant effect of inhaled vaporized cannabis *vs.* CTRL on heart rate, presumably due to the relatively low plasma levels of THC.

Methodological considerations. The generalizability of our results is restricted to a small and relatively homogeneous group of clinically stable and symptomatic adults with advanced COPD. Larger randomized clinical trials with more participants are needed to draw definitive conclusions regarding the effect of inhaled vaporized cannabis on exertional breathlessness, EET and cardiopulmonary physiologic parameters in adults with COPD.

We caution against the extrapolation of our results to other doses, modes (e.g. smoked, oral), types (e.g. various THC:CBD ratios) and regimes (e.g. repeat-dose) of cannabis dispensation in this patient population.

In our study, inhaled vaporized cannabis had a modest but significant within-treatment effect on some measures of psychoactivity. Future studies should utilize existing cannabinoid preparations (e.g., CBD) that do not affect psychoactivity but act on cannabinoid receptors to assess changes in airway function, exertional breathlessness and EET in COPD.

The dried herbal cannabis material used in the CTRL arm of our trial may not have represented a "true" placebo as it contained trace amounts of CBD (<1%) that were detected in the plasma 2-min following vaporization. Furthermore, 12 of the 16 participants correctly identified the visit at which they received cannabis, with 4 of these 12 participants citing a noticeable difference in taste/smell of the inhaled vapour between cannabis and CTRL visits. Thus, a placebo devoid of

THC and CBD and with the same taste and smell as the active cannabis should be identified for use in future trials.

Conclusions. In 2015, the American Thoracic Society Marijuana Workgroup highlighted a need for controlled studies to evaluate the clinical effects of inhaled vaporized cannabis on lung disease, sleep and critical illness (440). In response to this call for research, our randomized controlled trial is the first to demonstrate that 35 mg of inhaled vaporized cannabis containing 18.2% THC had no clinically meaningful positive or negative effect on exertional breathlessness, exercise endurance and airway function in symptomatic adults with advanced COPD receiving dual or triple inhalation therapy for management of their underlying pulmonary pathophysiology.

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Author contributions.

S.J.A., B.M.S., M.A.W., J.B. and D.J. contributed to the conception of the study and data collection, analysis and interpretation. M.M. contributed to data collection. P.L. contributed to data analysis. S.J.A and D.J. wrote the manuscript with critical input from all authors. All authors read and approved the final version of the manuscript.

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Table 7.1. Baseline participant characteristics.

Parameter	Value
Male:Female, n	10 : 6
Age, yrs	65.4 ± 7.7 [66; 47-77]
Height, cm	165.6 ± 7.3 [168; 150-175]
Body mass, kg	70.9 ± 11.7 [72; 50-89]
Body mass index, kg · m^{-2}	25.8 ± 11.8 [26.7; 18.6-33.5]
Cigarette smoking history, pack years	63 ± 28 [60; 21-127]
Cannabis smoking history, joint years	34 ± 99 [0; 0-392]
Post-bronchodilator pulmonary function	
FEV_1, L (% predicted)	0.88 ± 0.28 (36±11) [0.98; 0.51-1.53]
FEV_1/FVC, %	31 ± 7 [31; 20-47]
TLC, L (% predicted)	8.10 ± 2.08 (143±42) [7.86; 5.81-13.56]
RV, L (% predicted)	5.04 ± 2.51 (242±123) [4.41; 2.31-11.64]
FRC, L (% predicted)	6.40 ± 2.17 (210±78) [5.85; 4.24-12.32]
IC, L (% predicted)	1.70 ± 0.43 (64±13) [1.79; 0.92-2.24]
D_LCO, ml · min^{-1} · $mmHg^{-2}$ (% predicted)	11.9 ± 3.9 (62±4) [11.7; 4.0-18.8]
sRaw, cmH_2O · L^{-1} · sec^{-2} (% predicted)	40.4 ± 17.3 (900±478) [34.9; 20.5-78.7]
Impulse Oscillometry	
R_5, kPa · L^{-1} · sec	0.51 ± 0.13 [0.49; 0.27-0.82]
R_{20}, kPa · L^{-1} · sec	0.32 ± 0.07 [0.32; 0.24-0.50]
X_5, kPa · L^{-1} · sec	-0.28 ± 0.12 [-0.27; -0.55 - -0.76]
F_{res}, 1 · sec^{-1}	22.83 ± 3.49 [22.43; 17.73-28.06]
A_X, kPa · L^{-1}	2.29 ± 1.11 [2.14; 1.1-4.8]
Breathlessness and health status	
mMRC score, 0-4	2.8 ± 0.5 [3; 2-3]
BDI focal score, out of 12	4.1 ± 1.8 [3; 1-7]
Oxygen cost diagram, % full scale	44 ± 17 [38; 23-81]
CAT score, out of 40	15.7 ± 7.8 [16; 4-28]
HADS score, out of 42	12.3 ± 8.1 [13; 0-31]
COPD medication summary	
LABA + LAMA, n	7
LABA + LAMA + ICS, n	9

Values are mean±SD [median; range]. Cannabis smoking history was calculated as number of joints per day x number of years smoking. GOLD, Global Initiative for Obstructive Lung Disease; FEV_1, forced expiratory volume in 1-sec; FEV_1/FVC, FEV_1-to-forced vital capacity ratio; TLC, total lung capacity; RV, residual volume; FRC, functional residual capacity; IC, inspiratory capacity; D_LCO, diffusing capacity of the lung for carbon monoxide; sRaw, specific airway resistance; R_5 and R_{20}, resistance at 5 Hz and 20 Hz, respectively; X_5, reactance at 5Hz; F_{res}, resonant frequency; A_X, area of reactance; mMRC, modified Medical Research Council Dyspnoea Scale; BDI, Baseline Dyspnoea Index; CAT, Chronic Obstructive Pulmonary Disease Assessment Test; HADS, Hospital Anxiety and Depression Scale; LABA, long-acting β_2 agonist; LAMA, long-acting muscarinic antagonist; ICS, inhaled corticosteroid.

Table 7.2. Physiological and perceptual responses at the symptom-limited peak of incremental cycle exercise testing in adults with advanced chronic obstructive pulmonary disease.

Parameter	Value
$\dot{V}O_2$, ml · kg · min^{-1} (% predicted)	10.9 ± 2.9 (48±13)
HR, beats · min^{-1} (% predicted)	117 ± 13 (67±13)
Breathlessness intensity, Borg 0-10 units	5.2 ± 2.2
Breathlessness unpleasantness, Borg 0-10 units	5.4 ± 2.6
Leg discomfort, Borg units	4.7 ± 1.9
$\dot{V}_E$, L · min^{-1} (% estimated MVV)	29.4 ± 10.5 (96±23)
V_T, L	1.06 ± 0.29
f_R, breaths · min^{-1}	27.9 ± 7.2
Δ IC from rest, L	-0.67 ± 0.40
IRV, L	0.36 ± 0.20
$\dot{V}_E/\dot{V}CO_2$	38.1 ± 5.7
$P_{ET}CO_2$, mmHg	41.8 ± 15.9
SpO_2, %	93 ± 3
Δ SpO_2 from rest, %	-2.2 ± 1.4
Reasons for stopping exercise	
Breathlessness, n	6
Leg discomfort, n	2
Breathlessness and leg discomfort, n	7
Other, n	1

Values are mean±SD. $\dot{V}O_2$, rate of oxygen uptake; HR, heart rate; $\dot{V}_E$, minute ventilation; MVV, maximal voluntary ventilation (estimated as forced expiratory volume in 1-sec x 35); V_T, tidal volume, f_R, breathing frequency; Δ, exercise-induced change; IC, inspiratory capacity; IRV, inspiratory reserve volume; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent for carbon dioxide; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; SpO_2, oxygen saturation by pulse oximetry.

Table 7.3 Effect of inhaled vaporized cannabis (35 mg of dried herbal cannabis containing 18.2% delta-9-tetrahdyrocannabinol (THC) and <0.1% cannabidiol (CBD)) *versus* control (35 mg of dried herbal cannabis containing 0.33% THC and 0.99% CBD) on physiological and perceptual responses at rest, at a standardized submaximal time (isotime) during constant-load cycle exercise testing, and at the symptom-limited peak of constant-load cycle exercise testing in adults with advanced chronic obstructive pulmonary disease.

	Rest		Isotime		Peak	
	Control	**Cannabis**	**Control**	**Cannabis**	**Control**	**Cannabis**
Cycle exercise time, min	-	-	2.4±0.8	2.4±0.8	4.2±1.9	3.8±1.9
Breathlessness intensity, Borg 0-10 units	0.4±0.4	0.7±1.1	2.6±1.3	2.7±1.2	5.1±1.8	5.4±2.0
Breathlessness unpleasantness, Borg 0-10 units	0.5±0.8	0.5±1.0	2.6±1.2	2.8±1.8	5.3±2.2	5.1±2.4
Leg discomfort, Borg units	0.4±0.6	0.7±1.0	2.4±1.7	2.9±1.9	4.6±2.4	4.4±2.6
$\dot{V}O_2$, ml · kg · min^{-1}	4.0±0.6	4.3±0.8	9.8±2.2	9.8±2.5	11.3±2.1	11.0±2.9
$\dot{V}CO_2$, ml · kg · min^{-1}	3.7±0.5	4.0±0.8	9.5 ±3.3	9.8±3.5	11.6±3.0	11.3±3.8
HR, beats · min^{-1}	84±12	86±12	104±12	107±14	112±13	114±18
O_2 pulse, ml O_2 · $beat^{-1}$	3.4±0.5	4.0±2.3	6.7±1.6	7.4±4.5	7.1±1.6	7.7±4.4
$\dot{V}_E$, L · min^{-1}	13.4±2.7	14.3±3.4	26.1±10.1	26.4±8.9	29.5±9.6	29.6±10.0
V_T, L	0.81±0.24	0.77±0.16	1.05±0.26	1.07±0.29	1.11±0.28	1.08±0.30
f_R, breaths · min^{-1}	17.9±6.4	19.6±6.0	25.6±7.4	25.6±7.2	26.8±5.7	27.9±6.7
IC, L	2.08±0.51	2.04±0.60	1.54±0.40	1.48±0.41	1.44±0.44	1.41±0.44
Δ IC from rest, L	-	-	-0.54±0.34	-0.56±0.28	-0.65±0.34	-0.63±0.38
IRV, L	1.27±0.40	1.27±0.46	0.49±0.29	0.41±0.26	0.32±0.24	0.33±0.23
$\dot{V}_E/\dot{V}CO_2$	51.7±6.1	51.6±5.7	39.0±5.0	38.9±4.5	36.2±5.3	37.2±5.1
$P_{ET}CO_2$, mmHg	32.8±3.2	33.1±3.2	37.2±5.0	37.4±4.3	39.0±5.9	38.4±5.3
SpO_2, %	94±5	96±2	93±3	93±3	92±4	93±3
Reasons for stopping exercise						
Breathlessness, n (% contribution)	-	-	-	-	8 (62±34)	7 (61±33)
Leg discomfort, n (% contribution)	-	-	-	-	2 (27±28)	3 (13±29)
Breathlessness and leg discomfort, n	-	-	-	-	4	4
Other, n	-	-	-	-	2	2

Values are mean±SD. $\dot{V}O_2$, rate of oxygen uptake; $\dot{V}CO_2$, rate of carbon dioxide production; HR, heart rate; $\dot{V}_E$, minute ventilation; V_T, tidal volume; f_R, breathing frequency; IC, inspiratory capacity; Δ, exercise-induced change; IRV, inspiratory reserve volume; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent for carbon dioxide; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; SpO_2, oxygen saturation by pulse oximetry.

Table 7.4. Effect of inhaled vaporized cannabis (35 mg of dried herbal cannabis containing 18.2% delta-9-tetrahdyrocannabinol (THC) and <0.1% cannabidiol (CBD)) *versus* control (35 mg of dried herbal cannabis containing 0.33% THC and 0.99% CBD) on spirometry and impulse oscillometry-derived pulmonary function test parameters at rest in adults with advanced chronic obstructive pulmonary disease.

	Control		Cannabis	
	Pre-treatment	**Post-treatment**	**Pre-treatment**	**Post-treatment**
Spirometry				
FVC, L	2.87±0.91	2.93±0.85	2.94±0.91	2.90±0.90
FEV_1, L	0.89±0.26	0.89±0.26	0.89±0.25	0.89±0.24
FEV_1/FVC, %	32±8	31±7	32±9	32±6
$FEF_{25-75\%}$, L · sec^{-1}	0.26±0.06	0.26±0.07	0.26±0.07	0.26±0.07
PEF, L · sec^{-1}	2.81±0.82	2.59±0.87	2.60±0.78	2.62±0.84
Impulse Oscillometry				
R_5, kPa · L^{-1} · sec	0.60±0.18	0.59±0.24	0.60±0.14	0.58±0.17
R_{20}, kPa · L^{-1} · sec	0.34±0.08	0.34±0.13	0.34±0.05	0.33±0.06
X_5, kPa · L^{-1} · sec	-0.35±0.15	-0.34±0.16	-0.35±0.15	-0.34±0.16
F_{res}, 1 · sec^{-1}	23.9±4.1	24.0±5.1	22.6±3.7	23.3±4.2
A_X, kPa · L^{-1}	3.04±1.76	3.04±1.99	2.85±1.55	2.88±1.81

Values are mean±SD. FVC, forced vital capacity; FEV_1, forced expiratory volume in 1-sec; FEV_1/FVC, FEV_1-to-forced vital capacity ratio; $FEF_{25-75\%}$, forced expiratory flow at 25-75% of the FVC maneuver; PEF, peak expiratory flow; R_5 and R_{20}, resistance at 5 Hz and 20 Hz, respectively; X_5, reactance at 5Hz; F_{res}, resonant frequency; A_X, area of reactance.

Table 7.5. Pharmacokinetics of inhaled vaporized cannabis (35 mg of dried herbal cannabis containing 18.2% delta-9-tetrahdyrocannabinol (THC) and <0.1% cannabidiol (CBD)) *versus* control (35 mg of dried herbal cannabis containing 0.33% THC and 0.99% CBD) in adults with advanced chronic obstructive pulmonary disease.

Metabolite	Control					Cannabis				
	Pre-treatment	2-min	30-min	75-min	180-min	Pre-treatment	2-min	30-min	75-min	180-min
THC, ng · mL^{-1}	-	0.82±0.55	0.05±0.10	-	-	-	13.91±6.16**	2.18±0.96*	0.79±0.44	0.14±0.16
THCA, ng · mL^{-1}	-	-	-	-	-	-	0.70±0.27	0.30±0.15	0.09±0.12	-
11-OH-THC, ng · mL^{-1}	-	-	-	-	-	-	0.87±0.71	0.56±0.41	0.29±0.22	0.06±0.10
TH-COOH, ng · mL^{-1}	-	0.08±0.22	0.21±0.28	0.14±0.23	0.10±0.21	-	1.54±1.38**	3.05±1.95**	2.23±1.57**	1.37±1.04**
CBD, ng · mL^{-1}	-	1.36±0.84	0.19±0.19	0.04±0.10	-	-	-	-	-	-

Values are mean±SD. THC, Δ^9-tetrahydrocannabinol; THCA, trans-Δ^9-tetrahydrocannabinol-9-acid A; 11-OH-THC, 11-hydroxy-Δ^9-tetrahydrocannabinol; TH-COOH, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol; CBD, cannabidiol. **p<0.0001 and *p<0.01 in control *vs.* cannabis.

Table 7.6. Effect of inhaled vaporized cannabis (35 mg of dried herbal cannabis containing 18.2% delta-9-tetrahdyrocannabinol (THC) and <0.1% cannabidiol (CBD)) *versus* control (35 mg of dried herbal cannabis containing 0.33% THC and 0.99% CBD) on cognitive function, mood and psychoactivity in adults with advanced chronic obstructive pulmonary disease.

Parameter	Control		Cannabis	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Mini-Mental State Exam, out of 30	29.6±0.5	29.7±0.6	29.4±0.9	29.6±0.8
Mood Effects, 100-mm VAS				
Sad/Happy	89.5±13.5	87.9±12.4	89.1±13.9	89.9±13.3
Anxious/Relaxed	83.7±22.6	89.4±10.8	80.5±23.3	84.7±25.3
Jittery/Calm	85.2±17.8	91.5±8.1	81.4±22.9	86.9±21.1
Bad/Good	91.2±10.4	89.9±10.6	90.1±10.2	89.9±12.5
Paranoid/Self-assured	94.0±6.5	92.8±8.0	90.4±12.8	91.6±11.4
Fearful/Unafraid	90.9±15.3	93.1±8.1	91.9±11.4	94.1±6.4
Psychoactive Effects, 100-mm VAS				
Down	13.2±19.1	11.6±19.3	15.0±19.8	12.4±2.5
Anxious	9.8±12.4	9.1±13.8	17.6±18.0	8.2±1.2*
Hungry	13.2±16.9	16.2±18.9	12.6±16.2	11.4±1.5
Sedated	8.6±17.0	8.5±14.3	8.6±18.1	8.4±8.7
Impaired	5.2±7.9	4.4±4.6	5.5±7.9	8.4±1.2
Drunk	2.5±2.8	3.2±3.7	1.6±1.6	4.5±4.3*
Stoned	2.7±3.1	3.7±3.6	1.6±1.5	6.3±5.6*
High	2.8±3.4	3.8±3.9	1.9±2.1	4.8±4.5*
Good drug effects	2.4±3.2	5.3±7.0	1.8±2.1	17.6±27.8*
Bad drug effects	2.1±3.0	3.2±3.3	1.5±1.8	4.0±4.6*
Do you like the drug effects	2.1±3.1	6.9±12.0	2.1±2.8	15.3±27.7*

Values are mean±SD. VAS, visual analogue scale. *p<0.05 *vs.* pre-treatment within condition.

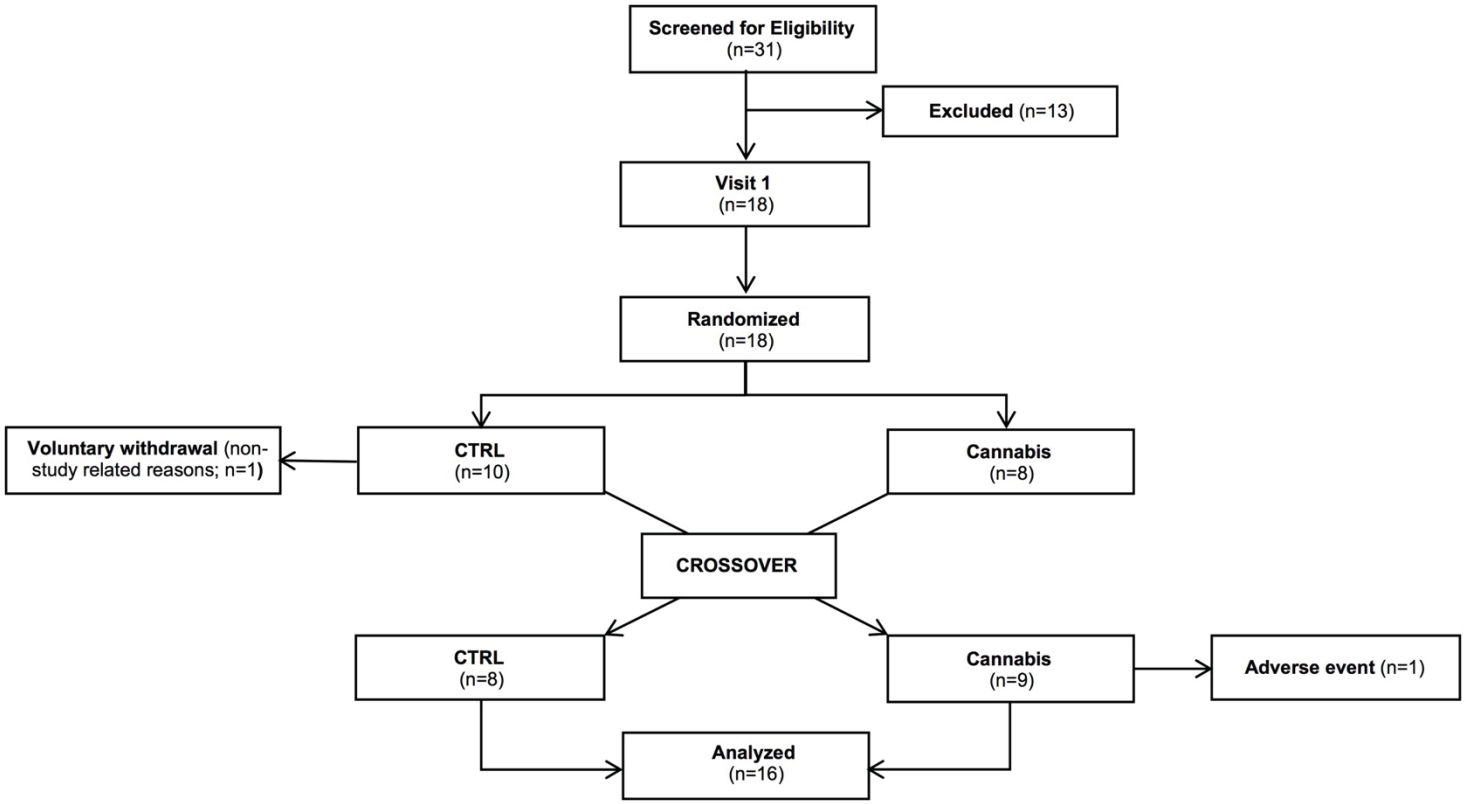

Figure 7.1. Consort diagram of the study population.

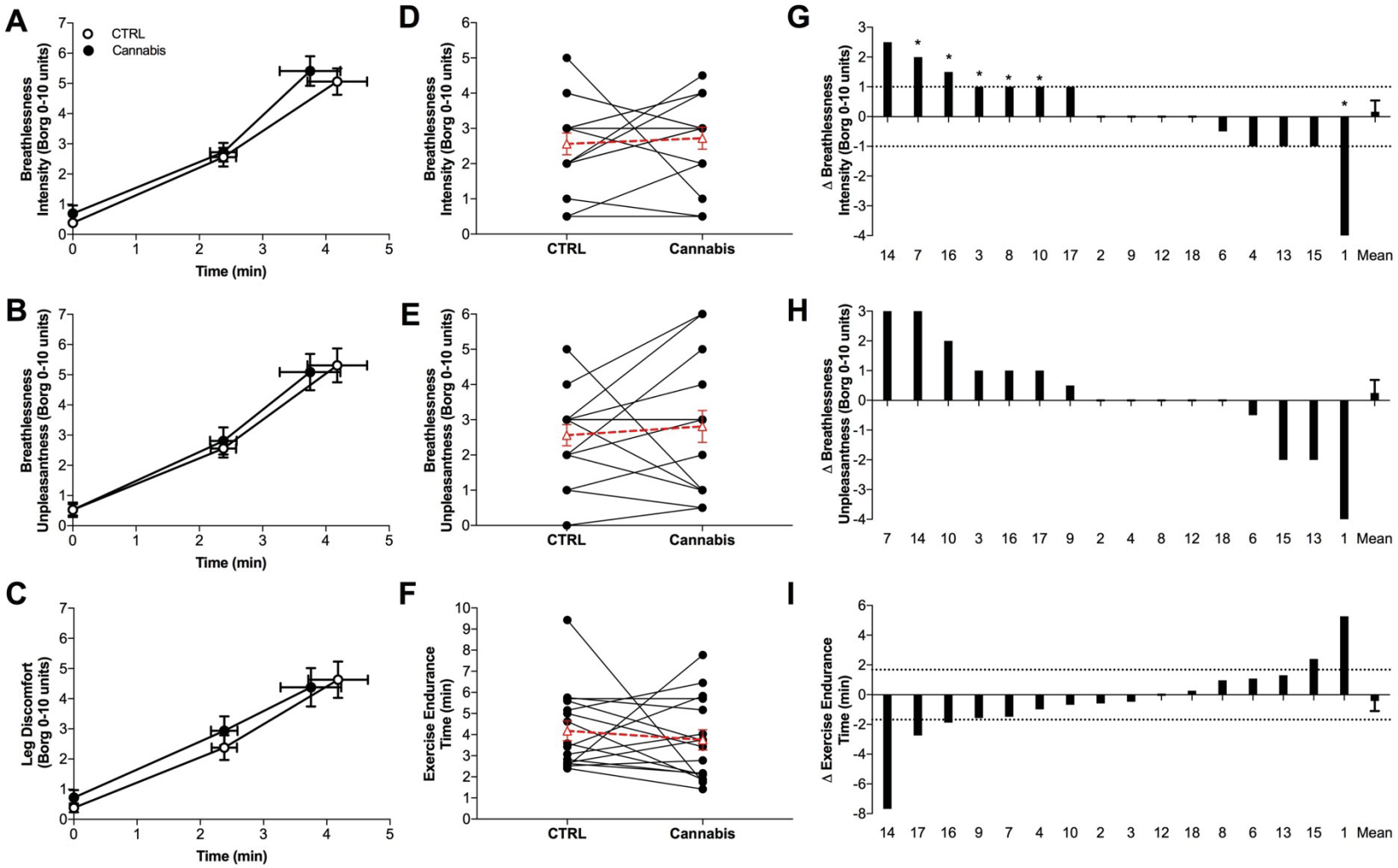

Figure 7.2. Effect of inhaled vaporized cannabis *versus* control (CTRL) on exertional breathlessness and exercise endurance in adults with advanced chronic obstructive pulmonary disease. Mean±SEM breathlessness intensity ratings (**A**), breathlessness unpleasantness ratings (**B**) and intensity ratings of leg discomfort (**C**) at rest and during symptom-limited constant-load cycle exercise testing at 75% of peak incremental power output. Individual participant post-treatment values and post-treatment differences in breathlessness intensity ratings during exercise at isotime (**D and G**), breathlessness unpleasantness ratings during exercise at isotime (**E and H**) and exercise endurance time (**F and I**), where red symbols with dashed horizontal lines in panels **D, E** and **F** denote mean±SEM. Dashed horizontal lines in panels **G** and **I** denote minimally clinically important differences for breathlessness intensity (204) and exercise endurance time (205). Δ, post-treatment difference (cannabis *minus* CTRL). *denotes participants that coughed following inhalation of vaporized cannabis.

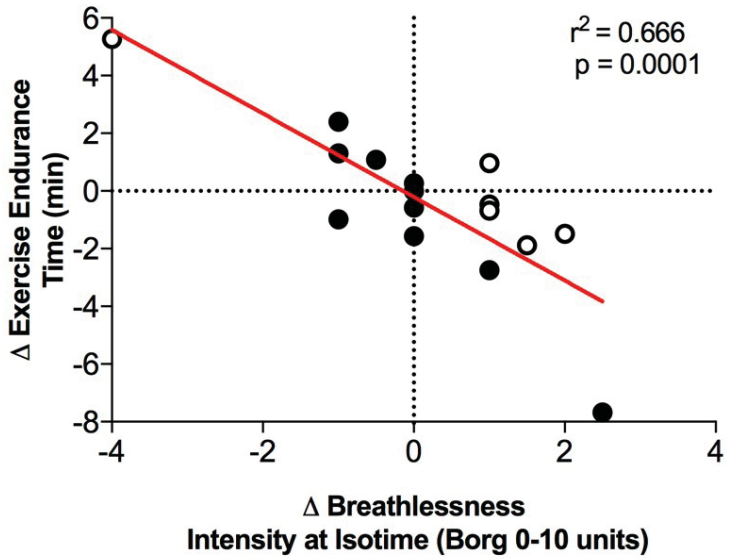

Figure 7.3. Relationship between post-treatment differences in breathlessness intensity ratings during exercise at isotime and exercise endurance time in adults with advanced chronic obstructive pulmonary disease. Open circles denote participants that coughed following inhalation of vaporized cannabis. Δ, post-treatment difference (cannabis *minus* control).

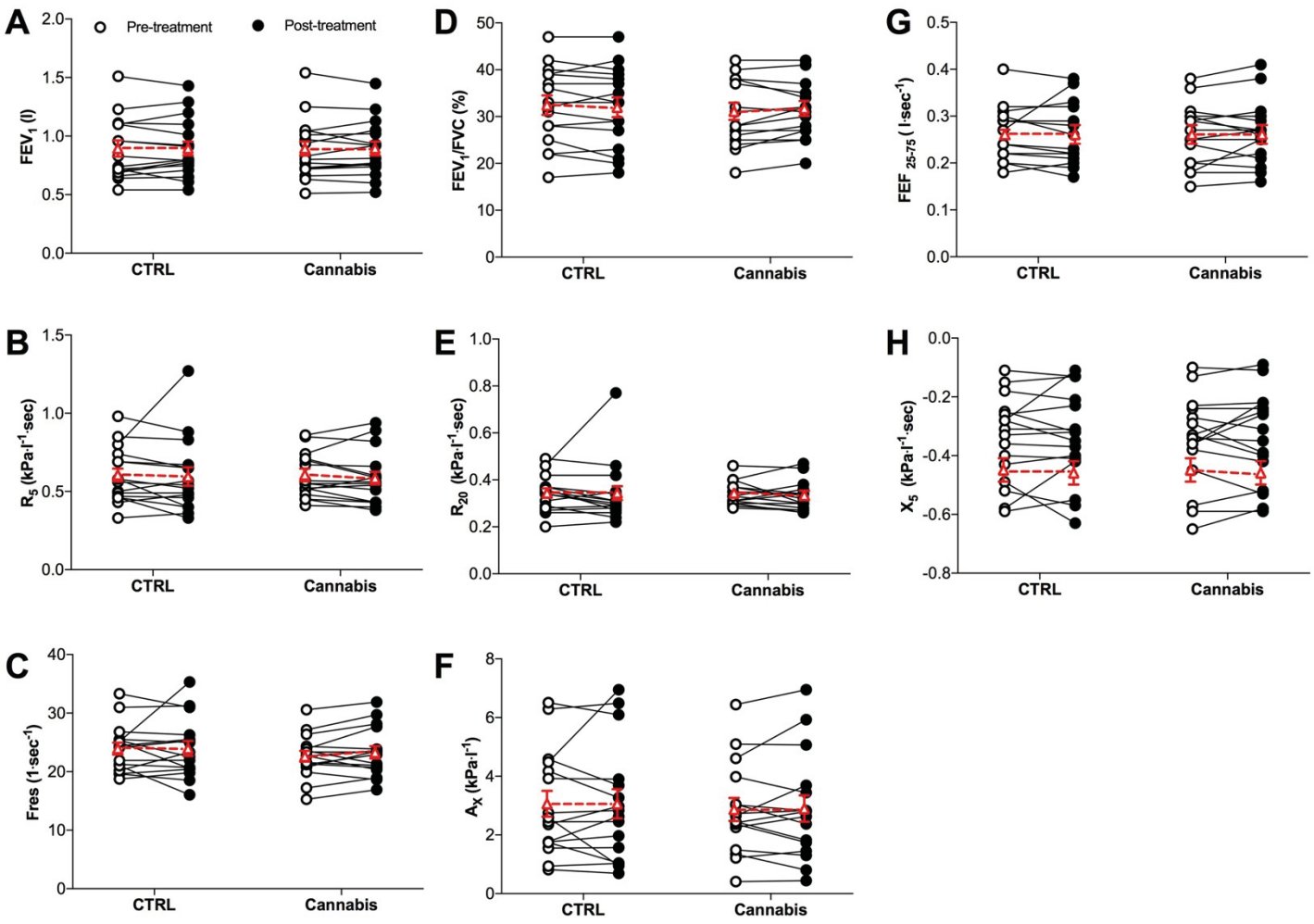

Figure 7.4. Effect of inhaled vaporized cannabis *versus* control (CTRL) on spirometry and impulse oscillometry-derived pulmonary function test parameters in adults with advanced chronic obstructive pulmonary disease. Tests were performed at rest immediately before and after inhalation of vaporized cannabis and CTRL. Values are presented as individual responses, where red symbols with dashed horizontal lines denote mean±SEM. FEV_1, forced expiratory volume in 1-sec; FEV_1/FVC, FEV_1-to-forced vital capacity ratio; FEF_{25-75}, forced expiratory flow at 25-75% of the FVC maneuver; R_5, resistance at 5 Hz; R_{20}, resistance at 20 Hz; X_5, reactance at 5 Hz; F_{res}, resonant frequency; Ax, area of reactance.

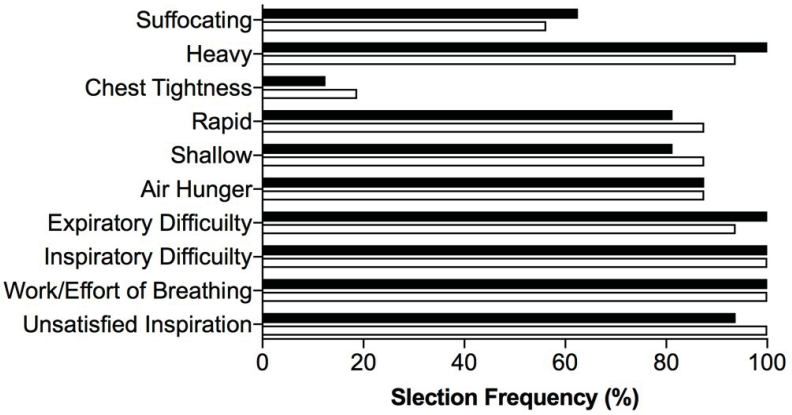

Figure 7.5. Effect of inhaled vaporized cannabis (closed bars) *versus* control (CTRL; open bars) on the selection frequency of breathlessness descriptors at the symptom-limited peak of constant-load cycle exercise testing at 75% of peak incremental power output in adults with advanced chronic obstructive pulmonary disease.

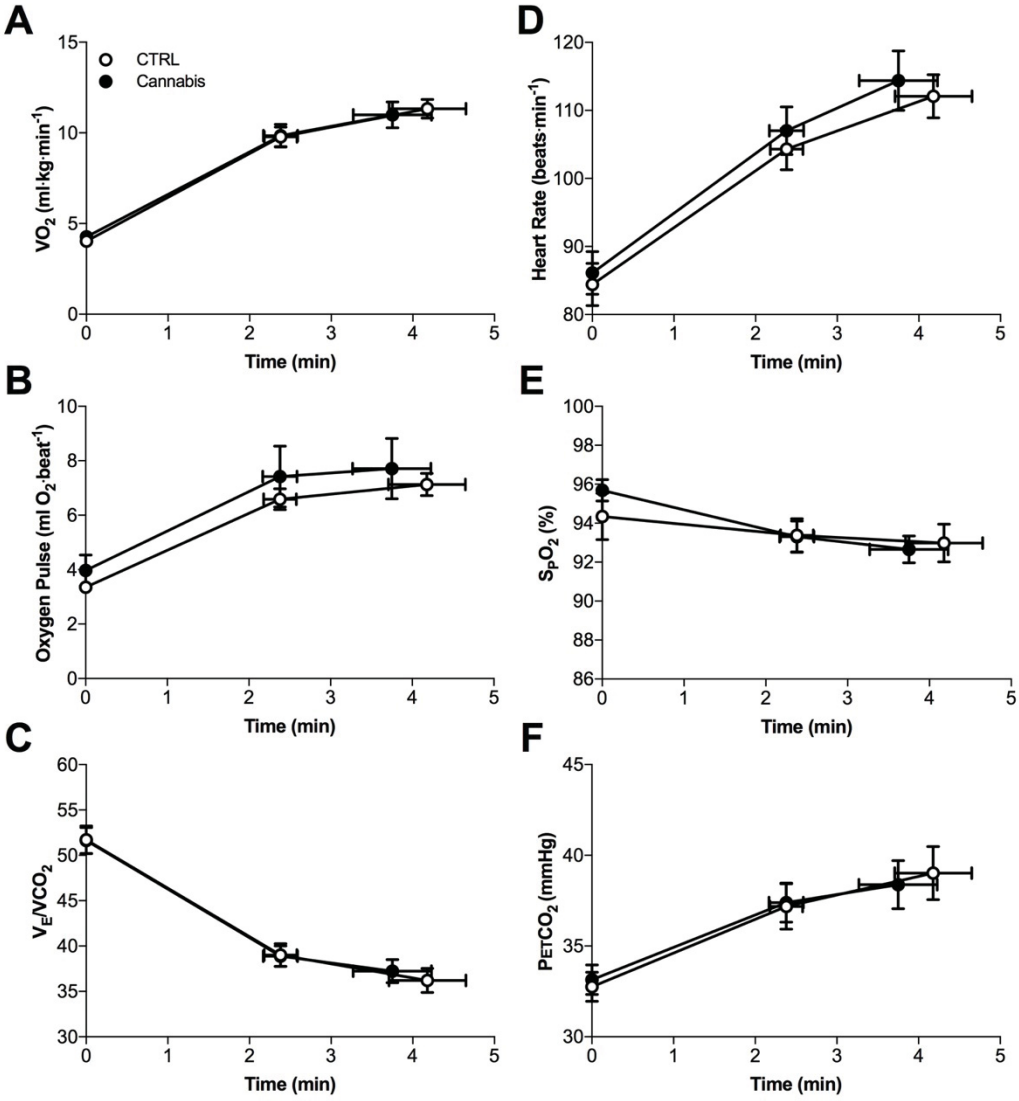

Figure 7.6. Effect of inhaled vaporized cannabis *versus* control (CTRL) on oxygen consumption (**A**), oxygen pulse (**B**), the ventilatory equivalent for carbon dioxide (**C**), heart rate (**D**), oxygen saturation (**E**) and the partial pressure of end-tidal carbon dioxide (**F**) during symptom-limited constant-load cycle exercise testing at 75% of peak incremental power output in adults with advanced chronic obstructive pulmonary disease. Data are presented as mean±SEM.

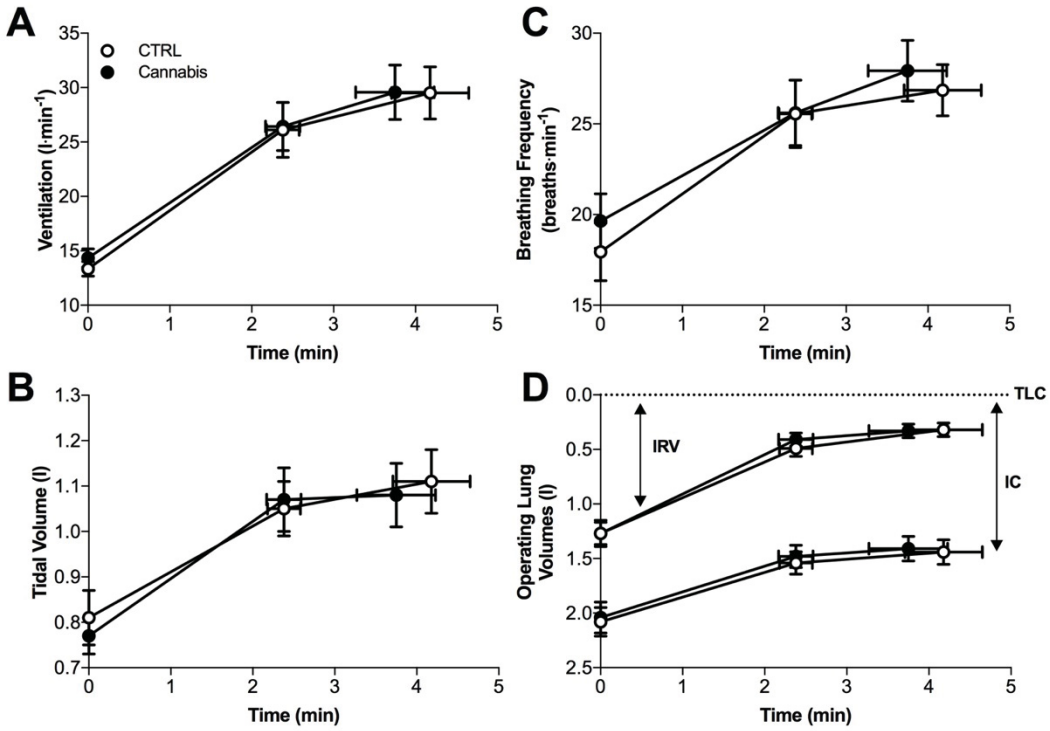

Figure 7.7. Effect of inhaled vaporized cannabis *versus* control (CTRL) on minute ventilation (**A**), tidal volume (**B**), breathing frequency (**C**) and dynamic operating lung volume (**D**) responses during symptom-limited constant-load cycle exercise testing at 75% of peak incremental power output in adults with advanced chronic obstructive pulmonary disease. Data are presented as mean±SEM. IRV, inspiratory reserve volume; IC, inspiratory capacity; TLC, total lung capacity.

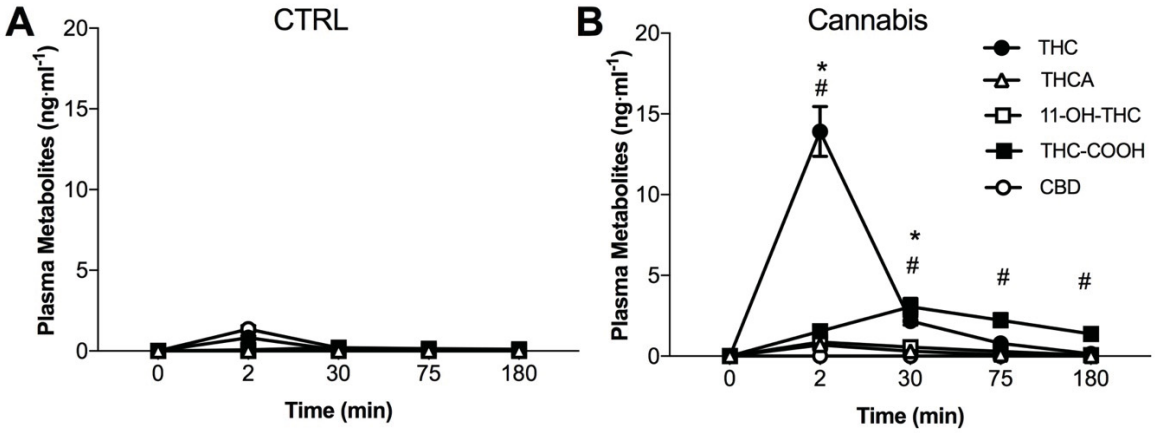

Figure 7.8. Effect of inhaled vaporized cannabis and control (CTRL) on blood biochemistry in adults with advanced chronic obstructive pulmonary disease. Data are presented as mean±SEM. THC, Δ^9-tetrahydrocannabinol; THCA, trans-Δ^9-tetrahydrocannabinol-9-acid A; 11-OH-THC, 11-hydroxy-Δ^9-tetrahydrocannabinol; THC-COOH, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol; CBD, cannabidiol. * and # denote significant difference in CTRL *vs.* THC for plasma THC and THC-COOH, respectively ($p<0.05$).

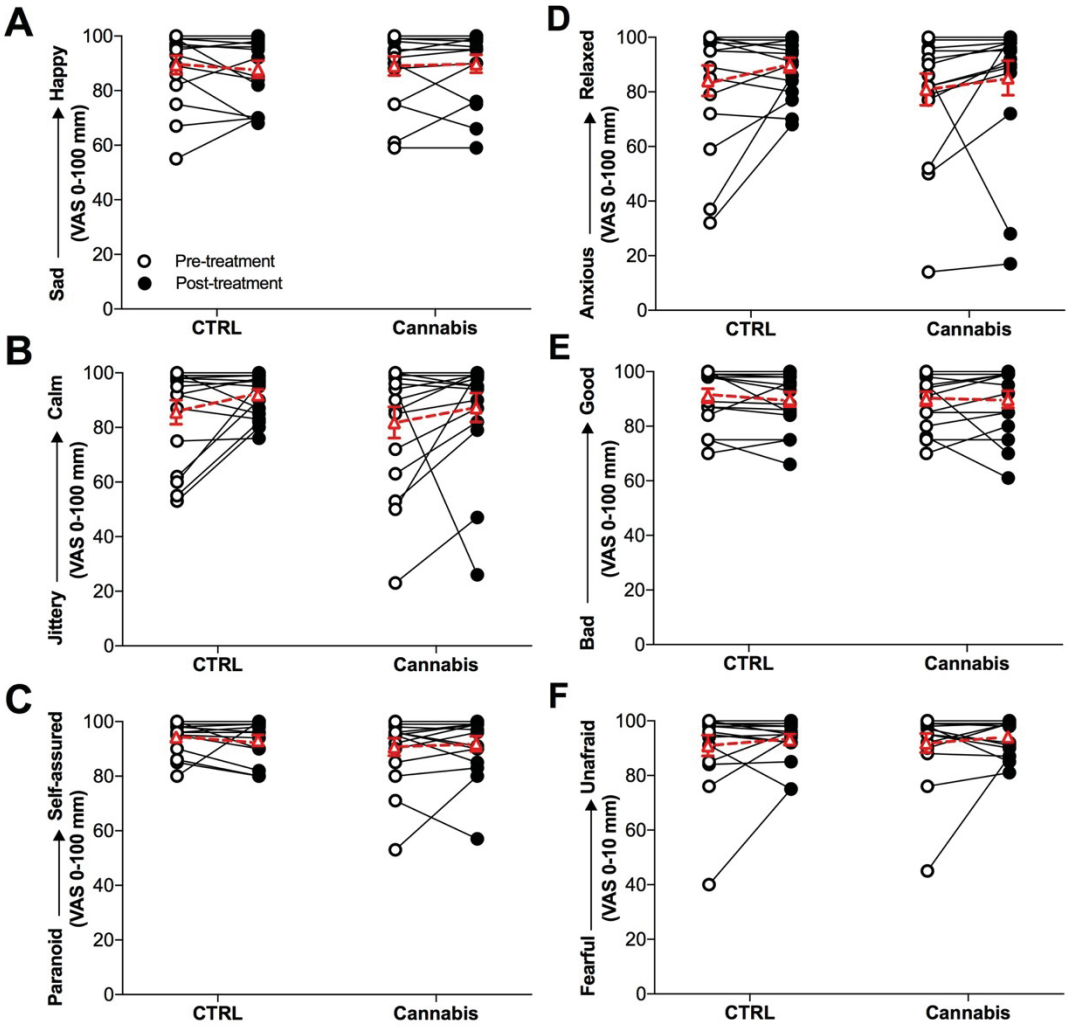

Figure 7.9. Effect of inhaled vaporized cannabis *versus* control (CTRL) on mood ratings in adults with advanced chronic obstructive pulmonary disease. Mood was assessed at rest immediately before and after inhalation of vaporized cannabis and CTRL. Individual participant pre- and post-treatment ratings are presented as sold black horizontal lines, while red symbols with dashed horizontal lines denote mean±SEM. VAS, visual analogue scale.

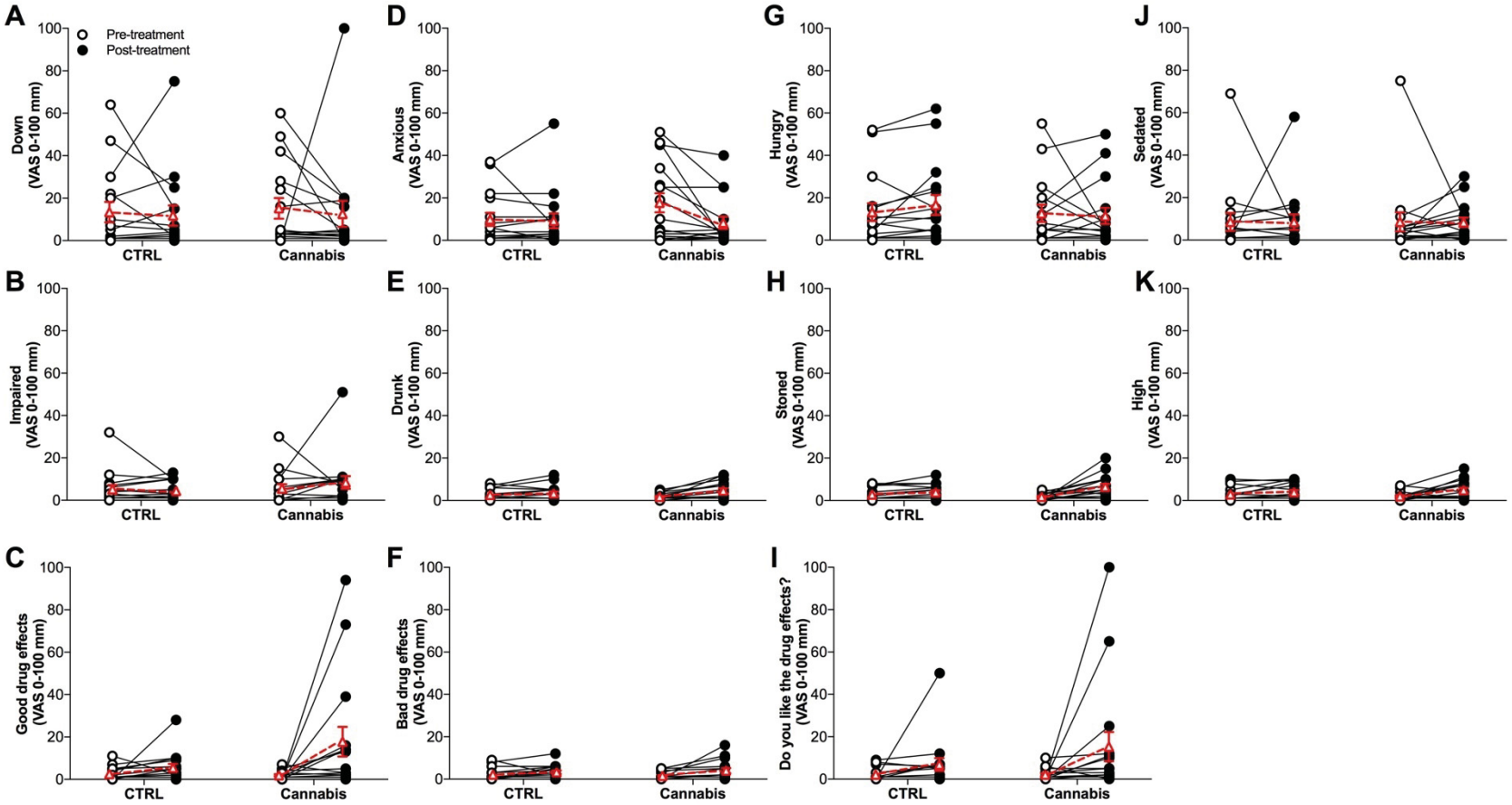

Figure 7.10. Effect of inhaled vaporized cannabis *versus* control (CTRL) on psychoactivity ratings in adults with advanced chronic obstructive pulmonary disease. Psychoactivity was assessed at rest immediately before and after inhalation of vaporized cannabis and CTRL. Individual participant pre- and post-treatment ratings are presented as sold black horizontal lines, while red symbols with dashed horizontal lines denote mean±SEM. VAS, visual analogue scale.

CHAPTER 8: SUMMARY OF FINDINGS, GENERAL DISCUSSION AND FUTURE DIRECTIONS

8.1 SUMMARY OF FINDINGS AND GENERAL DISCUSSION

8.1.1 Summary of findings

The overall aims of this thesis were to evaluate the efficacy and physiological mechanisms of action of novel and/or poorly studied therapies for the management of breathlessness and exercise intolerance in adults with COPD, particularly in those individuals suffering from chronic breathlessness syndrome. Pharmacological and non-pharmacological therapies were carefully selected to target any one or combination of the neurophysiological pathways mediating breathlessness in adults with COPD (**Figure 3.1**). Specifically, AB was selected to target neuromuscular efficiency of the diaphragm. Immediate-release oral morphine was selected to target central neural processing, neural respiratory drive and central corollary discharge. Inhaled vaporized cannabis was selected to target the load on the respiratory muscles (i.e., hyperinflation), and by extension neural respiratory drive and neuromechanical coupling of the respiratory system. It was hypothesized that, compared to the respective control condition, adults with COPD would experience relief of exertional breathlessness and improved exercise endurance following application of AB and administration of immediate-release oral morphine and inhaled vaporized cannabis by positively altering the intended neurophysiological target/pathways(s).

With regards to **Study 2**, we found that AB-induced enhancement of diaphragmatic neuromuscular efficiency did not have an effect on exertional breathlessness and exercise endurance in adults with mild-to-moderate COPD (6). In keeping with the neurophysiological construct of breathlessness illustrated in **Figure 3.1**, these results suggested that the relief of breathlessness extends beyond

acute and isolated improvements in the capacity of the respiratory muscles, and that relief of breathlessness is likely associated with any one or combination of: altered central neural processing; decreased neural respiratory drive and central corollary discharge; and/or enhanced neuromechanical coupling of the respiratory system via improved respiratory mechanics (i.e., a decrease in the load on the respiratory muscles).

With regards to **Study 3**, we found that morphine-induced improvements in exertional breathlessness and exercise endurance were associated with modest but significant decreases in $\dot{V}_E$ and f_R, which were accompanied by a concomitant decrease in neural respiratory drive (i.e., EMGdi) during exercise at isotime in adults with severe-to-very severe COPD and chronic breathlessness syndrome (7). These results supported our hypothesis that opioid-induced relief of exertional breathlessness and improvements in exercise tolerance reflected, at least in part, awareness of reduced neural respiratory drive as sensed by reduced central corollary discharge. Given the established effects of opioid on various brain centers, we further speculated that relief of exertional breathlessness with oral morphine may have been achieved by altering central neural processing either by reducing neural respiratory drive and/or by blunting the activity of various cortico-limbic regions independent of neural respiratory drive.

With regards to **Study 4**, we found, for the first time, that inhaled vaporized cannabis did not have a significant effect on static and dynamic airway function (i.e., the load on the respiratory muscle) nor an effect on exertional breathlessness and exercise endurance in symptomatic adults with advanced COPD receiving dual or triple inhalation therapy for management of their underlying pulmonary pathophysiology (8). These results are not surprising when considered in the context

of the neurophysiological construct of breathlessness presented earlier (**Figure 3.1**). Without decreasing the mechanical load on the respiratory muscles, inhaled vaporized cannabis did not decrease neural respiratory drive and improve neuromechanical coupling of the respiratory system. As a result, exertional breathlessness and exercise capacity remained unchanged, when compared to control condition.

The results of **Studies 2-4** provided critical new insights into the neurophysiological underpinnings of exertional breathlessness and exercise intolerance in symptomatic adults with COPD. Specifically, the collective results of **Studies 2 and 3** deemphasized the mechanistic role of diaphragmatic neuromuscular inefficiency and emphasized the contribution of neural respiratory drive and central corollary discharge in the etiology of exertional breathlessness in COPD (156, 181, 397-399). That is, despite improving the strength and efficiency of the diaphragm, AB had no effect on EMGdi or on exercise-induced changes in ratings of perceived breathlessness and exercise endurance. In contrast, decreases in neural respiratory drive and (presumably) central corollary discharge (as evidenced by reduced $\dot{V}_E$ and relatively preserved EMGdi-$\dot{V}_E$) following single-dose administration of immediate-release oral morphine were associated with clinically meaningful relief of exertional breathlessness and improvements in exercise endurance in the absence of changes in the capacity of the respiratory muscle (mean±SD Pdi,tidal at isotime in morphine *vs.* placebo: 19.5±7.7 and 18.3±4.3 cmH_2O; p=0.59). These results are consistent with the growing body of evidence emphasizing the role of neural respiratory drive and central corollary discharge in the neuromodulation of breathlessness in COPD (156, 181, 397-399).

8.1.2 General strength and limitations

Overall, the work constituting this thesis had several important strengths. First, the clinical studies included in this thesis each used a randomized controlled cross-over design and were adequately powered to detect clinically meaningful changes in breathlessness intensity during exercise at isotime and in exercise endurance time. In contrast to parallel group studies, a cross-over design [1] reduces the influence of cofounding variables, as each study subject serves as his or her own control; and [2] is more statistically efficient and therefore allows for a smaller sample size. Second, the constant-load cycle CPET, which is generally regarded as the most responsive exercise testing modality in the evaluation of interventional efficacy in COPD (234), was used in each of the studies presented in this thesis. Third, despite adhering to strict inclusion/exclusion criteria (a limitation discussed below), participants included in the **Studies 2, 3 and 4** were representative of a sub-population of individuals most likely to receive the experimental pharmacological and/or non-pharmacological therapy in the clinical care setting of COPD. For example, in the oral morphine study we included very symptomatic adults with advanced COPD in whom opioid therapy would be clinically indicated. In doing so, we increased the clinical relevance of our results.

The work presented within this thesis also had a few notable limitations. First, each of the pharmacological and/or non-pharmacological therapies assessed in this thesis were administered only once, as a single form (e.g., oral administration and/or vaporized form) and in a single dose/application. As a result, the interpretation of our findings cannot be generalized to other modes, types and regimens of the therapies selected. Second, we adhered to strict inclusion/exclusion criteria. Consequently, the generalizability of our findings is restricted to a

relatively small and homogenous group of clinically stable adults with COPD, with pre-defined clinical criteria as outlined in the Methods sections of **Studies 2-4**.

In addition to the above-mentioned strengths and limitations that apply to all studies included in the thesis, study-specific strengths and limitations do exist, and have been thoroughly discussed in their respective chapters.

8.2 CLINICAL RELEVANCE AND FUTURE WORK

The work presented within this thesis [1] provides important insights into the physiological mechanisms of exertional breathlessness in adults with COPD, and [2] has wide ranging implications on the management of breathlessness in adults with COPD.

In **Study 2**, AB enhanced diaphragmatic neuromuscular efficiency by increasing Pdi,tidal for a given EMGdi during submaximal exercise. These physiological alterations were not associated with relief of breathlessness or enhanced exercise endurance (6). As a result, acute application of AB should not be considered in the clinical management of breathlessness and exercise intolerance in adults with COPD.

The results of **Study 3** support the clinical utilization of immediate-release oral morphine for the management of exertional breathlessness and exercise intolerance in adults with severe-to-very severe COPD and chronic breathlessness syndrome. Therefore, we anticipate that the results of **Study 3** may be used to inform national and international clinical guidelines on the management of breathlessness in symptomatic adults with COPD. Importantly, in **Study 3**, we were able to

demonstrate that the locus of symptom-limitation on laboratory-based CPET could be used to predict which patients with advanced COPD are most likely to achieve clinically meaningful improvements in exertional breathlessness and exercise tolerance (7). Therefore, future studies should explore the predictive power of the locus of symptom-limitation on laboratory-based CPET in identifying opioid responders. Future studies should also explore physiological and phenotypic characteristics of opioid responsiveness, particularly as some adults with COPD may not be able to perform a CPET and/or CPET may not be readily available to all clinicians.

In a recent study, Dr. Kyle Pattinson's group demonstrated that opioids supress activity of the amygdala and hippocampus during anticipation of breathlessness in healthy adults (406). These brain centers are implicated in emotional processing of aversive stimuli; conditioned aversive learning; and memory consolidation (406). Based on this experimental evidence, we hypothesized that differences in anticipatory/learned behaviours may contribute to opioid responsiveness for breathlessness. In an effort to explore this hypothesis, I joined Dr. Kyle Pattinson's group at the University of Oxford (funded by the Canadian Institute of Health Research Michael Smith Foreign Study Supplement Award), where I worked closely with Dr. Olivia Faull on identifying neurophysiological predictors of opioid responsiveness in healthy adults and in adults with COPD. This collaboration produced a manuscript that is currently under review by the *European Respiratory Journal*. A brief overview of our rationale, methods and results are presented below. To review the full manuscript, please see **Appendix V**.

In the context of the Bayesian Brain Hypothesis, breathlessness perception is believed to be the balance between the brain's set of expectations and beliefs (collectively known as priors), and

incoming sensory information (457-459). Priors are shaped by previous experiences and learned behaviours; and the weight of priors may be influenced by various moderators, including negative affect (e.g., anxiety) (457-459). For example, in climbing a set of stairs an individual with anxiety may perceive breathlessness that is discordant from their objective physiology (i.e., "exaggerated breathlessness") when compared to an individual without anxiety. As a result, the individual with anxiety may have stronger priors and would therefore be more likely to "expect" to experience breathlessness during subsequent stair climbing, when compared to an individual without anxiety. In the context of this model, Dr. Faull and I sought to better understand the mechanisms underlying variability in opioid responsiveness for relief of breathlessness. To accomplish this task, we reanalyzed data from my **Study 3** along with behavioural and functional neuroimaging data from the fMRI study in healthy volunteers conducted by Dr. Pattinson's group (406).

In our reanalysis, we first performed a hierarchical cluster analyses to better understand the relationship between behavioural measures (i.e., measures of lung function; measures of negative affect, etc.) and opioid-induced relief of breathlessness in healthy adults and in adults with COPD. Across both datasets, we found that diminished opioid efficacy was more closely associated with negative affect than with other physiological and behavioural properties. Next, we explored the relationship between brain activity during anticipation of breathlessness and 'opioid-efficacy' for treatment of breathlessness (opioid-efficacy was defined by the 'response' cluster identified by the hierarchical cluster analysis, which included items that represented opioid-induced changes in physiological and subjective measures). Strikingly, we found that opioid unresponsiveness was directly correlated with brain activity in the anterior cingulate cortex and ventromedial prefrontal cortex (vmPFC) during anticipation of breathlessness. The anterior cingulate cortex and the

vmPFC are thought to be involved in the generation of predictions on emotional state and bodily awareness (and therefore priors) (406). Our results suggested that individuals with a higher co-existence of negative affective traits are less likely to experience opioid-induced relief of breathlessness. Moreover, individuals with greater brain activity in the anterior cingulate cortex and vmPFC during anticipation of breathlessness appeared to be more 'resistant' to opioid therapy. We speculated that in opioid non-responders, negative affective traits might increase the weight of priors within the brain's perceptual system, resulting in breathlessness perception that is shifted away from sensory-afferent inputs and towards priors (i.e., previous expectations). Consequently, individuals that are resistant to opioids for breathlessness may be more likely to benefit from therapies that reshape breathlessness expectations (e.g., pulmonary rehabilitation) (92). Additional studies are required to better understand the relationship between negative affect, breathlessness perception and the brain's perceptual network. These studies are likely to ascertain previously unexplored neurophysiological and neuropsychological underpinnings of breathlessness, which may provide clues for novel pharmacological and/or non-pharmacological therapies for the management of breathlessness across a range of malignant and non-malignant diagnoses, including COPD.

In addition to highlighting the need for predictors of opioid responsiveness for breathlessness, the results of **Study 3** suggested that immediate-release oral morphine may be an effective add-on pharmacotherapy to optimize the physiological and clinical benefits of a rehabilitative exercise training program in symptomatic adults with advanced COPD, particularly those who stop exercise due to intolerable breathlessness. That is, immediate-release oral morphine may allow patients to exercise at higher intensities for longer periods of time, which could translate into greater training

volume when exercise is performed three times per week as part of a rehabilitative exercise training program for lasting several weeks.

Available evidence suggests that "long-term" (i.e., 3-6 months) opioid therapy is beneficial for the management of breathlessness in adults with COPD (460). In these longitudinal studies, adults with COPD are typically required to rate their breathlessness at various time points throughout the day. These measures of breathlessness may underestimate the efficacy of opioid therapy for relief of breathlessness, as they do not take into account changes in functional capacity and/or physical activity levels. That is, during opioid therapy, adults with COPD may "feel better" and therefore "do more" (i.e., an increase in physical activity) when compared to the placebo condition (56). Without adjusting for changes in physical activity levels during opioid therapy, measures of breathlessness may be inaccurate. Therefore, future studies should evaluate the effect of repeat-dose opioid therapy on exertional breathlessness, functional capacity and physical activity levels.

The results of **Study 4** suggested that inhaled vaporized cannabis does not have a meaningful effect on exertional breathlessness or exercise endurance in adults with advanced COPD (8). Nevertheless, additional studies are needed before recommendations can be made on the clinical application of cannabis in adults with COPD. In moving forward, it may be important to first replicate the results of Tashkin, *et al.* (18-22), who demonstrated a bronchodilator effect of smoked cannabis in healthy adults and in adults with asthma. In the 40 years since Tashkin, *et al.* (18-22), published their findings, the potency of the cannabis plant along with its cannabinoid composition has changed (i.e., differences in THC to CBD ratio) (461). Therefore, it is important to identify the cannabis plant that can induce bronchodilatation in healthy adults and in adults with asthma

(when smoked). If the results of Tashkin, *et al.* (18-22) can be replicated, then the effects of smoked, vaporized, and orally ingested cannabis on airway function in adults with COPD should be further investigated. Such studies should evaluate the effects of cannabis in [1] newly diagnosed adults with COPD who have not received bronchodilator therapy and therefore the confounding effects of LABA and/or LAMA therapy is not of concern; [2] adults with COPD receiving LABA or LAMA monotherapy; and [3] adults with COPD receiving LABA and LAMA dual therapy. Of course, the physiological and psychological effects of cannabis should be compared to standard therapy (i.e., cannabis *vs.* LABA or LAMA monotherapy) and in addition to standard therapy (i.e., LABA or LAMA monotherapy *vs.* cannabis plus LABA or LAMA monotherapy). Finally, prospective longitudinal studies are urgently needed to appreciate the long-term effects of smoked, vaporized and orally ingested cannabis on human health, especially, lung health.

8.3 OVERALL CONCLUSIONS

This thesis evaluated the efficacy and physiological mechanisms of action of AB (sufficient to increase intra-abdominal pressures by 6.7±0.3 cmH_2O), immediate-release oral morphine (0.1 mg/kg body weight) and inhaled vaporized cannabis (35 mg containing 18.2% THC) in reducing exertional breathlessness and improving exercise endurance in adults with COPD. Isolated and acute improvements in diaphragmatic neuromuscular efficiency (i.e., enhanced capacity of the respiratory muscle) induced by AB did not translate into relief of exertional breathlessness or enhanced exercise endurance in adults with moderate-to-severe COPD (6). In contrast, immediate-release oral morphine was associated with statistically significant and clinically meaningful improvements in exertional breathlessness and exercise endurance in adults with severe-to-very severe COPD and chronic breathlessness syndrome (7). Opioid-induced relief of breathlessness and improvements in exercise endurance time were associated with reductions in $\dot{V}_E$ and f_R and

neural respiratory drive (and presumably central corollary discharge). Therefore, immediate-release oral morphine should be considered for the symptomatic management of chronic refractory breathlessness and exercise intolerance in selected adults with advanced COPD. Inhaled vaporized cannabis did not have a clinically meaningful positive or negative effect on exertional breathlessness, exercise endurance and/or airway function in symptomatic adults with advanced COPD receiving dual or triple inhalation therapy for management of their COPD (8). The collective results of **Studies 2-4** suggest that, to be clinically effective, adjunct therapies for the management of exertional breathlessness and exercise tolerance in adults with COPD should be targeted to [1] alter central neural processing of breathlessness, and [2] decrease neural respiratory drive and central corollary discharge.

REFERENCES

1. Mullerova H, Lu C, Li H, Tabberer M. Prevalence and burden of breathlessness in patients with chronic obstructive pulmonary disease managed in primary care. *Plos One* 2014; 9: e85540.
2. Oga T, Nishimura K, Tsukino M, Sato S, Hajiro T. Analysis of the factors related to mortality in chronic obstructive pulmonary disease: role of exercise capacity and health status. *Am J Respir Crit Care Med* 2003; 167: 544-549.
3. Sundh J, Ekstrom M. Persistent disabling breathlessness in chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis* 2016; 11: 2805-2812.
4. Johnson MJ, Yorke J, Hansen-Flaschen J, Lansing R, Ekstrom M, Similowski T, Currow DC. Towards an expert consensus to delineate a clinical syndrome of chronic breathlessness. *Eur Respir J* 2017; 49.
5. Abdallah SJ, Chan DS, Glicksman R, Mendonca CT, Luo Y, Bourbeau J, Smith BM, Jensen D. Abdominal Binding Improves Neuromuscular Efficiency of the Human Diaphragm during Exercise. *Front Physiol* 2017; 8: 345.
6. Abdallah SJ, Smith BM, Wilkinson-Maitland C, Li PZ, Bourbeau J, Jensen D. Effect of Abdominal Binding on Diaphragmatic Neuromuscular Efficiency, Exertional Breathlessness, and Exercise Endurance in Chronic Obstructive Pulmonary Disease. *Front Physiol* 2018; 9: 1618.
7. Abdallah SJ, Wilkinson-Maitland C, Saad N, Li PZ, Smith BM, Bourbeau J, Jensen D. Effect of morphine on breathlessness and exercise endurance in advanced COPD: a randomised crossover trial. *Eur Respir J* 2017; 50.
8. Abdallah SJ, Smith BM, Ware MA, Moore M, Li PZ, Bourbeau J, Jensen D. Effect of Vaporized Cannabis on Exertional Breathlessness and Exercise Endurance in Advanced Chronic Obstructive Pulmonary Disease. A Randomized Controlled Trial. *Ann Am Thorac Soc* 2018; 15: 1146-1158.
9. Laviolette L, Laveneziana P. Morphine to relieve exertional dyspnoea in COPD: myth, dream or reality? *Eur Respir J* 2017; 50.
10. Tashkin DP. Vaping Cannabis and Chronic Obstructive Pulmonary Disease. *Ann Am Thorac Soc* 2018; 15: 1137-1138.
11. Celli BR, Rassulo J, Berman JS, Make B. Respiratory consequences of abdominal hernia in a patient with severe chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1985; 131: 178-180.
12. Laghi F, Jubran A, Topeli A, Fahey PJ, Garrity ER, Jr., Arcidi JM, de Pinto DJ, Edwards LC, Tobin MJ. Effect of lung volume reduction surgery on neuromechanical coupling of the diaphragm. *Am J Respir Crit Care Med* 1998; 157: 475-483.
13. Laghi F, Jubran A, Topeli A, Fahey PJ, Garrity ER, Jr., de Pinto DJ, Tobin MJ, Loyola/Hines Lung Volume Reduction Surgery Research G. Effect of lung volume reduction surgery on diaphragmatic neuromechanical coupling at 2 years. *Chest* 2004; 125: 2188-2195.
14. Ekstrom M, Nilsson F, Abernethy AA, Currow DC. Effects of opioids on breathlessness and exercise capacity in chronic obstructive pulmonary disease. A systematic review. *Ann Am Thorac Soc* 2015; 12: 1079-1092.
15. Ahmadi Z, Bernelid E, Currow DC, Ekstrom M. Prescription of opioids for breathlessness in end-stage COPD: a national population-based study. *Int J Chron Obstruct Pulmon Dis* 2016; 11: 2651-2657.

16. Janssen DJ, de Hosson SM, bij de Vaate E, Mooren KJ, Baas AA. Attitudes toward opioids for refractory dyspnea in COPD among Dutch chest physicians. *Chron Respir Dis* 2015; 12: 85-92.
17. Young J, Donahue M, Farquhar M, Simpson C, Rocker G. Using opioids to treat dyspnea in advanced COPD: attitudes and experiences of family physicians and respiratory therapists. *Can Fam Physician* 2012; 58: e401-407.
18. Tashkin DP, Reiss S, Shapiro BJ, Calvarese B, Olsen JL, Lodge JW. Bronchial effects of aerosolized delta 9-tetrahydrocannabinol in healthy and asthmatic subjects. *Am Rev Respir Dis* 1977; 115: 57-65.
19. Tashkin DP, Shapiro BJ, Frank IM. Acute effects of smoked marijuana and oral delta9-tetrahydrocannabinol on specific airway conductance in asthmatic subjects. *Am Rev Respir Dis* 1974; 109: 420-428.
20. Tashkin DP, Shapiro BJ, Lee YE, Harper CE. Effects of smoked marijuana in experimentally induced asthma. *Am Rev Respir Dis* 1975; 112: 377-386.
21. Vachon L, Fitzgera.Mx, Solliday NH, Gould IA, Gaensler EA. Single-Dose Effect of Marihuana Smoke - Bronchial Dynamics and Respiratory-Center Sensitivity in Normal Subjects. *N Engl J Med* 1973; 288: 985-989.
22. Tashkin DP, Shapiro BJ, Frank IM. Acute pulmonary physiologic effects of smoked marijuana and oral 9 -tetrahydrocannabinol in healthy young men. *N Engl J Med* 1973; 289: 336-341.
23. Global strategy for the diagnosis, management, and prevention of Chronic Obstructive Pulmonary Disease; 2018.
24. Laveneziana P, Parker CM, O'Donnell DE. Ventilatory constraints and dyspnea during exercise in chronic obstructive pulmonary disease. *Appl Physiol Nutr Metab* 2007; 32: 1225-1238.
25. O'Donnell DE. Hyperinflation, dyspnea, and exercise intolerance in chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 2006; 3: 180-184.
26. O'Donnell DE, Banzett RB, Carrieri-Kohlman V, Casaburi R, Davenport PW, Gandevia SC, Gelb AF, Mahler DA, Webb KA. Pathophysiology of dyspnea in chronic obstructive pulmonary disease: a roundtable. *Proceedings of the American Thoracic Society* 2007; 4: 145-168.
27. Jones PW. Health status measurement in chronic obstructive pulmonary disease. *Thorax* 2001; 56: 880-887.
28. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Bin Abdulhak A, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahodwala N, De Leo D, Degenhardt L, Delossantos A, Denenberg J, Des Jarlais DC, Dharmaratne SD, Dorsey ER, Driscoll T, Duber H, Ebel B, Erwin PJ, Espindola P, Ezzati M, Feigin V, Flaxman AD, Forouzanfar MH, Fowkes FG, Franklin R, Fransen M, Freeman MK, Gabriel SE, Gakidou E, Gaspari F, Gillum RF, Gonzalez-Medina D, Halasa YA, Haring D, Harrison JE, Havmoeller R, Hay RJ, Hoen B, Hotez PJ, Hoy D, Jacobsen KH, James SL, Jasrasaria R, Jayaraman S, Johns N, Karthikeyan G, Kassebaum N, Keren A, Khoo JP, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lipnick M, Lipshultz SE, Ohno SL, Mabweijano J, MacIntyre MF, Mallinger L, March L, Marks GB, Marks R, Matsumori A,

Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGrath J, Mensah GA, Merriman TR, Michaud C, Miller M, Miller TR, Mock C, Mocumbi AO, Mokdad AA, Moran A, Mulholland K, Nair MN, Naldi L, Narayan KM, Nasseri K, Norman P, O'Donnell M, Omer SB, Ortblad K, Osborne R, Ozgediz D, Pahari B, Pandian JD, Rivero AP, Padilla RP, Perez-Ruiz F, Perico N, Phillips D, Pierce K, Pope CA, 3rd, Porrini E, Pourmalek F, Raju M, Ranganathan D, Rehm JT, Rein DB, Remuzzi G, Rivara FP, Roberts T, De Leon FR, Rosenfeld LC, Rushton L, Sacco RL, Salomon JA, Sampson U, Sanman E, Schwebel DC, Segui-Gomez M, Shepard DS, Singh D, Singleton J, Sliwa K, Smith E, Steer A, Taylor JA, Thomas B, Tleyjeh IM, Towbin JA, Truelsen T, Undurraga EA, Venketasubramanian N, Vijayakumar L, Vos T, Wagner GR, Wang M, Wang W, Watt K, Weinstock MA, Weintraub R, Wilkinson JD, Woolf AD, Wulf S, Yeh PH, Yip P, Zabetian A, Zheng ZJ, Lopez AD, Murray CJ, AlMazroa MA, Memish ZA. 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: 2095-2128.

29. Nishimura K, Izumi T, Tsukino M, Oga T. Dyspnea is a better predictor of 5-year survival than airway obstruction in patients with COPD. *Chest* 2002; 121: 1434-1440.
30. Ong KC, Earnest A, Lu SJ. A multidimensional grading system (BODE index) as predictor of hospitalization for COPD. *Chest* 2005; 128: 3810-3816.
31. Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M, Shibuya K, Salomon JA, Abdalla S, Aboyans V, Abraham J, Ackerman I, Aggarwal R, Ahn SY, Ali MK, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Bahalim AN, Barker-Collo S, Barrero LH, Bartels DH, Basanez MG, Baxter A, Bell ML, Benjamin EJ, Bennett D, Bernabe E, Bhalla K, Bhandari B, Bikbov B, Bin Abdulhak A, Birbeck G, Black JA, Blencowe H, Blore JD, Blyth F, Bolliger I, Bonaventure A, Boufous S, Bourne R, Boussinesq M, Braithwaite T, Brayne C, Bridgett L, Brooker S, Brooks P, Brugha TS, Bryan-Hancock C, Bucello C, Buchbinder R, Buckle G, Budke CM, Burch M, Burney P, Burstein R, Calabria B, Campbell B, Canter CE, Carabin H, Carapetis J, Carmona L, Cella C, Charlson F, Chen H, Cheng AT, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahiya M, Dahodwala N, Damsere-Derry J, Danaei G, Davis A, De Leo D, Degenhardt L, Dellavalle R, Delossantos A, Denenberg J, Derrett S, Des Jarlais DC, Dharmaratne SD, Dherani M, Diaz-Torne C, Dolk H, Dorsey ER, Driscoll T, Duber H, Ebel B, Edmond K, Elbaz A, Ali SE, Erskine H, Erwin PJ, Espindola P, Ewoigbokhan SE, Farzadfar F, Feigin V, Felson DT, Ferrari A, Ferri CP, Fevre EM, Finucane MM, Flaxman S, Flood L, Foreman K, Forouzanfar MH, Fowkes FG, Franklin R, Fransen M, Freeman MK, Gabbe BJ, Gabriel SE, Gakidou E, Ganatra HA, Garcia B, Gaspari F, Gillum RF, Gmel G, Gosselin R, Grainger R, Groeger J, Guillemin F, Gunnell D, Gupta R, Haagsma J, Hagan H, Halasa YA, Hall W, Haring D, Haro JM, Harrison JE, Havmoeller R, Hay RJ, Higashi H, Hill C, Hoen B, Hoffman H, Hotez PJ, Hoy D, Huang JJ, Ibeanusi SE, Jacobsen KH, James SL, Jarvis D, Jasrasaria R, Jayaraman S, Johns N, Jonas JB, Karthikeyan G, Kassebaum N, Kawakami N, Keren A, Khoo JP, King CH, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lalloo R, Laslett LL, Lathlean T, Leasher JL, Lee YY, Leigh J, Lim SS, Limb E, Lin JK, Lipnick M, Lipshultz SE, Liu W, Loane M, Ohno SL, Lyons R, Ma J, Mabweijano J, MacIntyre MF, Malekzadeh R, Mallinger L, Manivannan S, Marcenes W, March L, Margolis DJ, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM,

McAnulty JH, McDermott MM, McGill N, McGrath J, Medina-Mora ME, Meltzer M, Mensah GA, Merriman TR, Meyer AC, Miglioli V, Miller M, Miller TR, Mitchell PB, Mocumbi AO, Moffitt TE, Mokdad AA, Monasta L, Montico M, Moradi-Lakeh M, Moran A, Morawska L, Mori R, Murdoch ME, Mwaniki MK, Naidoo K, Nair MN, Naldi L, Narayan KM, Nelson PK, Nelson RG, Nevitt MC, Newton CR, Nolte S, Norman P, Norman R, O'Donnell M, O'Hanlon S, Olives C, Omer SB, Ortblad K, Osborne R, Ozgediz D, Page A, Pahari B, Pandian JD, Rivero AP, Patten SB, Pearce N, Padilla RP, Perez-Ruiz F, Perico N, Pesudovs K, Phillips D, Phillips MR, Pierce K, Pion S, Polanczyk GV, Polinder S, Pope CA, 3rd, Popova S, Porrini E, Pourmalek F, Prince M, Pullan RL, Ramaiah KD, Ranganathan D, Razavi H, Regan M, Rehm JT, Rein DB, Remuzzi G, Richardson K, Rivara FP, Roberts T, Robinson C, De Leon FR, Ronfani L, Room R, Rosenfeld LC, Rushton L, Sacco RL, Saha S, Sampson U, Sanchez-Riera L, Sanman E, Schwebel DC, Scott JG, Segui-Gomez M, Shahraz S, Shepard DS, Shin H, Shivakoti R, Singh D, Singh GM, Singh JA, Singleton J, Sleet DA, Sliwa K, Smith E, Smith JL, Stapelberg NJ, Steer A, Steiner T, Stolk WA, Stovner LJ, Sudfeld C, Syed S, Tamburlini G, Tavakkoli M, Taylor HR, Taylor JA, Taylor WJ, Thomas B, Thomson WM, Thurston GD, Tleyjeh IM, Tonelli M, Towbin JA, Truelsen T, Tsilimbaris MK, Ubeda C, Undurraga EA, van der Werf MJ, van Os J, Vavilala MS, Venketasubramanian N, Wang M, Wang W, Watt K, Weatherall DJ, Weinstock MA, Weintraub R, Weisskopf MG, Weissman MM, White RA, Whiteford H, Wiersma ST, Wilkinson JD, Williams HC, Williams SR, Witt E, Wolfe F, Woolf AD, Wulf S, Yeh PH, Zaidi AK, Zheng ZJ, Zonies D, Lopez AD, Murray CJ, AlMazroa MA, Memish ZA. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2012; 380: 2163-2196.

32. Johnson-Warrington V, Harrison S, Mitchell K, Steiner M, Morgan M, Singh S. Exercise capacity and physical activity in patients with COPD and healthy subjects classified as Medical Research Council dyspnea scale grade 2. *J Cardiopulm Rehabil Prev* 2014; 34: 150-154.
33. Troosters T, Sciurba F, Battaglia S, Langer D, Valluri SR, Martino L, Benzo R, Andre D, Weisman I, Decramer M. Physical inactivity in patients with COPD, a controlled multi-center pilot-study. *Respir Med* 2010; 104: 1005-1011.
34. Bourbeau J, Bhutani M, Hernandez P, Marciniuk DD, Aaron SD, Balter M, Beauchesne M-F, D'Urzo A, Goldstein R, Kaplan A, Maltais F, O'Donnell DE, Sin DD. CTS position statement: Pharmacotherapy in patients with COPD—An update. *Can J Respir Crit Care Med* 2017; 1: 222-241.
35. O'Donnell DE, Aaron S, Bourbeau J, Hernandez P, Marciniuk DD, Balter M, Ford G, Gervais A, Goldstein R, Hodder R, Kaplan A, Keenan S, Lacasse Y, Maltais F, Road J, Rocker G, Sin D, Sinuff T, Voduc N. Canadian Thoracic Society recommendations for management of chronic obstructive pulmonary disease - 2007 update. *Can Respir J* 2007; 14 Suppl B: 5B-32B.
36. O'Donnell DE, Elbehairy AF, Webb KA, Neder JA, Canadian Respiratory Research N. The Link between Reduced Inspiratory Capacity and Exercise Intolerance in Chronic Obstructive Pulmonary Disease. *Ann Am Thorac Soc* 2017; 14: S30-S39.
37. Jolley CJ, Moxham J. A physiological model of patient-reported breathlessness during daily activities in COPD. *Eur Respir Rev* 2009; 18: 66-79.

38. Chen Z, Eldridge FL. Inputs from upper airway affect firing of respiratory-associated midbrain neurons. *J Appl Physiol (1985)* 1997; 83: 196-203.
39. Chen Z, Eldridge FL, Wagner PG. Respiratory-associated rhythmic firing of midbrain neurones in cats: relation to level of respiratory drive. *J Physiol* 1991; 437: 305-325.
40. Chen Z, Eldridge FL, Wagner PG. Respiratory-associated thalamic activity is related to level of respiratory drive. *Respir Physiol* 1992; 90: 99-113.
41. Nishino T. Dyspnoea: underlying mechanisms and treatment. *Br J Anaesth* 2011; 106: 463-474.
42. Esser RW, Stoeckel MC, Kirsten A, Watz H, Taube K, Lehmann K, Magnussen H, Buchel C, von Leupoldt A. Brain Activation during Perception and Anticipation of Dyspnea in Chronic Obstructive Pulmonary Disease. *Front Physiol* 2017; 8: 617.
43. Herigstad M, Hayen A, Evans E, Hardinge FM, Davies RJ, Wiech K, Pattinson KTS. Dyspnea-related cues engage the prefrontal cortex: evidence from functional brain imaging in COPD. *Chest* 2015; 148: 953-961.
44. Belman MJ, Botnick WC, Shin JW. Inhaled bronchodilators reduce dynamic hyperinflation during exercise in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1996; 153: 967-975.
45. O'Donnell DE, Hamilton AL, Webb KA. Sensory-mechanical relationships during high-intensity, constant-work-rate exercise in COPD. *J Appl Physiol (1985)* 2006; 101: 1025-1035.
46. O'Donnell DE, Hong HH, Webb KA. Respiratory sensation during chest wall restriction and dead space loading in exercising men. *J Appl Physiol (1985)* 2000; 88: 1859-1869.
47. Atkinson RW, Carey IM, Kent AJ, van Staa TP, Anderson HR, Cook DG. Long-term exposure to outdoor air pollution and the incidence of chronic obstructive pulmonary disease in a national English cohort. *Occup Environ Med* 2015; 72: 42-48.
48. Schikowski T, Mills IC, Anderson HR, Cohen A, Hansell A, Kauffmann F, Kramer U, Marcon A, Perez L, Sunyer J, Probst-Hensch N, Kunzli N. Ambient air pollution: a cause of COPD? *Eur Respir J* 2014; 43: 250-263.
49. Collaborators GBDCRD. Global, regional, and national deaths, prevalence, disability-adjusted life years, and years lived with disability for chronic obstructive pulmonary disease and asthma, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Respir Med* 2017; 5: 691-706.
50. Statistics Canada. Fast facts about Chronic Obstructive Pulmonary Disease (COPD): data compiled from the 2011 Survey on Living with Chronic Diseases in Canada 2011.
51. Tan WC, Bourbeau J, FitzGerald JM, Cowie R, Chapman K, Hernandez P, Buist SA, Sin DD. Can age and sex explain the variation in COPD rates across large urban cities? A population study in Canada. *Int J Tuberc Lung D* 2011; 15: 1691-1696.
52. Canadian Institute of Health Information. Health Indicators 2008. 2008.
53. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med* 2006; 3: e442.
54. Marciniuk DD, Goodridge D, Hernandez P, Rocker G, Balter M, Bailey P, Ford G, Bourbeau J, O'Donnell DE, Maltais F, Mularski RA, Cave AJ, Mayers I, Kennedy V, Oliver TK, Brown C, Canadian Thoracic Society CCDEWG. Managing dyspnea in patients with advanced chronic obstructive pulmonary disease: a Canadian Thoracic Society clinical practice guideline. *Can Respir J* 2011; 18: 69-78.

55. Parshall MB, Schwartzstein RM, Adams L, Banzett RB, Manning HL, Bourbeau J, Calverley PM, Gift AG, Harver A, Lareau SC, Mahler DA, Meek PM, O'Donnell DE, American Thoracic Society Committee on D. An official American Thoracic Society statement: update on the mechanisms, assessment, and management of dyspnea. *Am J Respir Crit Care Med* 2012; 185: 435-452.
56. Pitta F, Troosters T, Spruit MA, Probst VS, Decramer M, Gosselink R. Characteristics of physical activities in daily life in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2005; 171: 972-977.
57. Zhang Y, Morgan RL, Alonso-Coello P, Wiercioch W, Bała MM, Jaeschke RR, Styczeń K, Pardo-Hernandez H, Selva A, Ara Begum H, Morgano GP, Waligóra M, Agarwal A, Ventresca M, Strzebońska K, Wasylewski MT, Blanco-Silvente L, Kerth J-L, Wang M, Zhang Y, Narsingam S, Fei Y, Guyatt G, Schünemann HJ. A systematic review of how patients value COPD outcomes. *Eur Respir J* 2018; 52.
58. Kunik ME, Roundy K, Veazey C, Souchek J, Richardson P, Wray NP, Stanley MA. Surprisingly high prevalence of anxiety and depression in chronic breathing disorders. *Chest* 2005; 127: 1205-1211.
59. Biswas D, Mukherjee S, Chakroborty R, Chatterjee S, Rath S, Das R, Begum S. Occurrence of Anxiety and Depression among Stable COPD Patients and its Impact on Functional Capability. *J Clin Diagn Res* 2017; 11: OC24-OC27.
60. Cleland JA, Lee AJ, Hall S. Associations of depression and anxiety with gender, age, health-related quality of life and symptoms in primary care COPD patients. *Fam Pract* 2007; 24: 217-223.
61. Light RW, Merrill EJ, Despars JA, Gordon GH, Mutalipassi LR. Prevalence of depression and anxiety in patients with COPD. Relationship to functional capacity. *Chest* 1985; 87: 35-38.
62. Xiao T, Qiu H, Chen Y, Zhou X, Wu K, Ruan X, Wang N, Fu C. Prevalence of anxiety and depression symptoms and their associated factors in mild COPD patients from community settings, Shanghai, China: a cross-sectional study. *BMC Psychiatry* 2018; 18: 89.
63. Yohannes AM, Alexopoulos GS. Depression and anxiety in patients with COPD. *Eur Respir Rev* 2014; 23: 345-349.
64. Barr RG, Bluemke DA, Ahmed FS, Carr JJ, Enright PL, Hoffman EA, Jiang R, Kawut SM, Kronmal RA, Lima JA, Shahar E, Smith LJ, Watson KE. Percent emphysema, airflow obstruction, and impaired left ventricular filling. *N Engl J Med* 2010; 362: 217-227.
65. Smith BM, Prince MR, Hoffman EA, Bluemke DA, Liu CY, Rabinowitz D, Hueper K, Parikh MA, Gomes AS, Michos ED, Lima JAC, Barr RG. Impaired left ventricular filling in COPD and emphysema: is it the heart or the lungs? The Multi-Ethnic Study of Atherosclerosis COPD Study. *Chest* 2013; 144: 1143-1151.
66. Maltais F, Decramer M, Casaburi R, Barreiro E, Burelle Y, Debigare R, Dekhuijzen PN, Franssen F, Gayan-Ramirez G, Gea J, Gosker HR, Gosselink R, Hayot M, Hussain SN, Janssens W, Polkey MI, Roca J, Saey D, Schols AM, Spruit MA, Steiner M, Taivassalo T, Troosters T, Vogiatzis I, Wagner PD, COPD AEAHCoLMDi. An official American Thoracic Society/European Respiratory Society statement: update on limb muscle dysfunction in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2014; 189: e15-62.
67. Barreiro E, Gea J. Molecular and biological pathways of skeletal muscle dysfunction in chronic obstructive pulmonary disease. *Chron Respir Dis* 2016; 13: 297-311.

68. Jaitovich A, Barreiro E. Skeletal Muscle Dysfunction in Chronic Obstructive Pulmonary Disease. What We Know and Can Do for Our Patients. *Am J Respir Crit Care Med* 2018; 198: 175-186.
69. Elliott MW, Adams L, Cockcroft A, MacRae KD, Murphy K, Guz A. The language of breathlessness. Use of verbal descriptors by patients with cardiopulmonary disease. *Am Rev Respir Dis* 1991; 144: 826-832.
70. Lansing RW, Gracely RH, Banzett RB. The multiple dimensions of dyspnea: review and hypotheses. *Respir Physiol Neurobiol* 2009; 167: 53-60.
71. Lansing RW, Im BS, Thwing JI, Legedza AT, Banzett RB. The perception of respiratory work and effort can be independent of the perception of air hunger. *Am J Respir Crit Care Med* 2000; 162: 1690-1696.
72. Simon PM, Schwartzstein RM, Weiss JW, Fencl V, Teghtsoonian M, Weinberger SE. Distinguishable types of dyspnea in patients with shortness of breath. *Am Rev Respir Dis* 1990; 142: 1009-1014.
73. Simon PM, Schwartzstein RM, Weiss JW, Lahive K, Fencl V, Teghtsoonian M, Weinberger SE. Distinguishable sensations of breathlessness induced in normal volunteers. *Am Rev Respir Dis* 1989; 140: 1021-1027.
74. Banzett RB, Lansing RW, Brown R, Topulos GP, Yager D, Steele SM, Londono B, Loring SH, Reid MB, Adams L, et al. 'Air hunger' from increased PCO2 persists after complete neuromuscular block in humans. *Respir Physiol* 1990; 81: 1-17.
75. Banzett RB, Pedersen SH, Schwartzstein RM, Lansing RW. The affective dimension of laboratory dyspnea: air hunger is more unpleasant than work/effort. *Am J Respir Crit Care Med* 2008; 177: 1384-1390.
76. Bloch-Salisbury E, Spengler CM, Brown R, Banzett RB. Self-control and external control of mechanical ventilation give equal air hunger relief. *Am J Respir Crit Care Med* 1998; 157: 415-420.
77. Manning HL, Shea SA, Schwartzstein RM, Lansing RW, Brown R, Banzett RB. Reduced tidal volume increases 'air hunger' at fixed PCO2 in ventilated quadriplegics. *Respir Physiol* 1992; 90: 19-30.
78. Kotrach HG, Bourbeau J, Jensen D. Does nebulized fentanyl relieve dyspnea during exercise in healthy man? *J Appl Physiol (1985)* 2015; 118: 1406-1414.
79. Mendonca CT, Schaeffer MR, Riley P, Jensen D. Physiological mechanisms of dyspnea during exercise with external thoracic restriction: role of increased neural respiratory drive. *J Appl Physiol (1985)* 2014; 116: 570-581.
80. Larson JL. Functional performance and physical activity in chronic obstructive pulmonary disease: theoretical perspectives. *Copd* 2007; 4: 237-242.
81. O'Donnell DE, Elbehairy AF, Faisal A, Webb KA, Neder JA, Mahler DA. Exertional dyspnoea in COPD: the clinical utility of cardiopulmonary exercise testing. *Eur Respir Rev* 2016; 25: 333-347.
82. Vorrink SN, Kort HS, Troosters T, Lammers JW. Level of daily physical activity in individuals with COPD compared with healthy controls. *Respir Res* 2011; 12: 33.
83. O'Donnell DE, Revill SM, Webb KA. Dynamic hyperinflation and exercise intolerance in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001; 164: 770-777.
84. Durr S, Zogg S, Miedinger D, Steveling EH, Maier S, Leuppi JD. Daily physical activity, functional capacity and quality of life in patients with COPD. *Copd* 2014; 11: 689-696.

85. Agrawal SR, Joshi R, Jain A. Correlation of severity of chronic obstructive pulmonary disease with health-related quality of life and six-minute walk test in a rural hospital of central India. *Lung India* 2015; 32: 233-240.
86. Verhage TL, Vercoulen JH, van Helvoort HA, Peters JB, Molema J, Dekhuijzen PN, Heijdra YF. Maximal exercise capacity in chronic obstructive pulmonary disease: a limited indicator of the health status. *Respiration* 2010; 80: 453-462.
87. Jackson BE, Coultas DB, Ashmore J, Russo R, Peoples J, Uhm M, Singh KP, Bae S. Domain-specific self-efficacy is associated with measures of functional capacity and quality of life among patients with moderate to severe chronic obstructive pulmonary disease. *Ann Am Thorac Soc* 2014; 11: 310-315.
88. Banzett RB, Mulnier HE, Murphy K, Rosen SD, Wise RJ, Adams L. Breathlessness in humans activates insular cortex. *Neuroreport* 2000; 11: 2117-2120.
89. Brannan S, Liotti M, Egan G, Shade R, Madden L, Robillard R, Abplanalp B, Stofer K, Denton D, Fox PT. Neuroimaging of cerebral activations and deactivations associated with hypercapnia and hunger for air. *Proc Natl Acad Sci U S A* 2001; 98: 2029-2034.
90. Evans KC. Cortico-limbic circuitry and the airways: insights from functional neuroimaging of respiratory afferents and efferents. *Biol Psychol* 2010; 84: 13-25.
91. Evans KC, Banzett RB, Adams L, McKay L, Frackowiak RS, Corfield DR. BOLD fMRI identifies limbic, paralimbic, and cerebellar activation during air hunger. *J Neurophysiol* 2002; 88: 1500-1511.
92. Herigstad M, Faull OK, Hayen A, Evans E, Hardinge FM, Wiech K, Pattinson KTS. Treating breathlessness via the brain: changes in brain activity over a course of pulmonary rehabilitation. *Eur Respir J* 2017; 50.
93. 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: 440-449.
94. Peiffer C, Poline JB, Thivard L, Aubier M, Samson Y. Neural substrates for the perception of acutely induced dyspnea. *Am J Respir Crit Care Med* 2001; 163: 951-957.
95. von Leupoldt A, Sommer T, Kegat S, Eippert F, Baumann HJ, Klose H, Dahme B, Buchel C. Down-regulation of insular cortex responses to dyspnea and pain in asthma. *Am J Respir Crit Care Med* 2009; 180: 232-238.
96. von Leupoldt A, Sommer T, Kegat S, Baumann HJ, Klose H, Dahme B, Buchel C. Dyspnea and pain share emotion-related brain network. *Neuroimage* 2009; 48: 200-206.
97. von Leupoldt A, Sommer T, Kegat S, Baumann HJ, Klose H, Dahme B, Buchel C. The unpleasantness of perceived dyspnea is processed in the anterior insula and amygdala. *Am J Respir Crit Care Med* 2008; 177: 1026-1032.
98. Pattinson KTS, Governo RJ, MacIntosh BJ, Russell EC, Corfield DR, Tracey I, Wise RG. Opioids Depress Cortical Centers Responsible for the Volitional Control of Respiration. *J Neurosci* 2009; 29: 8177-8186.
99. Herigstad M, Hayen A, Reinecke A, Pattinson KT. Development of a dyspnoea word cue set for studies of emotional processing in COPD. *Respir Physiol Neurobiol* 2016; 223: 37-42.
100. von Leupoldt A, Dahme B. Cortical substrates for the perception of dyspnea. *Chest* 2005; 128: 345-354.
101. Jensen D, Schaeffer MR, Guenette JA. Pathophysiological mechanisms of exertional breathlessness in chronic obstructive pulmonary disease and interstitial lung disease. *Curr Opin Support Palliat Care* 2018; 12: 237-245.

102. Mahler DA, O'Donnell DE. Recent advances in dyspnea. *Chest* 2015; 147: 232-241.
103. Shea SA, Andres LP, Shannon DC, Guz A, Banzett RB. Respiratory sensations in subjects who lack a ventilatory response to CO2. *Respir Physiol* 1993; 93: 203-219.
104. O'Donnell DE, Ora J, Webb KA, Laveneziana P, Jensen D. Mechanisms of activity-related dyspnea in pulmonary diseases. *Respir Physiol Neurobiol* 2009; 167: 116-132.
105. Krogh A, Lindhard J. The regulation of respiration and circulation during the initial stages of muscular work. *J Physiol* 1913; 47: 112-136.
106. Forster HV, Haouzi P, Dempsey JA. Control of breathing during exercise. *Compr Physiol* 2012; 2: 743-777.
107. Guyenet PG. Regulation of breathing and autonomic outflows by chemoreceptors. *Compr Physiol* 2014; 4: 1511-1562.
108. Smith JC, Abdala AP, Borgmann A, Rybak IA, Paton JF. Brainstem respiratory networks: building blocks and microcircuits. *Trends Neurosci* 2013; 36: 152-162.
109. Smith JC, Abdala AP, Rybak IA, Paton JF. Structural and functional architecture of respiratory networks in the mammalian brainstem. *Philos Trans R Soc Lond B Biol Sci* 2009; 364: 2577-2587.
110. Smith CA, Forster HV, Blain GM, Dempsey JA. An interdependent model of central/peripheral chemoreception: evidence and implications for ventilatory control. *Respir Physiol Neurobiol* 2010; 173: 288-297.
111. Chronos N, Adams L, Guz A. Effect of hyperoxia and hypoxia on exercise-induced breathlessness in normal subjects. *Clin Sci (Lond)* 1988; 74: 531-537.
112. Moosavi SH, Golestanian E, Binks AP, Lansing RW, Brown R, Banzett RB. Hypoxic and hypercapnic drives to breathe generate equivalent levels of air hunger in humans. *J Appl Physiol (1985)* 2003; 94: 141-154.
113. 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: 892-898.
114. Lee LY, Yu J. Sensory nerves in lung and airways. *Compr Physiol* 2014; 4: 287-324.
115. Lee LY, Pisarri TE. Afferent properties and reflex functions of bronchopulmonary C-fibers. *Respir Physiol* 2001; 125: 47-65.
116. Moreira TS, Takakura AC, Colombari E, West GH, Guyenet PG. Inhibitory input from slowly adapting lung stretch receptors to retrotrapezoid nucleus chemoreceptors. *J Physiol* 2007; 580: 285-300.
117. Green JF, Schertel ER, Coleridge HM, Coleridge JC. Effect of pulmonary arterial PCO2 on slowly adapting pulmonary stretch receptors. *J Appl Physiol (1985)* 1986; 60: 2048-2055.
118. Sant'Ambrogio G, Widdicombe J. Reflexes from airway rapidly adapting receptors. *Respir Physiol* 2001; 125: 33-45.
119. Coleridge JC, Coleridge HM, Schelegle ES, Green JF. Acute inhalation of ozone stimulates bronchial C-fibers and rapidly adapting receptors in dogs. *J Appl Physiol (1985)* 1993; 74: 2345-2352.
120. Perez de Vega MJ, Gomez-Monterrey I, Ferrer-Montiel A, Gonzalez-Muniz R. Transient Receptor Potential Melastatin 8 Channel (TRPM8) Modulation: Cool Entryway for Treating Pain and Cancer. *J Med Chem* 2016; 59: 10006-10029.
121. Kanezaki M, Ebihara S. Effect of the cooling sensation induced by olfactory stimulation by L-menthol on dyspnoea: a pilot study. *Eur Respir J* 2017; 49.

122. Nishino T, Tagaito Y, Sakurai Y. Nasal inhalation of l-menthol reduces respiratory discomfort associated with loaded breathing. *Am J Respir Crit Care Med* 1997; 156: 309-313.
123. Schwartzstein RM, Lahive K, Pope A, Weinberger SE, Weiss JW. Cold facial stimulation reduces breathlessness induced in normal subjects. *Am Rev Respir Dis* 1987; 136: 58-61.
124. Spence DP, Graham DR, Ahmed J, Rees K, Pearson MG, Calverley PM. Does cold air affect exercise capacity and dyspnea in stable chronic obstructive pulmonary disease? *Chest* 1993; 103: 693-696.
125. Sant'Ambrogio G, Mathew OP, Fisher JT, Sant'Ambrogio FB. Laryngeal receptors responding to transmural pressure, airflow and local muscle activity. *Respir Physiol* 1983; 54: 317-330.
126. Burki NK, Lee LY. Mechanisms of dyspnea. *Chest* 2010; 138: 1196-1201.
127. Coleridge JC, Coleridge HM. Afferent vagal C fibre innervation of the lungs and airways and its functional significance. *Rev Physiol Biochem Pharmacol* 1984; 99: 1-110.
128. Flume PA, Eldridge FL, Edwards LJ, Mattison LE. Relief of the 'air hunger' of breathholding - A role for pulmonary stretch receptors. *Respir Physiol* 1996; 103: 221-232.
129. Flume PA, Eldridge FL, Edwards LJ, Houser LM. The Fowler Breathholding Study Revisited - Continuous Rating of Respiratory Sensation. *Respir Physiol* 1994; 95: 53-66.
130. Vovk A, Binks AP. Raising end-expiratory volume relieves air hunger in mechanically ventilated healthy adults. *J Appl Physiol (1985)* 2007; 103: 779-786.
131. Davis HL, Fowler WS, Lambert EH. Effect of volume and rate of inflation and deflation on transpulmonary pressure and response of pulmonary stretch receptors. *Am J Physiol* 1956; 187: 558-566.
132. Banzett RB, Mulnier HE, Murphy K, Rosen SD, Wise RJS, Adams L. Breathlessness in humans activates insular cortex. *Neuroreport* 2000; 11: 2117-2120.
133. Dallak MA, Pirie LJ, Davies A. The influence of pulmonary receptors on respiratory drive in a rabbit model of pulmonary emphysema. *Respir Physiol Neurobiol* 2007; 156: 33-39.
134. Pack AI, DeLaney RG. Response of pulmonary rapidly adapting receptors during lung inflation. *J Appl Physiol Respir Environ Exerc Physiol* 1983; 55: 955-963.
135. Sellick H, Widdicombe JG. The activity of lung irritant receptors during pneumothorax, hyperpnoea and pulmonary vascular congestion. *J Physiol* 1969; 203: 359-381.
136. Burki NK, Alam M, Lee LY. The pulmonary effects of intravenous adenosine in asthmatic subjects. *Respir Res* 2006; 7.
137. Burki NK, Dale WJ, Lee LY. Intravenous adenosine and dyspnea in humans. *J Appl Physiol (1985)* 2005; 98: 180-185.
138. Burki NK, Sheatt M, Lee LY. Effects of airway anesthesia on dyspnea and ventilatory response to intravenous injection of adenosine in healthy human subjects. *Pulm Pharmacol Ther* 2008; 21: 208-213.
139. Taguchi O, Kikuchi Y, Hida W, Iwase N, Okabe S, Chonan T, Takishima T. Prostaglandin E2 inhalation increases the sensation of dyspnea during exercise. *Am Rev Respir Dis* 1992; 145: 1346-1349.
140. Nishino T. Dyspnoea: underlying mechanisms and treatment. *Brit J Anaesth* 2011; 106: 463-474.
141. Gandevia SC, Macefield G. Projection of low-threshold afferents from human intercostal muscles to the cerebral cortex. *Respir Physiol* 1989; 77: 203-214.
142. Remmers JE, Marttila I. Action of intercostal muscle afferents on the respiratory rhythm of anesthetized cats. *Respir Physiol* 1975; 24: 31-41.

143. Road JD. Phrenic Afferents and Ventilatory Control. *Lung* 1990; 168: 137-149.
144. Remmers JE. Inhibition of inspiratory activity by intercostal muscle afferents. *Respir Physiol* 1970; 10: 358-383.
145. Edo H, Kimura H, Niijima M, Sakabe H, Shibuya M, Kanamaru A, Homma I, Kuriyama T. Effects of chest wall vibration on breathlessness during hypercapnic ventilatory response. *J Appl Physiol (1985)* 1998; 84: 1487-1491.
146. Homma I, Obata T, Sibuya M, Uchida M. Gate mechanism in breathlessness caused by chest wall vibration in humans. *J Appl Physiol Respir Environ Exerc Physiol* 1984; 56: 8-11.
147. Manning HL, Basner R, Ringler J, Rand C, Fencl V, Weinberger SE, Weiss JW, Schwartzstein RM. Effect of chest wall vibration on breathlessness in normal subjects. *J Appl Physiol (1985)* 1991; 71: 175-181.
148. Sibuya M, Yamada M, Kanamaru A, Tanaka K, Suzuki H, Noguchi E, Altose MD, Homma I. Effect of chest wall vibration on dyspnea in patients with chronic respiratory disease. *Am J Respir Crit Care Med* 1994; 149: 1235-1240.
149. Amann M. Significance of Group III and IV muscle afferents for the endurance exercising human. *Clin Exp Pharmacol Physiol* 2012; 39: 831-835.
150. Amann M, Sidhu SK, Weavil JC, Mangum TS, Venturelli M. Autonomic responses to exercise: group III/IV muscle afferents and fatigue. *Auton Neurosci* 2015; 188: 19-23.
151. Dempsey JA. New perspectives concerning feedback influences on cardiorespiratory control during rhythmic exercise and on exercise performance. *J Physiol* 2012; 590: 4129-4144.
152. Dempsey JA, Blain GM, Amann M. Are type III-IV muscle afferents required for a normal steady-state exercise hyperpnoea in humans? *J Physiol* 2014; 592: 463-474.
153. Kaufman MP, Forster HV. Reflexes controlling circulatory, ventilatory and airway responses to exercise. *Compr Physiol* 1996.
154. Road JD, West NH, Vanvliet BN. Ventilatory Effects of Stimulation of Phrenic Afferents. *J Appl Physiol (1985)* 1987; 63: 1063-1069.
155. Ward ME, Eidelman D, Stubbing DG, Bellemare F, Macklem PT. Respiratory Sensation and Pattern of Respiratory Muscle Activation during Diaphragm Fatigue. *J Appl Physiol (1985)* 1988; 65: 2181-2289.
156. Jolley CJ, Luo YM, Steier J, Rafferty GF, Polkey MI, Moxham J. Neural respiratory drive and breathlessness in COPD. *Eur Respir J* 2015; 45: 355-364.
157. Brusasco V, Martinez F. Chronic obstructive pulmonary disease. *Compr Physiol* 2014; 4: 1-31.
158. 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: 731-749.
159. 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: 663-668.
160. O'Donnell DE, Laveneziana P. Dyspnea and activity limitation in COPD: mechanical factors. *Copd* 2007; 4: 225-236.
161. O'Donnell DE, Revill SM, Webb KA. Dynamic hyperinflation and exercise intolerance in chronic obstructive pulmonary disease. *Am J Resp Crit Care* 2001; 164: 770-777.
162. Pepin V, Saey D, Laviolette L, Maltais F. Exercise capacity in chronic obstructive pulmonary disease: mechanisms of limitation. *Copd* 2007; 4: 195-204.

163. Thomas M, Decramer M, O'Donnell DE. No room to breathe: the importance of lung hyperinflation in COPD. *Prim Care Respir J* 2013; 22: 101-111.
164. Vogiatzis I, Zakynthinos S. Factors limiting exercise tolerance in chronic lung diseases. *Compr Physiol* 2012; 2: 1779-1817.
165. Brusasco V, Martinez F. Chronic Obstructive Pulmonary Disease. *Comprehensive Physiology* 2014; 4: 1-31.
166. Sharma P, Morris NR, Adams L. Effect of experimental modulation of mood on perception of exertional dyspnea in healthy subjects. *J Appl Physiol (1985)* 2016; 120: 114-120.
167. von Leupoldt A, Sommer T, Kegat S, Baumann HJ, Klose H, Dahme B, Buchel C. The unpleasantness of perceived dyspnea is processed in the anterior insula and amygdala. *Am J Resp Crit Care* 2008; 177: 1026-1032.
168. Aldhafeeri FM, Mackenzie I, Kay T, Alghamdi J, Sluming V. Regional brain responses to pleasant and unpleasant IAPS pictures: different networks. *Neurosci Lett* 2012; 512: 94-98.
169. O'Donnell DE, Laveneziana P. Physiology and consequences of lung hyperinflation in COPD. *Eur Respir Rev* 2006; 15: 61-67.
170. Neder JA, Berton DC, Muller PT, Elbehairy AF, Rocha A, Palange P, O'Donnell DE, Canadian Respiratory Research N. Ventilatory Inefficiency and Exertional Dyspnea in Early Chronic Obstructive Pulmonary Disease. *Ann Am Thorac Soc* 2017; 14: S22-S29.
171. Reilly CC, Ward K, Jolley CJ, Lunt AC, Steier J, Elston C, Polkey MI, Rafferty GF, Moxham J. Neural respiratory drive, pulmonary mechanics and breathlessness in patients with cystic fibrosis. *Thorax* 2011; 66: 240-246.
172. 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: 427-441.
173. Schaeffer MR, Ryerson CJ, Ramsook AH, Molgat-Seon Y, Wilkie SS, Dhillon SS, Mitchell RA, Sheel AW, Khalil N, Camp PG, Guenette JA. Neurophysiological mechanisms of exertional dyspnoea in fibrotic interstitial lung disease. *Eur Respir J* 2018; 51.
174. O'Donnell DE, Travers J, Webb KA, He Z, Lam YM, Hamilton A, Kesten S, Maltais F, Magnussen H. Reliability of ventilatory parameters during cycle ergometry in multicentre trials in COPD. *Eur Respir J* 2009; 34: 866-874.
175. Neder JA, Berton DC, Arbex FF, Alencar MC, Rocha A, Sperandio PA, Palange P, O'Donnell DE. Physiological and clinical relevance of exercise ventilatory efficiency in COPD. *Eur Respir J* 2017; 49.
176. Cassart M, Pettiaux N, Gevenois PA, Paiva M, Estenne M. Effect of chronic hyperinflation on diaphragm length and surface area. *Am J Respir Crit Care Med* 1997; 156: 504-508.
177. Kabitz HJ, Walterspacher S, Walker D, Windisch W. Inspiratory muscle strength in chronic obstructive pulmonary disease depending on disease severity. *Clin Sci (Lond)* 2007; 113: 243-249.
178. Laghi F, Tobin MJ. Disorders of the respiratory muscles. *Am J Respir Crit Care Med* 2003; 168: 10-48.
179. Sinderby C, Spahija J, Beck J, Kaminski D, Yan S, Comtois N, Sliwinski P. Diaphragm activation during exercise in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001; 163: 1637-1641.

180. Neder JA, Alharbi A, Berton DC, Alencar MC, Arbex FF, Hirai DM, Webb KA, O'Donnell DE. Exercise Ventilatory Inefficiency Adds to Lung Function in Predicting Mortality in COPD. *Copd* 2016; 13: 416-424.
181. Guenette JA, Chin RC, Cheng S, Dominelli PB, Raghavan N, Webb KA, Neder JA, O'Donnell DE. Mechanisms of exercise intolerance in global initiative for chronic obstructive lung disease grade 1 COPD. *Eur Respir J* 2014; 44: 1177-1187.
182. Faisal A, Alghamdi BJ, Ciavaglia CE, Elbehairy AF, Webb KA, Ora J, Neder JA, O'Donnell DE. Common Mechanisms of Dyspnea in Chronic Interstitial and Obstructive Lung Disorders. *Am J Respir Crit Care Med* 2016; 193: 299-309.
183. Holverda S, Rietema H, Westerhof N, Marcus JT, Gan CTJ, Postmus PE, Vonk-Noordegraaf A. Stroke volume increase to exercise in chronic obstructive pulmonary disease is limited by increased pulmonary artery pressure. *Heart* 2009; 95: 137-141.
184. Light RW, Mintz HM, Linden GS, Brown SE. Hemodynamics of Patients with Severe Chronic Obstructive Pulmonary-Disease during Progressive Upright Exercise. *American Review of Respiratory Disease* 1984; 130: 391-395.
185. Tzani P, Aiello M, Elia D, Boracchia L, Marangio E, Olivieri D, Clini E, Chetta A. Dynamic Hyperinflation Is Associated with a Poor Cardiovascular Response to Exercise in Copd Patients. *Thorax* 2011; 66: A120-A120.
186. Vassaux C, Torre-Bouscoulet L, Zeineldine S, Cortopassi F, Paz-Diaz H, Celli BR, Pinto-Plata VM. Effects of hyperinflation on the oxygen pulse as a marker of cardiac performance in COPD. *Eur Respir J* 2008; 32: 1275-1282.
187. O'Donnell DE, Laveneziana P, Webb K, Neder JA. Chronic obstructive pulmonary disease: clinical integrative physiology. *Clin Chest Med* 2014; 35: 51-69.
188. Butcher SJ, Lagerquist O, Marciniuk DD, Petersen SR, Collins DF, Jones RL. Relationship between ventilatory constraint and muscle fatigue during exercise in COPD. *Eur Respir J* 2009; 33: 763-770.
189. Amann M, Regan MS, Kobitary M, Eldridge MW, Boutellier U, Pegelow DF, Dempsey JA. Impact of pulmonary system limitations on locomotor muscle fatigue in patients with COPD. *Am J Physiol Regul Integr Comp Physiol* 2010; 299: R314-324.
190. Saey D, Debigare R, LeBlanc P, Mador MJ, Cote CH, Jobin J, Maltais F. Contractile leg fatigue after cycle exercise: a factor limiting exercise in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2003; 168: 425-430.
191. Ofir D, Laveneziana P, Webb KA, Lam YM, O'Donnell DE. Mechanisms of dyspnea during cycle exercise in symptomatic patients with GOLD stage I chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2008; 177: 622-629.
192. Hamilton AL, Killian KJ, Summers E, Jones NL. Symptom intensity and subjective limitation to exercise in patients with cardiorespiratory disorders. *Chest* 1996; 110: 1255-1263.
193. Page CP, Barnes PJ. Pharmacology and Therapeutics of Asthma and COPD. 2004.
194. Nardini S, Camiciottoli G, Locicero S, Maselli R, Pasqua F, Passalacqua G, Pela R, Pesci A, Sebastiani A, Vatrella A. COPD: maximization of bronchodilation. *Multidiscip Respir Med* 2014; 9: 50.
195. Matera MG, Page CP, Cazzola M. Novel bronchodilators for the treatment of chronic obstructive pulmonary disease. *Trends Pharmacol Sci* 2011; 32: 495-506.
196. Cazzola M, Rogliani P, Ruggeri P, Segreti A, Proietto A, Picciolo S, Matera MG. Chronic treatment with indacaterol and airway response to salbutamol in stable COPD. *Respir Med* 2013; 107: 848-853.

197. Sestini P, Renzoni E, Robinson S, Poole P, Ram FS. Short-acting beta 2 agonists for stable chronic obstructive pulmonary disease. *Cochrane Database Syst Rev* 2002: CD001495.
198. Di Marco F, Sotgiu G, Santus P, O'Donnell DE, Beeh KM, Dore S, Roggi MA, Giuliani L, Blasi F, Centanni S. Long-acting bronchodilators improve exercise capacity in COPD patients: a systematic review and meta-analysis. *Respir Res* 2018; 19: 18.
199. Maltais F, Hamilton A, Marciniuk D, Hernandez P, Sciurba FC, Richter K, Kesten S, O'Donnell D. Improvements in symptom-limited exercise performance over 8 h with once-daily tiotropium in patients with COPD. *Chest* 2005; 128: 1168-1178.
200. O'Donnell DE, Fluge T, Gerken F, Hamilton A, Webb K, Aguilaniu B, Make B, Magnussen H. Effects of tiotropium on lung hyperinflation, dyspnoea and exercise tolerance in COPD. *Eur Respir J* 2004; 23: 832-840.
201. O'Donnell DE, Lam M, Webb KA. Spirometric correlates of improvement in exercise performance after anticholinergic therapy in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999; 160: 542-549.
202. O'Donnell DE, Voduc N, Fitzpatrick M, Webb KA. Effect of salmeterol on the ventilatory response to exercise in chronic obstructive pulmonary disease. *Eur Respir J* 2004; 24: 86-94.
203. Qin YY, Li RF, Wu GF, Zhu Z, Liu J, Zhou CZ, Guan WJ, Luo JY, Yu XX, Ou YM, Jiang M, Zhong NS, Luo YM. Effect of tiotropium on neural respiratory drive during exercise in severe COPD. *Pulm Pharmacol Ther* 2015; 30: 51-56.
204. Ries AL. Minimally clinically important difference for the UCSD Shortness of Breath Questionnaire, Borg Scale, and Visual Analog Scale. *Copd* 2005; 2: 105-110.
205. Puente-Maestu L, Villar F, de Miguel J, Stringer WW, Sanz P, Sanz ML, de Pedro JG, Martinez-Abad Y. Clinical relevance of constant power exercise duration changes in COPD. *Eur Respir J* 2009; 34: 340-345.
206. Rabe KF, Magnussen H, Dent G. Theophylline and selective PDE inhibitors as bronchodilators and smooth muscle relaxants. *Eur Respir J* 1995; 8: 637-642.
207. Biaggioni I, Paul S, Puckett A, Arzubiaga C. Caffeine and theophylline as adenosine receptor antagonists in humans. *J Pharmacol Exp Ther* 1991; 258: 588-593.
208. Barnes PJ. Theophylline in chronic obstructive pulmonary disease: new horizons. *Proc Am Thorac Soc* 2005; 2: 334-339; discussion 340-331.
209. Polosa R, Blackburn MR. Adenosine receptors as targets for therapeutic intervention in asthma and chronic obstructive pulmonary disease. *Trends Pharmacol Sci* 2009; 30: 528-535.
210. Barnes PJ. Theophylline. *Pharmaceuticals (Basel)* 2010; 3: 725-747.
211. Mahler DA, Matthay RA, Snyder PE, Wells CK, Loke J. Sustained-Release Theophylline Reduces Dyspnea in Nonreversible Obstructive Airway Disease. *Am Rev Respir Dis* 1985; 131: 22-25.
212. McKay SE, Howie CA, Thomson AH, Whiting B, Addis GJ. Value of theophylline treatment in patients handicapped by chronic obstructive lung disease. *Thorax* 1993; 48: 227-232.
213. Murciano D, Aubier M, Lecocguic Y, Pariente R. Effects of Theophylline on Diaphragmatic Strength and Fatigue in Patients with Chronic Obstructive Pulmonary-Disease. *N Engl J Med* 1984; 311: 349-353.
214. Murciano D, Auclair MH, Pariente R, Aubier M. A randomized, controlled trial of theophylline in patients with severe chronic obstructive pulmonary disease. *N Engl J Med* 1989; 320: 1521-1525.

215. Tsukino M, Nishimura K, Ikeda A, Hajiro T, Koyama H, Izumi T. Effects of theophylline and ipratropium bromide on exercise performance in patients with stable chronic obstructive pulmonary disease. *Thorax* 1998; 53: 269-273.
216. Voduc N, Alvarez GG, Amjadi K, Tessier C, Sabri E, Aaron SD. Effect of theophylline on exercise capacity in COPD patients treated with combination long-acting bronchodilator therapy: a pilot study. *Int J Chronic Obstr* 2012; 7: 245-252.
217. Fink G, Kaye C, Sulkes J, Gabbay U, Spitzer SA. Effect of Theophylline on Exercise Performance in Patients with Severe Chronic Obstructive Pulmonary-Disease. *Thorax* 1994; 49: 332-334.
218. Ram FSF, Jardin JR, Atallah A, Castro AA, Mazzini R, Goldstein R, Lacasse Y, Cendon S. Efficacy of theophylline in people with stable chronic obstructive pulmonary disease: a systematic review and meta-analysis. *Resp Med* 2005; 99: 135-144.
219. Voduc N, Alvarez GG, Amjadi K, Tessier C, Sabri E, Aaron SD. Effect of theophylline on exercise capacity in COPD patients treated with combination long-acting bronchodilator therapy: a pilot study. *Int J Chron Obstruct Pulmon Dis* 2012; 7: 245-252.
220. Aubier M. Effect of theophylline on diaphragmatic and other skeletal muscle function. *J Allergy Clin Immunol* 1986; 78: 787-792.
221. Nietrzeba RM, Elliott CG, Adams TD, Yeh MP, Yanowitz FG. Effects of Aminophylline Upon the Exercise Performance of Patients with Stable Chronic Air-Flow Obstruction. *B Eur Physiopath Res* 1984; 20: 361-367.
222. Moxham J, Miller J, Wiles CM, Morris AJ, Green M. Effect of aminophylline on the human diaphragm. *Thorax* 1985; 40: 288-292.
223. Kongragunta VR, Druz WS, Sharp JT. Dyspnea and diaphragmatic fatigue in patients with chronic obstructive pulmonary disease. Responses to theophylline. *Am Rev Respir Dis* 1988; 137: 662-667.
224. Barnes PJ. How corticosteroids control inflammation: Quintiles Prize Lecture 2005. *Br J Pharmacol* 2006; 148: 245-254.
225. Barnes PJ. Cellular and molecular mechanisms of chronic obstructive pulmonary disease. *Clin Chest Med* 2014; 35: 71-86.
226. Barnes PJ, Shapiro SD, Pauwels RA. Chronic obstructive pulmonary disease: molecular and cellular mechanisms. *Eur Respir J* 2003; 22: 672-688.
227. Ito K, Lim S, Caramori G, Chung KF, Barnes PJ, Adcock IM. Cigarette smoking reduces histone deacetylase 2 expression, enhances cytokine expression, and inhibits glucocorticoid actions in alveolar macrophages. *FASEB J* 2001; 15: 1110-1112.
228. Roche N, Marthan R, Berger P, Chambellan A, Chanez P, Aguilaniu B, Brillet PY, Burgel PR, Chaouat A, Devillier P, Escamilla R, Louis R, Mal H, Muir JF, Perez T, Similowski T, Wallaert B, Aubier M. Beyond corticosteroids: future prospects in the management of inflammation in COPD. *Eur Respir Rev* 2011; 20: 175-182.
229. Bourbeau J, Rouleau MY, Boucher S. Randomised controlled trial of inhaled corticosteroids in patients with chronic obstructive pulmonary disease. *Thorax* 1998; 53: 477-482.
230. Guenette JA, Raghavan N, Harris-McAllister V, Preston ME, Webb KA, O'Donnell DE. Effect of adjunct fluticasone propionate on airway physiology during rest and exercise in COPD. *Respir Med* 2011; 105: 1836-1845.
231. Guenette JA, Webb KA, O'Donnell DE. Effect of fluticasone/salmeterol combination on dyspnea and respiratory mechanics in mild-to-moderate COPD. *Respir Med* 2013; 107: 708-716.

232. Paggiaro PL, Dahle R, Bakran I, Frith L, Hollingworth K, Efthimiou J. Multicentre randomised placebo-controlled trial of inhaled fluticasone propionate in patients with chronic obstructive pulmonary disease. International COPD Study Group. *Lancet* 1998; 351: 773-780.
233. Yang IA, Clarke MS, Sim EH, Fong KM. Inhaled corticosteroids for stable chronic obstructive pulmonary disease. *Cochrane Database Syst Rev* 2012: CD002991.
234. Puente-Maestu L, Palange P, Casaburi R, Laveneziana P, Maltais F, Neder JA, O'Donnell DE, Onorati P, Porszasz J, Rabinovich R, Rossiter HB, Singh S, Troosters T, Ward S. Use of exercise testing in the evaluation of interventional efficacy: an official ERS statement. *Eur Respir J* 2016; 47: 429-460.
235. O'Donnell DE, Sciurba F, Celli B, Mahler DA, Webb KA, Kalberg CJ, Knobil K. Effect of fluticasone propionate/salmeterol on lung hyperinflation and exercise endurance in COPD. *Chest* 2006; 130: 647-656.
236. Worth H, Forster K, Eriksson G, Nihlen U, Peterson S, Magnussen H. Budesonide added to formoterol contributes to improved exercise tolerance in patients with COPD. *Respir Med* 2010; 104: 1450-1459.
237. Chong J, Leung B, Poole P. Phosphodiesterase 4 inhibitors for chronic obstructive pulmonary disease. *Cochrane Database Syst Rev* 2017; 9: CD002309.
238. Shen LF, Lv XD, Chen WY, Yang Q, Fang ZX, Lu WF. Effect of roflumilast on chronic obstructive pulmonary disease: a systematic review and meta-analysis. *Ir J Med Sci* 2018.
239. Rabe KF. Update on roflumilast, a phosphodiesterase 4 inhibitor for the treatment of chronic obstructive pulmonary disease. *Br J Pharmacol* 2011; 163: 53-67.
240. Kawamatawong T. Roles of roflumilast, a selective phosphodiesterase 4 inhibitor, in airway diseases. *J Thorac Dis* 2017; 9: 1144-1154.
241. Polkey MI, Spruit MA, Edwards LD, Watkins ML, Pinto-Plata V, Vestbo J, Calverley PM, Tal-Singer R, Agusti A, Bakke PS, Coxson HO, Lomas DA, MacNee W, Rennard S, Silverman EK, Miller BE, Crim C, Yates J, Wouters EF, Celli B, Evaluation of CLtIPSESI. Six-minute-walk test in chronic obstructive pulmonary disease: minimal clinically important difference for death or hospitalization. *Am J Respir Crit Care Med* 2013; 187: 382-386.
242. Grootendorst DC, Gauw SA, Baan R, Kelly J, Murdoch RD, Sterk PJ, Rabe KF. Does a single dose of the phosphodiesterase 4 inhibitor, cilomilast (15 mg), induce bronchodilation in patients with chronic obstructive pulmonary disease? *Pulm Pharmacol Ther* 2003; 16: 115-120.
243. O'Donnell DE, Bredenbroker D, Brose M, Webb KA. Physiological effects of roflumilast at rest and during exercise in COPD. *Eur Respir J* 2012; 39: 1104-1112.
244. Borson S, McDonald GJ, Gayle T, Deffebach M, Lakshminarayan S, VanTuinen C. Improvement in mood, physical symptoms, and function with nortriptyline for depression in patients with chronic obstructive pulmonary disease. *Psychosomatics* 1992; 33: 190-201.
245. Currow DC, Ekstrm M, Louw S, Hill J, Fazekas B, Clark K, Davidson PM, McDonald C, Sajkov D, McCaffrey N, Doogue M, Abernethy AP, Agar M. Sertraline in symptomatic chronic breathlessness: a double blind, randomised trial. *Eur Respir J* 2018.
246. Greene JG, Pucino F, Carlson JD, Storsved M, Strommen GL. Effects of alprazolam on respiratory drive, anxiety, and dyspnea in chronic airflow obstruction: a case study. *Pharmacotherapy* 1989; 9: 34-38.

247. Light RW, Merrill EJ, Despars J, Gordon GH, Mutalipassi LR. Doxepin treatment of depressed patients with chronic obstructive pulmonary disease. *Arch Intern Med* 1986; 146: 1377-1380.
248. Man GC, Hsu K, Sproule BJ. Effect of alprazolam on exercise and dyspnea in patients with chronic obstructive pulmonary disease. *Chest* 1986; 90: 832-836.
249. Mitchell-Heggs P, Murphy K, Minty K, Guz A, Patterson SC, Minty PS, Rosser RM. Diazepam in the treatment of dyspnoea in the 'Pink Puffer' syndrome. *Q J Med* 1980; 49: 9-20.
250. Rudolf M, Geddes DM, Turner JA, Saunders KB. Depression of central respiratory drive by nitrazepam. *Thorax* 1978; 33: 97-100.
251. Simon ST, Higginson IJ, Booth S, Harding R, Weingartner V, Bausewein C. Benzodiazepines for the relief of breathlessness in advanced malignant and non-malignant diseases in adults. *Cochrane Database Syst Rev* 2016; 10: CD007354.
252. Singh NP, Despars JA, Stansbury DW, Avalos K, Light RW. Effects of buspirone on anxiety levels and exercise tolerance in patients with chronic airflow obstruction and mild anxiety. *Chest* 1993; 103: 800-804.
253. Smoller JW, Pollack MH, Systrom D, Kradin RL. Sertraline effects on dyspnea in patients with obstructive airways disease. *Psychosomatics* 1998; 39: 24-29.
254. Strom K, Boman G, Pehrsson K, Alton M, Singer J, Rydstrom PO, Uddenfeldt M, Ericsson CH, Ostholm B, Morlin C. Effect of protriptyline, 10 mg daily, on chronic hypoxaemia in chronic obstructive pulmonary disease. *Eur Respir J* 1995; 8: 425-429.
255. Woodcock AA, Gross ER, Geddes DM. Drug treatment of breathlessness: contrasting effects of diazepam and promethazine in pink puffers. *Br Med J (Clin Res Ed)* 1981; 283: 343-346.
256. Griffin CE, 3rd, Kaye AM, Bueno FR, Kaye AD. Benzodiazepine pharmacology and central nervous system-mediated effects. *Ochsner J* 2013; 13: 214-223.
257. Hayen A, Herigstad M, Pattinson KTS. Understanding dyspnea as a complex individual experience. *Maturitas* 2013; 76: 45-50.
258. Inokuchi R, Aoki A, Aoki Y, Yahagi N. Effectiveness of inhaled furosemide for acute asthma exacerbation: a meta-analysis. *Critical care* 2014; 18: 621.
259. Laveneziana P, Galarducci A, Binazzi B, Stendardi L, Duranti R, Scano G. Inhaled furosemide does not alleviate respiratory effort during flow-limited exercise in healthy subjects. *Pulm Pharmacol Ther* 2008; 21: 196-200.
260. Moosavi SH, Binks AP, Lansing RW, Toplos GP, Banzett RB, Schwartzstein RM. Effect of inhaled furosemide on air hunger induced in healthy humans. *Resp Physiol Neurobi* 2007; 156: 1-8.
261. Waskiw-Ford M, Wu AN, Mainra A, Marchand N, Alhuzaim A, Bourbeau J, Smith BM, Jensen D. Effect of Inhaled Nebulized Furosemide (40 and 120 mg) on Breathlessness during Exercise in the Presence of External Thoracic Restriction in Healthy Men. *Front Physiol* 2018; 9.
262. Nishino T, Ide T, Sudo T, Sato J. Inhaled furosemide greatly alleviates the sensation of experimentally induced dyspnea. *Am J Resp Crit Care* 2000; 161: 1963-1967.
263. Banzett RB, Schwartzstein RM, Lansing RW, O'Donnell CR. Aerosol furosemide for dyspnea: High-dose controlled delivery does not improve effectiveness. *Respir Physiol Neurobiol* 2018; 247: 24-30.

264. Morelot-Panzini C, O'Donnell CR, Lansing RW, Schwartzstein RM, Banzett RB. Aerosol furosemide for dyspnea: Controlled delivery does not improve effectiveness. *Respir Physiol Neurobiol* 2018; 247: 146-155.
265. Ben-Aharon I, Gafter-Gvili A, Leibovici L, Stemmer SM. Interventions for alleviating cancer-related dyspnea: a systematic review and meta-analysis. *Acta oncologica* 2012; 51: 996-1008.
266. Boyden JY, Connor SR, Otolorin L, Nathan SD, Fine PG, Davis MS, Muir JC. Nebulized medications for the treatment of dyspnea: a literature review. *J Aerosol Med Pulm Drug Deliv* 2015; 28: 1-19.
267. Jeba J, George R, Pease N. Nebulised furosemide in the palliation of dyspnoea in cancer: a systematic review. *BMJ Support Palliat Care* 2014; 4: 132-139.
268. Newton PJ, Davidson PM, Macdonald P, Ollerton R, Krum H. Nebulized furosemide for the management of dyspnea: does the evidence support its use? *J Pain Symptom Manage* 2008; 36: 424-441.
269. Jensen D, Amjadi K, Harris-McAllister V, Webb KA, O'Donnell DE. Mechanisms of dyspnoea relief and improved exercise endurance after furosemide inhalation in COPD. *Thorax* 2008; 63: 606-613.
270. Ong KC, Kor AC, Chong WF, Earnest A, Wang YT. Effects of inhaled furosemide on exertional dyspnea in chronic obstructive pulmonary disease. *Am J Resp Crit Care* 2004; 169: 1028-1033.
271. Sudo T, Hayashi F, Nishino T. Responses of tracheobronchial receptors to inhaled furosemide in anesthetized rats. *Am J Resp Crit Care* 2000; 162: 971-975.
272. Eldridge FL, Chen ZB. Respiratory-Associated Rhythmic Firing of Midbrain Neurons Is Modulated by Vagal Input. *Respir Physiol* 1992; 90: 31-46.
273. Hill K, Vogiatzis I, Burtin C. The importance of components of pulmonary rehabilitation, other than exercise training, in COPD. *Eur Respir Rev* 2013; 22: 405-413.
274. Marciniuk DD, Brooks D, Butcher S, Debigare R, Dechman G, Ford G, Pepin V, Reid D, Sheel AW, Stickland MK, Todd DC, Walker SL, Aaron SD, Balter M, Bourbeau J, Hernandez P, Maltais F, O'Donnell DE, Bleakney D, Carlin B, Goldstein R, Muthuri SK, Canadian Thoracic Society CCEWG. Optimizing pulmonary rehabilitation in chronic obstructive pulmonary disease--practical issues: a Canadian Thoracic Society Clinical Practice Guideline. *Can Respir J* 2010; 17: 159-168.
275. McCarthy B, Casey D, Devane D, Murphy K, Murphy E, Lacasse Y. Pulmonary rehabilitation for chronic obstructive pulmonary disease. *Cochrane Database Syst Rev* 2015: CD003793.
276. Spruit MA, Singh SJ, Garvey C, ZuWallack R, Nici L, Rochester C, Hill K, Holland AE, Lareau SC, Man WDC, Pitta F, Sewell L, Raskin J, Bourbeau J, Crouch R, Franssen FME, Casaburi R, Vercoulen JH, Vogiatzis I, Gosselink R, Clini EM, Effing TW, Maltais F, van der Palen J, Troosters T, Janssen DJA, Collins E, Garcia-Aymerich J, Brooks D, Fahy BF, Puhan MA, Hoogendoorn M, Garrod R, Schols AMWJ, Carlin B, Benzo R, Meek P, Morgan M, Rutten-van Molken MPMH, Ries AL, Make B, Goldstein RS, Dowson CA, Brozek JL, Donner CF, Wouters EFM, Rehabil AETFP. An Official American Thoracic Society/European Respiratory Society Statement: Key Concepts and Advances in Pulmonary Rehabilitation. *Am J Resp Crit Care* 2013; 188: E13-E64.
277. Wadell K, Webb KA, Preston ME, Amornputtisathaporn N, Samis L, Patelli J, Guenette JA, O'Donnell DE. Impact of pulmonary rehabilitation on the major dimensions of dyspnea in COPD. *Copd* 2013; 10: 425-435.

278. McKeough ZJ, Alison JA, Bye PT, Trenell MI, Sachinwalla T, Thompson CH, Kemp GJ. Exercise capacity and quadriceps muscle metabolism following training in subjects with COPD. *Respir Med* 2006; 100: 1817-1825.
279. Vogiatzis I, Simoes DC, Stratakos G, Kourepini E, Terzis G, Manta P, Athanasopoulos D, Roussos C, Wagner PD, Zakynthinos S. Effect of pulmonary rehabilitation on muscle remodelling in cachectic patients with COPD. *Eur Respir J* 2010; 36: 301-310.
280. Vogiatzis I, Terzis G, Nanas S, Stratakos G, Simoes DC, Georgiadou O, Zakynthinos S, Roussos C. Skeletal muscle adaptations to interval training in patients with advanced COPD. *Chest* 2005; 128: 3838-3845.
281. Vogiatzis I, Terzis G, Stratakos G, Cherouveim E, Athanasopoulos D, Spetsioti S, Nasis I, Manta P, Roussos C, Zakynthinos S. Effect of pulmonary rehabilitation on peripheral muscle fiber remodeling in patients with COPD in GOLD stages II to IV. *Chest* 2011; 140: 744-752.
282. Maltais F, LeBlanc P, Simard C, Jobin J, Berube C, Bruneau J, Carrier L, Belleau R. Skeletal muscle adaptation to endurance training in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1996; 154: 442-447.
283. Sala E, Roca J, Marrades RM, Alonso J, Gonzalez De Suso JM, Moreno A, Barbera JA, Nadal J, de Jover L, Rodriguez-Roisin R, Wagner PD. Effects of endurance training on skeletal muscle bioenergetics in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999; 159: 1726-1734.
284. Casaburi R, Patessio A, Ioli F, Zanaboni S, Donner CF, Wasserman K. Reductions in exercise lactic acidosis and ventilation as a result of exercise training in patients with obstructive lung disease. *Am Rev Respir Dis* 1991; 143: 9-18.
285. Maltais F, LeBlanc P, Jobin J, Berube C, Bruneau J, Carrier L, Breton MJ, Falardeau G, Belleau R. Intensity of training and physiologic adaptation in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1997; 155: 555-561.
286. Bernard S, Whittom F, Leblanc P, Jobin J, Belleau R, Berube C, Carrier G, Maltais F. Aerobic and strength training in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999; 159: 896-901.
287. Strasser B, Siebert U, Schobersberger W. Effects of resistance training on respiratory function in patients with chronic obstructive pulmonary disease: a systematic review and meta-analysis. *Sleep Breath* 2013; 17: 217-226.
288. O'Donnell DE, McGuire M, Samis L, Webb KA. The impact of exercise reconditioning on breathlessness in severe chronic airflow limitation. *Am J Respir Crit Care Med* 1995; 152: 2005-2013.
289. Osterling K, MacFadyen K, Gilbert R, Dechman G. The effects of high intensity exercise during pulmonary rehabilitation on ventilatory parameters in people with moderate to severe stable COPD: a systematic review. *Int J Chron Obstruct Pulmon Dis* 2014; 9: 1069-1078.
290. O'Donnell DE, McGuire M, Samis L, Webb KA. General exercise training improves ventilatory and peripheral muscle strength and endurance in chronic airflow limitation. *Am J Respir Crit Care Med* 1998; 157: 1489-1497.
291. Brooks D, Krip B, Mangovski-Alzamora S, Goldstein RS. The effect of postrehabilitation programmes among individuals with chronic obstructive pulmonary disease. *Eur Respir J* 2002; 20: 20-29.

292. Ries AL, Kaplan RM, Myers R, Prewitt LM. Maintenance after pulmonary rehabilitation in chronic lung disease: a randomized trial. *Am J Respir Crit Care Med* 2003; 167: 880-888.
293. Camp PG, Hernandez P, Bourbeau J, Kirkham A, Debigare R, Stickland MK, Goodridge D, Marciniuk DD, Road JD, Bhutani M, Dechman G. Pulmonary rehabilitation in Canada: A report from the Canadian Thoracic Society COPD Clinical Assembly. *Can Respir J* 2015; 22: 147-152.
294. Rochester CL, Vogiatzis I, Powell P, Masefield S, Spruit MA. Patients' perspective on pulmonary rehabilitation: experiences of European and American individuals with chronic respiratory diseases. *ERJ Open Res* 2018; 4.
295. Bradley JM, Lasserson T, Elborn S, Macmahon J, O'Neill B. A systematic review of randomized controlled trials examining the short-term benefit of ambulatory oxygen in COPD. *Chest* 2007; 131: 278-285.
296. Ekstrom M, Ahmadi Z, Bornefalk-Hermansson A, Abernethy A, Currow D. Oxygen for breathlessness in patients with chronic obstructive pulmonary disease who do not qualify for home oxygen therapy. *Cochrane Database Syst Rev* 2016; 11: CD006429.
297. Gagnon P, Bussieres JS, Ribeiro F, Gagnon SL, Saey D, Gagne N, Provencher S, Maltais F. Influences of spinal anesthesia on exercise tolerance in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2012; 186: 606-615.
298. Jolly EC, Di Boscio V, Aguirre L, Luna CM, Berensztein S, Gene RJ. Effects of supplemental oxygen during activity in patients with advanced COPD without severe resting hypoxemia. *Chest* 2001; 120: 437-443.
299. Louvaris Z, Vogiatzis I, Dimitris A, Vasilis A, Panagiotis A, Harrieth W, Habazettl H, Roussos C, Wagner P, Zakynthinos S. Does cerebral blood flow & oxygenation limit maximal exercise performance in healthy humans? *Faseb Journal* 2010; 24.
300. Maltais F, Simon M, Jobin J, Desmeules M, Sullivan MJ, Belanger M, Leblanc P. Effects of oxygen on lower limb blood flow and O2 uptake during exercise in COPD. *Med Sci Sports Exerc* 2001; 33: 916-922.
301. Miki K, Maekura R, Hiraga T, Kitada S, Miki M, Yoshimura K, Tateishi Y. Effects of oxygen on exertional dyspnoea and exercise performance in patients with chronic obstructive pulmonary disease. *Respirology* 2012; 17: 149-154.
302. O'Donnell DE, Bain DJ, Webb KA. Factors contributing to relief of exertional breathlessness during hyperoxia in chronic airflow limitation. *Am J Respir Crit Care Med* 1997; 155: 530-535.
303. O'Donnell DE, D'Arsigny C, Webb KA. Effects of hyperoxia on ventilatory limitation during exercise in advanced chronic obstructive pulmonary disease. *Am J Resp Crit Care* 2001; 163: 892-898.
304. Stein DA, Bradley BL, Miller WC. Mechanisms of oxygen effects on exercise in patients with chronic obstructive pulmonary disease. *Chest* 1982; 81: 6-10.
305. Louvaris Z, Zakynthinos S, Aliverti A, Habazettl H, Vasilopoulou M, Andrianopoulos V, Wagner H, Wagner P, Vogiatzis I. Heliox increases quadriceps muscle oxygen delivery during exercise in COPD patients with and without dynamic hyperinflation. *J Appl Physiol (1985)* 2012; 113: 1012-1023.
306. Hunt T, Williams MT, Frith P, Schembri D. Heliox, dyspnoea and exercise in COPD. *Eur Respir Rev* 2010; 19: 30-38.
307. Moga AM, de Marchie M, Saey D, Spahija J. Mechanisms of non-pharmacologic adjunct therapies used during exercise in COPD. *Respir Med* 2012; 106: 614-626.

308. Chiappa GR, Queiroga F, Jr., Meda E, Ferreira LF, Diefenthaeler F, Nunes M, Vaz MA, Machado MC, Nery LE, Neder JA. Heliox improves oxygen delivery and utilization during dynamic exercise in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2009; 179: 1004-1010.
309. 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: 763-771.
310. Laude EA, Duffy NC, Baveystock C, Dougill B, Campbell MJ, Lawson R, Jones PW, Calverley PM. The effect of helium and oxygen on exercise performance in chronic obstructive pulmonary disease: a randomized crossover trial. *Am J Respir Crit Care Med* 2006; 173: 865-870.
311. Louvaris Z, Vogiatzis I, Aliverti A, Habazettl H, Wagner H, Wagner P, Zakynthinos S. Blood flow does not redistribute from respiratory to leg muscles during exercise breathing heliox or oxygen in COPD. *J Appl Physiol (1985)* 2014; 117: 267-276.
312. Palange P, Valli G, Onorati P, Antonucci R, Paoletti P, Rosato A, Manfredi F, Serra P. Effect of heliox on lung dynamic hyperinflation, dyspnea, and exercise endurance capacity in COPD patients. *J Appl Physiol (1985)* 2004; 97: 1637-1642.
313. Queiroga F, Jr., Nunes M, Meda E, Chiappa G, Machado MC, Nery LE, Neder JA. Exercise tolerance with helium-hyperoxia versus hyperoxia in hypoxaemic patients with COPD. *Eur Respir J* 2013; 42: 362-370.
314. Vogiatzis I, Habazettl H, Aliverti A, Athanasopoulos D, Louvaris Z, LoMauro A, Wagner H, Roussos C, Wagner PD, Zakynthinos S. Effect of helium breathing on intercostal and quadriceps muscle blood flow during exercise in COPD patients. *Am J Physiol-Reg I* 2011; 300: 1549-1559.
315. van 't Hul A, Kwakkel G, Gosselink R. The acute effects of noninvasive ventilatory support during exercise on exercise endurance and dyspnea in patients with chronic obstructive pulmonary disease: a systematic review. *J Cardiopulm Rehabil* 2002; 22: 290-297.
316. Hernandez P, Maltais F, Gursahaney A, Leblanc P, Gottfried SB. Proportional assist ventilation may improve exercise performance in severe chronic obstructive pulmonary disease. *J Cardiopulm Rehabil* 2001; 21: 135-142.
317. Kyroussis D, Polkey MI, Hamnegard CH, Mills GH, Green M, Moxham J. Respiratory muscle activity in patients with COPD walking to exhaustion with and without pressure support. *Eur Respir J* 2000; 15: 649-655.
318. O'Donnell DE, Sanii R, Younes M. Improvement in exercise endurance in patients with chronic airflow limitation using continuous positive airway pressure. *Am Rev Respir Dis* 1988; 138: 1510-1514.
319. 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: 179-188.
320. 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: 1027-1033.
321. Porszasz J, Cao R, Morishige R, van Eykern LA, Stenzler A, Casaburi R. Physiologic effects of an ambulatory ventilation system in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2013; 188: 334-342.
322. McKim DA, Road J, Avendano M, Abdool S, Cote F, Duguid N, Fraser J, Maltais F, Morrison DL, O'Connell C, Petrof BJ, Rimmer K, Skomro R, Canadian Thoracic Society Home

Mechanical Ventilation C. Home mechanical ventilation: a Canadian Thoracic Society clinical practice guideline. *Can Respir J* 2011; 18: 197-215.
323. Neder JA, O'Donnell DE. Update on Nonsurgical Lung Volume Reduction Procedures. *Can Respir J* 2016; 2016: 6462352.
324. Criner GJ, Scharf SM, Falk JA, Gaughan JP, Sternberg AL, Patel NB, Fessler HE, Minai OA, Fishman AP, National Emphysema Treatment Trial Research G. Effect of lung volume reduction surgery on resting pulmonary hemodynamics in severe emphysema. *Am J Respir Crit Care Med* 2007; 176: 253-260.
325. Gorman RB, McKenzie DK, Butler JE, Tolman JF, Gandevia SC. Diaphragm length and neural drive after lung volume reduction surgery. *Am J Respir Crit Care Med* 2005; 172: 1259-1266.
326. Lando Y, Boiselle PM, Shade D, Furukawa S, Kuzma AM, Travaline JM, Criner GJ. Effect of lung volume reduction surgery on diaphragm length in severe chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999; 159: 796-805.
327. Lederer DJ, Thomashow BM, Ginsburg ME, Austin JH, Bartels MN, Yip CK, Jellen PA, Brogan FL, Kawut SM, Maxfield RA, DiMango AM, Simonelli PF, Gorenstein LA, Pearson GD, Sonett JR. Lung-volume reduction surgery for pulmonary emphysema: Improvement in body mass index, airflow obstruction, dyspnea, and exercise capacity index after 1 year. *J Thorac Cardiovasc Surg* 2007; 133: 1434-1438.
328. Martinez FJ, de Oca MM, Whyte RI, Stetz J, Gay SE, Celli BR. Lung-volume reduction improves dyspnea, dynamic hyperinflation, and respiratory muscle function. *Am J Respir Crit Care Med* 1997; 155: 1984-1990.
329. Sciurba FC, Rogers RM, Keenan RJ, Slivka WA, Gorcsan J, 3rd, Ferson PF, Holbert JM, Brown ML, Landreneau RJ. Improvement in pulmonary function and elastic recoil after lung-reduction surgery for diffuse emphysema. *N Engl J Med* 1996; 334: 1095-1099.
330. van Agteren JE, Carson KV, Tiong LU, Smith BJ. Lung volume reduction surgery for diffuse emphysema. *Cochrane Database Syst Rev* 2016; 10: CD001001.
331. van Agteren JE, Hnin K, Grosser D, Carson KV, Smith BJ. Bronchoscopic lung volume reduction procedures for chronic obstructive pulmonary disease. *Cochrane Database Syst Rev* 2017; 2: CD012158.
332. Armstrong HF, Dussault NE, Thirapatarapong W, Lemieux RS, Thomashow BM, Bartels MN. Ventilatory efficiency before and after lung volume reduction surgery. *Respir Care* 2015; 60: 63-71.
333. Hussain SN, Rabinovitch B, Macklem PT, Pardy RL. Effects of separate rib cage and abdominal restriction on exercise performance in normal humans. *J Appl Physiol (1985)* 1985; 58: 2020-2026.
334. West CR, Campbell IG, Goosey-Tolfrey VL, Mason BS, Romer LM. Effects of abdominal binding on field-based exercise responses in Paralympic athletes with cervical spinal cord injury. *J Sci Med Sport* 2014; 17: 351-355.
335. West CR, Campbell IG, Shave RE, Romer LM. Effects of abdominal binding on cardiorespiratory function in cervical spinal cord injury. *Respir Physiol Neurobiol* 2012; 180: 275-282.
336. West CR, Goosey-Tolfrey VL, Campbell IG, Romer LM. Effect of abdominal binding on respiratory mechanics during exercise in athletes with cervical spinal cord injury. *J Appl Physiol (1985)* 2014; 117: 36-45.

337. Baydur A. Respiratory muscle strength and control of ventilation in patients with neuromuscular disease. *Chest* 1991; 99: 330-338.
338. Baydur A, Alsalek M, Louie SG, Sharma OP. Respiratory muscle strength, lung function, and dyspnea in patients with sarcoidosis. *Chest* 2001; 120: 102-108.
339. Brown R, DiMarco AF, Hoit JD, Garshick E. Respiratory dysfunction and management in spinal cord injury. *Respir Care* 2006; 51: 853-868;discussion 869-870.
340. Kabitz HJ, Lang F, Walterspacher S, Sorichter S, Muller-Quernheim J, Windisch W. Impact of impaired inspiratory muscle strength on dyspnea and walking capacity in sarcoidosis. *Chest* 2006; 130: 1496-1502.
341. Meyer FJ, Borst MM, Zugck C, Kirschke A, Schellberg D, Kubler W, Haass M. Respiratory muscle dysfunction in congestive heart failure: clinical correlation and prognostic significance. *Circulation* 2001; 103: 2153-2158.
342. Nishimura Y, Maeda H, Tanaka K, Nakamura H, Hashimoto Y, Yokoyama M. Respiratory muscle strength and hemodynamics in chronic heart failure. *Chest* 1994; 105: 355-359.
343. Petrof BJ, Jaber S, Matecki S. Ventilator-induced diaphragmatic dysfunction. *Curr Opin Crit Care* 2010; 16: 19-25.
344. Polkey MI, Kyroussis D, Hamnegard CH, Mills GH, Green M, Moxham J. Diaphragm strength in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1996; 154: 1310-1317.
345. Tantucci C, Massucci M, Piperno R, Betti L, Grassi V, Sorbini CA. Control of breathing and respiratory muscle strength in patients with multiple sclerosis. *Chest* 1994; 105: 1163-1170.
346. West CR, Campbell IG, Shave RE, Romer LM. Resting cardiopulmonary function in Paralympic athletes with cervical spinal cord injury. *Med Sci Sports Exerc* 2012; 44: 323-329.
347. Berlowitz DJ, Tamplin J. Respiratory muscle training for cervical spinal cord injury. *Cochrane Database Syst Rev* 2013: CD008507.
348. Budweiser S, Moertl M, Jorres RA, Windisch W, Heinemann F, Pfeifer M. Respiratory muscle training in restrictive thoracic disease: a randomized controlled trial. *Arch Phys Med Rehabil* 2006; 87: 1559-1565.
349. Geddes EL, O'Brien K, Reid WD, Brooks D, Crowe J. Inspiratory muscle training in adults with chronic obstructive pulmonary disease: an update of a systematic review. *Respir Med* 2008; 102: 1715-1729.
350. Martin-Valero R, Zamora-Pascual N, Armenta-Peinado JA. Training of respiratory muscles in patients with multiple sclerosis: a systematic review. *Respir Care* 2014; 59: 1764-1772.
351. Moodie L, Reeve J, Elkins M. Inspiratory muscle training increases inspiratory muscle strength in patients weaning from mechanical ventilation: a systematic review. *J Physiother* 2011; 57: 213-221.
352. Smart NA, Giallauria F, Dieberg G. Efficacy of inspiratory muscle training in chronic heart failure patients: a systematic review and meta-analysis. *Int J Cardiol* 2013; 167: 1502-1507.
353. Doorduin J, Sinderby CA, Beck J, Stegeman DF, van Hees HW, van der Hoeven JG, Heunks LM. The calcium sensitizer levosimendan improves human diaphragm function. *Am J Respir Crit Care Med* 2012; 185: 90-95.

354. 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: 41-47.
355. Koulouris N, Mulvey DA, Laroche CM, Goldstone J, Moxham J, Green M. The effect of posture and abdominal binding on respiratory pressures. *Eur Respir J* 1989; 2: 961-965.
356. Goldman JM, Rose LS, Williams SJ, Silver JR, Denison DM. Effect of abdominal binders on breathing in tetraplegic patients. *Thorax* 1986; 41: 940-945.
357. Hart N, Laffont I, de la Sota AP, Lejaille M, Macadou G, Polkey MI, Denys P, Lofaso F. Respiratory effects of combined truncal and abdominal support in patients with spinal cord injury. *Arch Phys Med Rehabil* 2005; 86: 1447-1451.
358. Koo P, Gartman EJ, Sethi JM, McCool FD. Physiology in Medicine: physiological basis of diaphragmatic dysfunction with abdominal hernias-implications for therapy. *J Appl Physiol (1985)* 2015; 118: 142-147.
359. McCool FD, Pichurko BM, Slutsky AS, Sarkarati M, Rossier A, Brown R. Changes in lung volume and rib cage configuration with abdominal binding in quadriplegia. *J Appl Physiol (1985)* 1986; 60: 1198-1202.
360. Vanmeenen MT, Ghesquiere J, Demedts M. Effects of thoracic or abdominal strapping on exercise performance. *Bull Eur Physiopathol Respir* 1984; 20: 127-132.
361. Harty HR, Corfield DR, Schwartzstein RM, Adams L. External thoracic restriction, respiratory sensation, and ventilation during exercise in men. *J Appl Physiol (1985)* 1999; 86: 1142-1150.
362. Miller JD, Beck KC, Joyner MJ, Brice AG, Johnson BD. Cardiorespiratory effects of inelastic chest wall restriction. *J Appl Physiol (1985)* 2002; 92: 2419-2428.
363. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J, Force AET. Standardisation of spirometry. *Eur Respir J* 2005; 26: 319-338.
364. Charloux A, Lonsdorfer-Wolf E, Richard R, Lampert E, Oswald-Mammosser M, Mettauer B, Geny B, Lonsdorfer J. A new impedance cardiograph device for the non-invasive evaluation of cardiac output at rest and during exercise: comparison with the "direct" Fick method. *Eur J Appl Physiol* 2000; 82: 313-320.
365. Richard R, Lonsdorfer-Wolf E, Charloux A, Doutreleau S, Buchheit M, Oswald-Mammosser M, Lampert E, Mettauer B, Geny B, Lonsdorfer J. Non-invasive cardiac output evaluation during a maximal progressive exercise test, using a new impedance cardiograph device. *Eur J Appl Physiol* 2001; 85: 202-207.
366. Stubbing DG, Pengelly LD, Morse JL, Jones NL. Pulmonary mechanics during exercise in normal males. *J Appl Physiol Respir Environ Exerc Physiol* 1980; 49: 506-510.
367. Borg GA. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 1982; 14: 377-381.
368. Jones NL, Makrides L, Hitchcock C, Chypchar T, McCartney N. Normal standards for an incremental progressive cycle ergometer test. *Am Rev Respir Dis* 1985; 131: 700-708.
369. Wilson TA, De Troyer A. Effects of the insertional and appositional forces of the canine diaphragm on the lower ribs. *J Physiol* 2013; 591: 3539-3548.
370. De Troyer A. Mechanical role of the abdominal muscles in relation to posture. *Respir Physiol* 1983; 53: 341-353.

371. Vivier E, Metton O, Piriou V, Lhuillier F, Cottet-Emard JM, Branche P, Duperret S, Viale JP. Effects of increased intra-abdominal pressure on central circulation. *Br J Anaesth* 2006; 96: 701-707.
372. Aliverti A, Bovio D, Fullin I, Dellaca RL, Lo Mauro A, Pedotti A, Macklem PT. The abdominal circulatory pump. *Plos One* 2009; 4: e5550.
373. Aliverti A, Uva B, Laviola M, Bovio D, Lo Mauro A, Tarperi C, Colombo E, Loomas B, Pedotti A, Similowski T, Macklem PT. Concomitant ventilatory and circulatory functions of the diaphragm and abdominal muscles. *J Appl Physiol (1985)* 2010; 109: 1432-1440.
374. Uva B, Aliverti A, Bovio D, Kayser B. The "Abdominal Circulatory Pump": An Auxiliary Heart during Exercise? *Front Physiol* 2015; 6: 411.
375. Finucane KE, Singh B. Diaphragm efficiency estimated as power output relative to activation in chronic obstructive pulmonary disease. *J Appl Physiol (1985)* 2012; 113: 1567-1575.
376. Alexander H, Kontz W. Symptomatic relief of emphysema by an abdominal belt. *Am J Med Sci* 1934; 187: 687-691.
377. Gordon B. The mechanism and use of abdominal supports and the treatment of pulmonary diseases. *Am J Med Sci* 1934; 187: 692-699.
378. 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: 581-586.
379. 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: 751-758.
380. McGavin CR, Artvinli M, Naoe H, McHardy GJ. Dyspnoea, disability, and distance walked: comparison of estimates of exercise performance in respiratory disease. *Br Med J* 1978; 2: 241-243.
381. Jones PW, Harding G, Berry P, Wiklund I, Chen WH, Kline Leidy N. Development and first validation of the COPD Assessment Test. *Eur Respir J* 2009; 34: 648-654.
382. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand* 1983; 67: 361-370.
383. Jensen D, O'Donnell DE, Li R, Luo YM. Effects of dead space loading on neuro-muscular and neuro-ventilatory coupling of the respiratory system during exercise in healthy adults: implications for dyspnea and exercise tolerance. *Respir Physiol Neurobiol* 2011; 179: 219-226.
384. Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, Celli BR, Chen R, Decramer M, Fabbri LM, Frith P, Halpin DM, Lopez Varela MV, Nishimura M, Roche N, Rodriguez-Roisin R, Sin DD, Singh D, Stockley R, Vestbo J, Wedzicha JA, Agusti A. 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: 557-582.
385. Wanger J, Clausen JL, Coates A, Pedersen OF, Brusasco V, Burgos F, Casaburi R, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Hankinson J, Jensen R, Johnson D, Macintyre N, McKay R, Miller MR, Navajas D, Pellegrino R, Viegi G. Standardisation of the measurement of lung volumes. *Eur Respir J* 2005; 26: 511-522.
386. Miller MR, Crapo R, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas

D, Pedersen OF, Pellegrino R, Viegi G, Wanger J, Force AET. General considerations for lung function testing. *Eur Respir J* 2005; 26: 153-161.
387. Macintyre N, Crapo RO, Viegi G, Johnson DC, van der Grinten CP, Brusasco V, Burgos F, Casaburi R, Coates A, Enright P, Gustafsson P, Hankinson J, Jensen R, McKay R, Miller MR, Navajas D, Pedersen OF, Pellegrino R, Wanger J. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J* 2005; 26: 720-735.
388. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med* 1999; 159: 179-187.
389. Crapo RO, Morris AH, Gardner RM. Reference Spirometric Values Using Techniques and Equipment That Meet Ats Recommendations. *Am Rev Respir Dis* 1981; 123: 659-664.
390. Briscoe WA, Dubois AB. The relationship between airway resistance, airway conductance and lung volume in subjects of different age and body size. *J Clin Invest* 1958; 37: 1279-1285.
391. Burrows B, Kasik JE, Niden AH, Barclay WR. Clinical usefulness of the single-breath pulmonucy diffusing capacity test. *Am Rev Respir Dis* 1961; 84: 789-806.
392. Guenette JA, Chin RC, Cory JM, Webb KA, O'Donnell DE. Inspiratory Capacity during Exercise: Measurement, Analysis, and Interpretation. *Pulm Med* 2013; 2013: 956081.
393. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behav Res Methods* 2009; 41: 1149-1160.
394. Dodd DS, Brancatisano TP, Engel LA. Effect of abdominal strapping on chest wall mechanics during exercise in patients with severe chronic air-flow obstruction. *Am Rev Respir Dis* 1985; 131: 816-821.
395. Lahrmann H, Wild M, Wanke T, Tschernko E, Wisser W, Klepetko W, Zwick H. Neural drive to the diaphragm after lung volume reduction surgery. *Chest* 1999; 116: 1593-1600.
396. Ciavaglia CE, Guenette JA, Langer D, Webb KA, Alberto Neder J, O'Donnell DE. Differences in respiratory muscle activity during cycling and walking do not influence dyspnea perception in obese patients with COPD. *J Appl Physiol (1985)* 2014; 117: 1292-1301.
397. Langer D, Ciavaglia CE, Faisal A, Webb KA, Neder JA, Gosselink R, Dacha S, Topalovic M, Ivanova A, O'Donnell DE. Inspiratory muscle training reduces diaphragm activation and dyspnea during exercise in COPD. *J Appl Physiol (1985)* 2018.
398. Elbehairy AF, Guenette JA, Faisal A, Ciavaglia CE, Webb KA, Jensen D, Ramsook AH, Neder JA, O'Donnell DE, Canadian Respiratory Research N. Mechanisms of exertional dyspnoea in symptomatic smokers without COPD. *Eur Respir J* 2016; 48: 694-705.
399. O'Donnell DE, Elbehairy AF, Faisal A, Neder JA, Webb KA, Canadian Respiratory Research N. Sensory-mechanical effects of a dual bronchodilator and its anticholinergic component in COPD. *Respir Physiol Neurobiol* 2018; 247: 116-125.
400. Guenette JA, Webb KA, O'Donnell DE. Does dynamic hyperinflation contribute to dyspnoea during exercise in patients with COPD? *Eur Respir J* 2012; 40: 322-329.
401. Pergolizzi J, Boger RH, Budd K, Dahan A, Erdine S, Hans G, Kress HG, Langford R, Likar R, Raffa RB, Sacerdote P. Opioids and the management of chronic severe pain in the elderly: consensus statement of an International Expert Panel with focus on the six clinically most often used World Health Organization Step III opioids (buprenorphine, fentanyl, hydromorphone, methadone, morphine, oxycodone). *Pain Pract* 2008; 8: 287-313.

402. Peng J, Sarkar S, Chang SL. Opioid receptor expression in human brain and peripheral tissues using absolute quantitative real-time RT-PCR. *Drug Alcohol Depend* 2012; 124: 223-228.
403. Zebraski SE, Kochenash SM, Raffa RB. Lung opioid receptors: pharmacology and possible target for nebulized morphine in dyspnea. *Life Sci* 2000; 66: 2221-2231.
404. Pattinson KTS. Opioids and the control of respiration. *Brit J Anaesth* 2008; 100: 747-758.
405. Ahmadi Z, Sandberg J, Shannon-Honson A, Vandersman Z, Currow DC, Ekstrom M. Is chronic breathlessness less recognised and treated compared with chronic pain? A case-based randomised controlled trial. *Eur Respir J* 2018; 52.
406. Hayen A, Wanigasekera V, Faull OK, Campbell SF, Garry PS, Raby SJ, Robertson J, Webster R, Wise RG, Herigstad M, Pattinson KT. Opioid suppression of conditioned anticipatory brain responses to breathlessness. *Neuroimage* 2017.
407. Woodcock AA, Gross ER, Gellert A, Shah S, Johnson M, Geddes DM. Effects of dihydrocodeine, alcohol, and caffeine on breathlessness and exercise tolerance in patients with chronic obstructive lung disease and normal blood gases. *N Engl J Med* 1981; 305: 1611-1616.
408. Woodcok AJ, M.; Geddes, D. Breathlessness, alcohol and opiates. *N Engl J Med* 1982; 306: 1363-1364.
409. Johnson MA, Woodcock AA, Geddes DM. Dihydrocodeine for breathlessness in "pink puffers". *Br Med J (Clin Res Ed)* 1983; 286: 675-677.
410. Rice KL, Kronenberg RS, Hedemark LL, Niewoehner DE. Effects of chronic administration of codeine and promethazine on breathlessness and exercise tolerance in patients with chronic airflow obstruction. *Br J Dis Chest* 1987; 81: 287-292.
411. Light RW, Muro JR, Sato RI, Stansbury DW, Fischer CE, Brown SE. Effects of oral morphine on breathlessness and exercise tolerance in patients with chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1989; 139: 126-133.
412. Munck LK, Christensen CB, Pedersen L, Larsen U, Branebjerg PE, Kampmann JP. Codeine in analgesic doses does not depress respiration in patients with severe chronic obstructive lung disease. *Pharmacol Toxicol* 1990; 66: 335-340.
413. Eiser N, Denman WT, West C, Luce P. Oral diamorphine: lack of effect on dyspnoea and exercise tolerance in the "pink puffer" syndrome. *Eur Respir J* 1991; 4: 926-931.
414. Light RW, Stansbury DW, Webster JS. Effect of 30 mg of morphine alone or with promethazine or prochlorperazine on the exercise capacity of patients with COPD. *Chest* 1996; 109: 975-981.
415. Poole PJ, Veale AG, Black PN. The effect of sustained-release morphine on breathlessness and quality of life in severe chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1998; 157: 1877-1880.
416. Abernethy AP, Currow DC, Frith P, Fazekas BS, McHugh A, Bui C. Randomised, double blind, placebo controlled crossover trial of sustained release morphine for the management of refractory dyspnoea. *BMJ* 2003; 327: 523-528.
417. Currow DC, McDonald C, Oaten S, Kenny B, Allcroft P, Frith P, Briffa M, Johnson MJ, Abernethy AP. Once-daily opioids for chronic dyspnea: a dose increment and pharmacovigilance study. *J Pain Symptom Manage* 2011; 42: 388-399.
418. Rocker GM, Simpson AC, Joanne Young B, Horton R, Sinuff T, Demmons J, Margaret Donahue MM, Hernandez P, Marciniuk D. Opioid therapy for refractory dyspnea in patients with advanced chronic obstructive pulmonary disease: patients' experiences and outcomes. *CMAJ open* 2013; 1: E27-36.

419. Agusti A, Edwards LD, Celli B, Macnee W, Calverley PM, Mullerova H, Lomas DA, Wouters E, Bakke P, Rennard S, Crim C, Miller BE, Coxson HO, Yates JC, Tal-Singer R, Vestbo J, Investigators E. Characteristics, stability and outcomes of the 2011 GOLD COPD groups in the ECLIPSE cohort. *Eur Respir J* 2013; 42: 636-646.
420. Small M, Holbrook T, Wood R, Mullerova H, Naya I, Punekar YS. Prevalence and burden of dyspnoea among COPD patients in Japan. *Int J Clin Pract* 2016; 70: 676-681.
421. Banzett RB, Adams L, O'Donnell CR, Gilman SA, Lansing RW, Schwartzstein RM. Using Laboratory Models to Test Treatment Morphine Reduces Dyspnea and Hypercapnic Ventilatory Response. *Am J Resp Crit Care* 2011; 184: 920-927.
422. Mahler DA, Selecky PA, Harrod CG, Benditt JO, Carrieri-Kohlman V, Curtis JR, Manning HL, Mularski RA, Varkey B, Campbell M, Carter ER, Chiong JR, Ely EW, Hansen-Flaschen J, O'Donnell DE, Waller A. American College of Chest Physicians Consensus Statement on the Management of Dyspnea in Patients With Advanced Lung or Heart Disease. *Chest* 2010; 137: 674-691.
423. Qaseem A, Wilt TJ, Weinberger SE, Hanania NA, Criner G, van der Molen T, Marciniuk DD, Denberg T, Schunemann H, Wedzicha W, MacDonald R, Shekelle P, Phys AC, Phys ACC, Soc AT, Soc ER. Diagnosis and Management of Stable Chronic Obstructive Pulmonary Disease: A Clinical Practice Guideline Update from the American College of Physicians, American College of Chest Physicians, American Thoracic Society, and European Respiratory Society. *Ann Intern Med* 2011; 155: 179-+.
424. Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, Celli BR, Chen R, Decramer M, Fabbri LM, Frith P, Halpin DM, Lopez Varela MV, Nishimura M, Roche N, Rodriguez-Roisin R, Sin DD, Singh D, Stockley R, Vestbo J, Wedzicha JA, Agusti A. 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.
425. Apfelbaum JL, Gan TJ, Zhao S, Hanna DB, Chen C. Reliability and validity of the perioperative opioid-related symptom distress scale. *Anesth Analg* 2004; 99: 699-709, table of contents.
426. Yadeau JT, Liu SS, Rade MC, Marcello D, Liguori GA. Performance characteristics and validation of the Opioid-Related Symptom Distress Scale for evaluation of analgesic side effects after orthopedic surgery. *Anesth Analg* 2011; 113: 369-377.
427. Jensen D, Alsuhail A, Viola R, Dudgeon DJ, Webb KA, O'Donnell DE. Inhaled fentanyl citrate improves exercise endurance during high-intensity constant work rate cycle exercise in chronic obstructive pulmonary disease. *J Pain Symptom Manage* 2012; 43: 706-719.
428. Baumgartner U, Buchholz HG, Bellosevich A, Magerl W, Siessmeier T, Rolke R, Hohnemann S, Piel M, Rosch F, Wester HJ, Henriksen G, Stoeter P, Bartenstein P, Treede RD, Schreckenberger M. High opiate receptor binding potential in the human lateral pain system. *Neuroimage* 2006; 30: 692-699.
429. Herigstad M, Hayen A, Evans E, Hardinge FM, Davies RJ, Wiech K, Pattinson KT. Dyspnea-related cues engage the prefrontal cortex: evidence from functional brain imaging in COPD. *Chest* 2015; 148: 953-961.
430. Currow DC, Quinn S, Ekstrom M, Kaasa S, Johnson MJ, Somogyi AA, Klepstad P. Can variability in the effect of opioids on refractory breathlessness be explained by genetic factors? *BMJ open* 2015; 5: e006818.

431. Deschenes D, Pepin V, Saey D, LeBlanc P, Maltais F. Locus of symptom limitation and exercise response to bronchodilation in chronic obstructive pulmonary disease. *J Cardiopulm Rehabil Prev* 2008; 28: 208-214.
432. Grotenhermen F, Muller-Vahl K. The therapeutic potential of cannabis and cannabinoids. *Deutsches Arzteblatt international* 2012; 109: 495-501.
433. Guzman M. Cannabinoids: potential anticancer agents. *Nat Rev Cancer* 2003; 3: 745-755.
434. Galiegue S, Mary S, Marchand J, Dussossoy D, Carriere D, Carayon P, Bouaboula M, Shire D, Lefur G, Casellas P. Expression of Central and Peripheral Cannabinoid Receptors in Human Immune Tissues and Leukocyte Subpopulations. *Eur J Biochem* 1995; 232: 54-61.
435. Munro S, Thomas KL, Abushaar M. Molecular Characterization of a Peripheral Receptor for Cannabinoids. *Nature* 1993; 365: 61-65.
436. Grassin-Delyle S, Naline E, Buenestado A, Faisy C, Alvarez JC, Salvator H, Abrial C, Advenier C, Zemoura L, Devillier P. Cannabinoids inhibit cholinergic contraction in human airways through prejunctional CB1 receptors. *Br J Pharmacol* 2014; 171: 2767-2777.
437. O'Donnell DE, Guenette JA, Maltais F, Webb KA. Decline of resting inspiratory capacity in COPD: the impact on breathing pattern, dyspnea, and ventilatory capacity during exercise. *Chest* 2012; 141: 753-762.
438. Mullerova H, Lu C, Li H, Tabberer M. Prevalence and Burden of Breathlessness in Patients with Chronic Obstructive Pulmonary Disease Managed in Primary Care. *Plos One* 2014; 9.
439. Chen S, Small M, Lindner L, Xu X. Symptomatic burden of COPD for patients receiving dual or triple therapy. *Int J Chron Obstruct Pulmon Dis* 2018; 13: 1365-1376.
440. Douglas IS, Albertson TE, Folan P, Hanania NA, Tashkin DP, Upson DJ, Leone FT. Implications of Marijuana Decriminalization on the Practice of Pulmonary, Critical Care, and Sleep Medicine. A Report of the American Thoracic Society Marijuana Workgroup. *Ann Am Thorac Soc* 2015; 12: 1700-1710.
441. Lynch ME, Ware MA. Cannabinoids for the Treatment of Chronic Non-Cancer Pain: An Updated Systematic Review of Randomized Controlled Trials. *J Neuroimmune Pharmacol* 2015; 10: 293-301.
442. Morris M.A., Jacobson S.R., Kinney G.L., Tashkin DP, Woodruff PG, Hoffman EA, Kanner RE, Cooper CB, D'rummond B, Barr RG, Oelsner EC, Make BJ, Han MK, Hansel NN, O'Neal WK, Bowler RP. Original Research Marijuana Use Associations with Pulmonary Symptoms and Function in Tobacco Smokers Enrolled in The Subpopulations and Intermediate Outcome Measures in COPD Study (SPIROMICS). *Chronic Obstr Pulm Dis* 2018; 5: 46-56.
443. Chait LD, Corwin RL, Johanson CE. A Cumulative Dosing Procedure for Administering Marijuana Smoke to Humans. *Pharmacol Biochem Be* 1988; 29: 553-557.
444. Kurlowicz L, Wallace M. The Mini Mental State Examination (MMSE). *Director* 1999; 7: 62.
445. Wilsey B, Marcotte T, Deutsch R, Gouaux B, Sakai S, Donaghe H. Low-dose vaporized cannabis significantly improves neuropathic pain. *J Pain* 2013; 14: 136-148.
446. Gong H, Jr., Tashkin DP, Simmons MS, Calvarese B, Shapiro BJ. Acute and subacute bronchial effects of oral cannabinoids. *Clin Pharmacol Ther* 1984; 35: 26-32.
447. Kirby M, Tanabe N, Tan WC, Zhou GH, Obeidat M, Hague CJ, Leipsic J, Bourbeau J, Sin DD, Hogg JC, Coxson HO, Grp CCR, Network CRR. Total Airway Count on Computed

Tomography and the Risk of Chronic Obstructive Pulmonary Disease Progression Findings from a Population-based Study. *Am J Resp Crit Care* 2018; 197: 56-65.
448. McDonough JE, Yuan R, Suzuki M, Seyednejad N, Elliott WM, Sanchez PG, Wright AC, Gefter WB, Litzky L, Coxson HO, Pare PD, Sin DD, Pierce RA, Woods JC, McWilliams AM, Mayo JR, Lam SC, Cooper JD, Hogg JC. Small-airway obstruction and emphysema in chronic obstructive pulmonary disease. *N Engl J Med* 2011; 365: 1567-1575.
449. Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, Cherniack RM, Rogers RM, Sciurba FC, Coxson HO, Pare PD. The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med* 2004; 350: 2645-2653.
450. Ware MA, Wang T, Shapiro S, Robinson A, Ducruet T, Huynh T, Gamsa A, Bennett GJ, Collet JP. Smoked cannabis for chronic neuropathic pain: a randomized controlled trial. *CMAJ* 2010; 182: E694-701.
451. Pomahacova B, Van der Kooy F, Verpoorte R. Cannabis smoke condensate III: The cannabinoid content of vaporised Cannabis sativa. *Inhal Toxicol* 2009; 21: 1108-1112.
452. Calignano A, Katona I, Desarnaud F, Giuffrida A, La Rana G, Mackie K, Freund TF, Piomelli D. Bidirectional control of airway responsiveness by endogenous cannabinoids. *Nature* 2000; 408: 96-101.
453. Bhattacharyya S, Morrison PD, Fusar-Poli P, Martin-Santos R, Borgwardt S, Winton-Brown T, Nosarti C, CM OC, Seal M, Allen P, Mehta MA, Stone JM, Tunstall N, Giampietro V, Kapur S, Murray RM, Zuardi AW, Crippa JA, Atakan Z, McGuire PK. Opposite effects of delta-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. *Neuropsychopharmacology* 2010; 35: 764-774.
454. Herigstad M, Hayen A, Wiech K, Pattinson KT. Dyspnoea and the brain. *Respir Med* 2011; 105: 809-817.
455. Pickering EE, Semple SJ, Nazir MS, Murphy K, Snow TM, Cummin AR, Moosavi SH, Guz A, Holdcroft A. Cannabinoid effects on ventilation and breathlessness: a pilot study of efficacy and safety. *Chron Respir Dis* 2011; 8: 109-118.
456. Banzett RB, Pedersen SH, Schwartzstein RM, Lansing RW. The affective dimension of laboratory dyspnea: Air hunger is more unpleasant than work/effort. *Am J Resp Crit Care* 2008; 177: 1384-1390.
457. Barrett LF, Simmons WK. Interoceptive predictions in the brain. *Nat Rev Neurosci* 2015; 16: 419-429.
458. Van den Bergh O, Witthoft M, Petersen S, Brown RJ. Symptoms and the body: Taking the inferential leap. *Neurosci Biobehav Rev* 2017; 74: 185-203.
459. Ongaro G, Kaptchuk TJ. Symptom perception, placebo effects, and the Bayesian brain. *Pain* 2019; 160: 1-4.
460. Rocker GM, Simpson AC, Horton R, Sinuff T, Demmons J, Hernandez P, Marciniuk D. Opioid therapy for refractory dyspnea in patients with advanced chronic obstructive pulmonary disease: patients' experiences and outcomes. *CMAJ Open* 2013; 1: E27-E36.
461. ElSohly MA, Mehmedic Z, Foster S, Gon C, Chandra S, Church JC. Changes in Cannabis Potency Over the Last 2 Decades (1995-2014): Analysis of Current Data in the United States. *Biol Psychiatry* 2016; 79: 613-619.

Appendix I

frontiers in Physiology

ORIGINAL RESEARCH
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Abdominal Binding Improves Neuromuscular Efficiency of the Human Diaphragm during Exercise

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We tested the hypothesis that elastic binding of the abdomen (AB) would enhance neuromuscular efficiency of the human diaphragm during exercise. Twelve healthy non-obese men aged 24.8 ± 1.7 years (mean ± *SE*) completed a symptom-limited constant-load cycle endurance exercise test at 85% of their peak incremental power output with diaphragmatic electromyography (EMGdi) and respiratory pressure measurements under two randomly assigned conditions: unbound control (CTRL) and AB sufficient to increase end-expiratory gastric pressure (Pga,ee) by 5–8 cmH_2O at rest. By design, AB increased Pga,ee by 6.6 ± 0.6 cmH_2O at rest. Compared to CTRL, AB significantly increased the transdiaphragmatic pressure swing-to-EMGdi ratio by 85–95% during exercise, reflecting enhanced neuromuscular efficiency of the diaphragm. By contrast, AB had no effect on spirometric parameters at rest, exercise endurance time or an effect on cardiac, metabolic, ventilatory, breathing pattern, dynamic operating lung volume, and perceptual responses during exercise. In conclusion, AB was associated with isolated and acute improvements in neuromuscular efficiency of the diaphragm during exercise in healthy men. The implications of our results are that AB may be an effective means of enhancing neuromuscular efficiency of the diaphragm in clinical populations with diaphragmatic weakness/dysfunction.

Keywords: breathlessness, exercise, abdominal binding, neuromuscular efficiency, diaphragm

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INTRODUCTION

Diaphragm muscle weakness/dysfunction is pervasive in many clinical populations, including chronic obstructive pulmonary disease (COPD), interstitial lung disease, heart failure, neuromuscular disease, critical illness and mechanical ventilation, and spinal cord injury (SCI; Baydur, 1991; Nishimura et al., 1994; Tantucci et al., 1994; Polkey et al., 1996; Baydur et al., 2001; Meyer et al., 2001; Laghi and Tobin, 2003; Brown et al., 2006; Kabitz et al., 2006, 2007; Petrof et al., 2010; West et al., 2012b). In these patient populations, diaphragm muscle weakness/dysfunction

has been linked to increased breathlessness, impaired exercise tolerance, prolonged and difficult weaning from mechanical ventilation, and adverse health outcomes, including quality of life and death (Laghi and Tobin, 2003). It follows that non-disease specific interventions capable of increasing the pressure generating capacity of the diaphragm may have important clinical and pathophysiological implications. With the exception of inspiratory muscle training (Budweiser et al., 2006; Geddes et al., 2008; Moodie et al., 2011; Berlowitz and Tamplin, 2013; Smart et al., 2013; Martin-Valero et al., 2014) and the Ca^{2+} sensitizing agent, Levosimendan (van Hees et al., 2009; Doorduin et al., 2012), few generalized interventions exist to improve the force generating capacity of the human diaphragm.

Accumulating evidence from studies in health (Koulouris et al., 1989; West et al., 2012a) and SCI (Goldman et al., 1986; Hart et al., 2005; West et al., 2012a) suggest that elastic binding of the abdomen (AB) significantly increases maximal voluntary (e.g., sniff) and involuntary (e.g., twitch) pressure generating capacity of the diaphragm, presumably by reducing abdominal wall compliance, improving the operating length of the diaphragm due to its ascent to a more mechanically advantageous (cephalad) end-expiratory position, increasing intra-abdominal pressure, increasing the area of diaphragmatic apposition to the rib cage and/or increasing diaphragm-rib cage insertional forces (McCool et al., 1986; Koo et al., 2015). A series of studies by West et al. (2012a, 2014a,b) recently reported that AB sufficient to increase end-expiratory gastric pressure (Pga,ee) by an average of ∼8 cmH_2O at rest in athletes with cervical SCI increased transdiaphragmatic twitch pressures by ∼40% relative to the unbound control condition. In those studies, AB-induced improvements in diaphragmatic function were associated with concurrent improvements in static lung volumes and capacities; cardiac output at rest; the behavior of dynamic operating lung volumes during exercise; and selected measures of field-based exercise performance.

To our knowledge, only two studies have examined the impact of AB on exercise physiological responses in healthy adults (Vanmeenen et al., 1984; Hussain et al., 1985). Vanmeenen et al. (1984) examined the effects of decreasing vital capacity by ∼30% through the application of an inelastic canvas corset around the abdomen (extending from the xyphoid process to the hips, thus encompassing the lower five ribs) on exercise physiological responses in 11 healthy men. In that study, AB impaired ventilatory and cardiovascular responses to exercise with attendant reductions in exercise performance, consistent with the established effects of external thoracic restriction on exercise physiological responses in healthy men (Harty et al., 1999; O'Donnell et al., 2000; Miller et al., 2002; Mendonca et al., 2014). A similar study by Hussain et al. (1985) found that applying an inelastic corset around the abdomen of five healthy men as tightly as possible while interfering minimally with ribcage movements, caused a "mild" restrictive lung deficit; significantly increased transdiaphragmatic pressure (Pdi) swings during exercise; and had no effect on exercise tolerance or an effect on ventilation ($\dot{V}_E$), breathing pattern and diaphragmatic electromyography (EMGdi) responses to exercise. While the study by Hussain et al. (1985) suggested that AB has the potential to enhance neuromuscular efficiency of the human diaphragm during exercise (i.e., increase ratio of Pdi-to-EMGdi), the authors did not (1) control for the degree of abdominal compression applied; (2) account for the possibility that the "mild" restrictive lung deficit imposed by AB may have offset the potential benefits of enhanced neuromuscular efficiency of the diaphragm on exercise tolerance; and/or (3) examine the simultaneous effect of AB on cardiac, metabolic, dynamic operating lung volume, and breathlessness responses to exercise.

The purpose of this study was to examine the effect of AB sufficient to increase Pga,ee by 5–8 cmH_2O at rest on cardiac, metabolic, ventilatory, breathing pattern, dynamic operating lung volume, EMGdi, respiratory pressure, and breathlessness responses during high-intensity constant-load cycle endurance exercise testing in healthy men.

MATERIALS AND METHODS

Study Design

This was a single-center, controlled, randomized, crossover study wherein eligible men participated in three testing visits over a period ≤2 weeks. Visit 1 included screening of medical history, spirometry, and a symptom-limited incremental cycle exercise test to determine peak power output (PPO). Visits 2 and 3 included spirometry and a symptom-limited constant-load cycle endurance exercise test at 85% of PPO with added measurements of EMGdi and respiratory pressures under two randomly assigned conditions: unbound control (CTRL) and AB. Although the conditions could not be blinded to the participants and investigators, the participants were naïve to the expected outcomes of the study. Visit 1–3 were separated by ≥24 h and conducted at the same time of day (±1 h) for each participant. Participants were instructed to avoid alcohol, caffeine, heavy meals, and strenuous exercise on each test day. The study was approved by the Institutional Review Board of the Faculty of Medicine at McGill University (A04-M42-12B) in accordance with the *Declaration of Helsinski*. Written informed consent was obtained from all participants prior to study initiation.

Participants

Participants included 12 non-smoking, non-obese men aged 18–40 years with normal spirometry [forced expiratory volume in 1 s (FEV_1) ≥80% predicted (Tan et al., 2011) and FEV_1-to-forced vital capacity ratio ≥70%] and no known or suspected cardiovascular, respiratory, metabolic, musculoskeletal, endocrine, and/or neuromuscular disorder(s).

Abdominal Binding

As described in detail elsewhere (West et al., 2012a), a binder made primarily of flexible neoprene (493R Universal Back Support; McDavid Inc., Woodridge, IL, USA) was individually sized and fitted with participants in the upright position and with the binder's upper edge below the costal margin so that it interfered minimally with rib-cage movement. The desired degree of abdominal compression—defined as an increase in Pga,ee of 5–8 cmH_2O during steady-state breathing while seated on a chair at rest prior to exercise—was achieved by tightening

Velcro fasteners at the front of the binder. An earlier study by West et al. (2012a) found that this level of abdominal compression optimized pulmonary function and twitch Pdi responses at rest in healthy adults and among individuals with cervical SCI.

Spirometry

Spirometry was performed using automated equipment (Vmax Encore™, CareFusion, Yorba Linda, CA, USA) according to recommended techniques (Miller et al., 2005).

Cardiopulmonary Exercise Testing

Symptom-limited exercise tests were performed on an electronically braked cycle ergometer (VIAsprint 150P; Ergoline, Bitz, Germany) using a cardiopulmonary exercise testing system (Vmax Encore™, CareFusion). Incremental exercise tests consisted of a steady-state resting period of ≥6 min, followed by 25 W increases in power output (starting at 25 W) every 2 min: PPO was defined as the highest power output that the participant was able to sustain for ≥30 s. Constant-load exercise endurance tests consisted of a steady-state resting period of ≥6 min followed by a step increase in power output to 85% PPO.

Standard cardiopulmonary exercise test parameters were collected breath-by-breath (Mendonca et al., 2014; Schaeffer et al., 2014), while heart rate (HR), stroke volume (SV), and cardiac output (CO) were assessed using an impedance cardiograph (PhysioFlow®; NewMeDx, Bristol, PA, USA) that provides an acceptable and non-invasive evaluation of CO during symptom-limited cycle exercise testing in both health and disease (Charloux et al., 2000; Richard et al., 2001). Inspiratory capacity (IC) maneuvers were performed at rest, within the last 30 s of every 2 min interval during exercise and at end-exercise (Mendonca et al., 2014; Schaeffer et al., 2014). Assuming that total lung capacity does not change during exercise with and without AB in normal males (Stubbing et al., 1980), changes in IC and inspiratory reserve volume [IRV = IC – tidal volume (V_T)] reflect changes in dynamic end-expiratory and end-inspiratory lung volume, respectively.

Breath-by-breath measures of the root mean square of EMGdi (EMGdi,rms) and of esophageal (Pes), gastric (Pga), and transdiaphragmatic pressure (Pdi = Pga – Pes) were recorded from a gastro-esophageal electrode-balloon catheter (Guangzhou Yinghui Medical Equipment Ltd., Guangzhou, China) and analyzed using published methods (Mendonca et al., 2014; Schaeffer et al., 2014). Maximum voluntary EMGdi,rms was identified as the largest of all EMGdi,rms values obtained from IC maneuvers performed either at rest or during exercise. Tidal swings in Pes (Pes,tidal), Pga (Pga,tidal), and Pdi (Pdi,tidal) were calculated as the difference between peak tidal inspiratory and peak tidal expiratory Pes, Pga, and Pdi, respectively. The ratio of Pdi,tidal-to-EMGdi,rms was used as an index of neuromuscular efficiency of the diaphragm.

Using Borg's 0–10 category ratio scale, participants rated the intensity of their breathing overall and the intensity of their leg discomfort at rest, within the last 30 s of every 2 min interval during exercise and at end-exercise (Borg, 1982). Breathing overall (hereafter referred to as breathlessness) was defined as "the global awareness of your breathing," which is consistent with the American Thoracic Society's recommendation that the definition of breathlessness should be neutral with respect to any particular quality of breathing (Parshall et al., 2012). Leg discomfort was defined as the "difficulty associated with pedaling." Participants were asked to verbalize their main reason(s) for stopping exercise; quantify the percentage contribution of breathlessness and leg discomfort to exercise cessation; and identify qualitative phrases that best described their breathlessness at end-exercise (O'Donnell et al., 2000).

Analysis of Exercise End-Points

All physiological parameters were averaged in 30 s intervals at rest and during exercise. These parameters, averaged over the first 30 s of every 2 min interval during exercise, were linked with IC and symptom measurements collected during the last 30 s of the same minute. Three main time points were used for the evaluation of measured parameters: (1) *pre-exercise rest*, defined as the average of the last 60 s of the steady-state period after ≥3 min of breathing on the mouthpiece while seated on the cycle ergometer before the start of exercise; (2) *isotime,* defined as the average of the first 30 s of the 2nd min of the highest equivalent 2 min interval of constant-load cycle exercise completed by a given participant with and without AB; and (3) *peak exercise*, defined as the average of the last 30 s of loaded pedaling. Exercise endurance time (EET) was the duration of loaded pedaling.

Statistical Analysis

Two-tailed paired *t*-tests were used to examine the effects of AB vs. CTRL on spirometric parameters, maximal voluntary EMGdi,rms, and the percentage contribution of breathlessness and leg discomfort to exercise cessation. A two-way repeated measures analysis of variance with Tukey's HSD *post-hoc* test was used to examine the effect of AB vs. CTRL on physiological and perceptual parameters measured at rest, at standardized submaximal time points during exercise (including isotime) and at peak exercise. All analyses were performed using SigmaStat®, version 3.5 (Systat® Software, San Jose, CA, USA) and statistical significance was set at $p < 0.05$. Data are presented as means ± SEM.

RESULTS

Participants, Abdominal Binding, and Spirometry

Participants were healthy, young (24.8 ± 1.7 years), non-obese (body mass index = 23.1 ± 0.6 kg $\times m^{-2}$) and non-smoking men with normal cardiorespiratory fitness: symptom-limited peak rate of O_2 consumption ($\dot{V}O_2$) of 55.1 ± 2.2 ml×kg×min^{-1} or 121 ± 6% predicted (Jones et al., 1985); and PPO of 267 ± 18 W or 109 ± 5% predicted (Jones et al., 1985). By design, AB increased Pga,ee by 6.6 ± 0.6 cmH_2O above its baseline value during the AB visit, but had no effect on spirometric parameters compared with CTRL (**Table 1**).

TABLE 1 | Effect of abdominal binding (AB) on spirometric pulmonary function test parameters at rest in healthy men.

Parameter	Control	AB
FVC, L	5.48 ± 0.22	5.35 ± 0.25
FEV_1, L (% predicted)	4.41 ± 0.19 (95 ± 3)	4.27 ± 0.21 (92 ± 4)
FEV_1/FVC, %	81 ± 2	80 ± 2
PEF, $L \times s^{-1}$	10.4 ± 0.5	9.8 ± 0.6
$FEF_{25-75\%}$, $L \times s^{-1}$	4.22 ± 0.33	4.04 ± 0.34

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

Physiological and Perceptual Responses to Exercise

The order of experimental conditions was balanced such that 7 of the 12 participants were randomized to exercise with AB at Visit 2. To rule out a potentially confounding order effect on exercise performance, we compared EET between Visits 2 and Visits 3, irrespective of experimental condition and found no statistically significant difference: 9.7 ± 1.0 vs. 9.0 ± 1.2 min, respectively ($p = 0.290$).

Compared to CTRL, AB had no effect on EET or an effect on cardiac, metabolic, perceptual, ventilatory, breathing pattern, and/or operating lung volume parameters at rest or during exercise (**Table 2**, **Figures 1**, **2**).

The relative contributions of breathlessness (AB, 46 ± 8% vs. CTRL, 40 ± 7%; $p = 0.592$) and leg discomfort (AB, 54 ± 8% vs. CTRL, 60 ± 7%; $p = 0.592$) to exercise cessation were not different under AB vs. CTRL conditions. The distribution of reasons for stopping exercise were also similar between-tests: Breathlessness: AB, $n = 1$ vs. CTRL, $n = 1$; Leg discomfort: AB, $n = 0$ vs. CTRL, $n = 1$; Combination of breathlessness and leg discomfort: AB, $n = 10$ vs. CTRL, $n = 9$. The majority of participants self-selected phrases alluding to a heightened sense of "*work/effort of breathing*" to describe their breathlessness at end-exercise under both AB and CTRL conditions; for example, "*My breathing is heavy*" (AB, 100% vs. CTRL, 92%) and "*My breathing requires more work*" (AB, 92% vs. CTRL, 100%).

Diaphragmatic EMG and Respiratory Pressures

Maximal voluntary EMGdi,rms was not significantly different under AB vs. CTRL conditions: 227 ± 19 vs. 234 ± 25 μV, respectively ($p = 0.727$). Peak inspiratory Pes values recorded during serial IC maneuvers did not change significantly from rest (AB, −34.2 ± 3.4 cmH_2O; CTRL, −34.5 ± 2.2 cmH_2O) and throughout exercise (e.g., AB at end-exercise, −34.8 ± 2.9 cmH_2O; CTRL at end-exercise, −36.1 ± 2.6 cmH_2O) both within and between conditions. Peak inspiratory Pdi values recorded during serial IC maneuvers (Pdi,IC) performed at rest and throughout exercise were significantly increased by 18.5–22.2 cmH_2O (or 43–53%) under AB vs. CTRL conditions; for example, AB, 70.3 ± 6.0 cmH_2O vs. CTRL, 48.5 ± 4.9 cmH_2O at rest ($p < 0.001$); and AB, 59.2 ± 4.0 cmH_2O vs. CTRL, 40.2 ± 2.8 cmH_2O at end-exercise ($p < 0.001$).

Compared with CTRL, AB had no effect on either EMGdi,rms (**Figure 3A**) or Pes (**Figure 3C**) responses during exercise (**Table 2**). As expected, peak tidal inspiratory Pga (Pga,inspir) and peak tidal expiratory Pga (Pga,expir) were consistency higher at rest and during exercise with vs. without AB (**Table 2**, **Figure 3E**). Compared with CTRL, AB increased Pdi,tidal and peak tidal inspiratory Pdi (Pdi,inspir) at rest and during exercise; for example, by +16.5 cmH_2O at rest and by +28.2 cmH_2O during exercise at isotime with vs. without AB (**Table 2**, **Figure 3B**). Furthermore, AB was associated with a marked increase in the magnitude of the exercise-induced rise in Pdi,tidal and Pdi,inspir (**Table 2**, **Figure 3B**): the respective increases in Pdi,tidal and Pdi,inspir from rest to isotime during exercise were ∼315 and 223% greater with vs. without AB. As illustrated in **Figure 3D**, Pdi,tidal and Pdi,inspir were much higher at any given EMGdi,rms during exercise with vs. without AB, indicating enhanced neuromuscular efficiency of the diaphragm. Indeed, AB increased the Pdi,tidal:EMGdi,rms ratio by an average of 85–95% at each measurement time during exercise (**Table 2**, **Figure 3F**).

DISCUSSION

The main finding of this study was that AB sufficient to increase intra-abdominal pressure by an average of 6.6 cmH_2O at rest enhanced neuromuscular efficiency of the diaphragm during exercise, but had no effect on exercise endurance nor an effect on cardiac, metabolic, ventilatory, breathing pattern, dynamic operating lung volume, and perceptual responses to exercise in healthy young men.

In keeping with the results of earlier AB studies in health (Hussain et al., 1985) and SCI (Hart et al., 2005; West et al., 2012a, 2014a,b), the increased Pdi,tidal, Pdi,inspir, and Pdi,IC responses observed at rest and during exercise with vs. without AB were mechanistically linked to increased intra-abdominal pressures (i.e., Pga,ee and Pga,expir). The increased intra-abdominal pressures associated with AB effectively shift the abdominal contents toward the diaphragm (cephalad), thereby increasing both insertional and appositional forces of the diaphragm on the lower rib cage (Wilson and De Troyer, 2013; Koo et al., 2015). By shifting the diaphragm cephalad, AB also lengthens diaphragm muscle fibers and optimizes its length-tension relationship (Koo et al., 2015). As a result, the diaphragm initiates its inspiratory contraction at a longer length, thus generating a greater pressure at any given level of muscle activation, reflecting enhanced diaphragmatic contractility (De Troyer, 1983). Abdominal binding may further enhance pressure-generating capacity of the diaphragm by improving (reducing) abdominal compliance, thus impeding diaphragmatic descent at the costal fibers during inspiration and minimizing muscle fiber shortening, i.e., maintaining the muscle length on a more favorable region of the length-tension curve (De Troyer, 1983; Hart et al., 2005; Koo et al., 2015). Finally, by increasing intra-abdominal pressures and decreasing abdominal compliance,

TABLE 2 | Effect of abdominal binding (AB) on physiological and perceptual responses to constant-load cycle endurance exercise testing at 85% of symptom-limited peak incremental power output (equivalent to 227 ± 17 W) in healthy men.

Parameter	REST		ISO-TIME		PEAK	
	Control	AB	Control	AB	Control	AB
Exercise time, min	0 ± 0	0 ± 0	7.5 ± 0.8	7.5 ± 0.8	9.9 ± 1.0	8.9 ± 1.1
Breathlessness, Borg 0–10 units	0.2 ± 0.2	0.3 ± 0.2	6.1 ± 0.6	6.8 ± 0.6	8.1 ± 0.7	8.3 ± 0.7
Leg Discomfort, Borg 0–10 units	0.0 ± 0.0	0.0 ± 0.0	6.9 ± 0.7	7.3 ± 0.6	8.1 ± 0.7	8.3 ± 0.5
$\dot{V}O_2$, ml×kg×min^{-1}	5.6 ± 0.2	5.1 ± 0.5	51.6 ± 2.3	51.5 ± 2.5	53.6 ± 2.1	52.4 ± 2.5
$\dot{V}CO_2$, ml×kg×min^{-1}	4.4 ± 0.2	4.7 ± 1.0	53.3 ± 1.9	53.8 ± 2.2	53.9 ± 1.7	53.9 ± 2.1
CO, L×min^{-1}	5.9 ± 0.3	5.5 ± 0.4	20.3 ± 1.0	20.6 ± 1.0	21.3 ± 1.1	21.3 ± 1.0
HR, beats×min^{-1}	78.4 ± 3.2	76.3 ± 3.2	172.7 ± 3.4	174.1 ± 3.3	182.6 ± 2.3	178.7 ± 2.9
SV, ml	75.6 ± 4.3	70.9 ± 3.8	117.9 ± 6.4	118.5 ± 6.1	116.6 ± 6.5	119.8 ± 6.0
$\dot{V}_E$, L×min^{-1}	12.5 ± 0.9	11.2 ± 0.9	116.4 ± 7.3	120.7 ± 7.7	133.4 ± 8.0	130.6 ± 8.6
V_T, L	0.90 ± 0.09	0.74 ± 0.06	2.96 ± 0.17	2.90 ± 0.21	2.72 ± 0.17	2.73 ± 0.19
f_R, breaths×min^{-1}	15.2 ± 1.1	15.7 ± 0.8	39.9 ± 2.0	42.8 ± 2.4	50.4 ± 3.6	49.8 ± 4.0
IC, L	3.38 ± 0.17	3.62 ± 0.16	3.84 ± 0.22	3.82 ± 0.20	3.68 ± 0.19	3.81 ± 0.23
IRV, L	2.48 ± 0.19	2.87 ± 0.15	0.89 ± 0.14	0.92 ± 0.18	0.96 ± 0.14	1.08 ± 0.17
EMGdi,rms, μV	22.5 ± 1.9	27.4 ± 3.7	129.2 ± 13.3	120.0 ± 11.8	150.7 ± 28.1	123.2 ± 14.7
EMGdi%max	10.4 ± 1.1	13.1 ± 2.1	56.0 ± 2.8	53.0 ± 2.9	61.9 ± 5.2	53.3 ± 3.4
End-expiratory Pes, cmH_2O	−7.3 ± 0.7	−5.0 ± 0.7	−5.4 ± 1.1	−4.3 ± 1.2	−6.0 ± 1.1	−5.3 ± 0.9
Pes,tidal, cmH_2O	6.1 ± 0.9	4.8 ± 0.6	31.6 ± 2.9	31.2 ± 2.6	35.0 ± 3.0	34.5 ± 2.8
Peak inspiratory Pes, cmH_2O	−11.7 ± 1.8	−8.4 ± 0.8	−23.6 ± 1.9	−21.5 ± 1.7	−24.3 ± 1.9	−22.0 ± 1.9
Peak expiratory Pes, cmH_2O	−5.6 ± 0.7	−3.5 ± 0.8	8.1 ± 2.3	9.8 ± 1.5	10.8 ± 2.2	12.5 ± 1.7
End-expiratory Pga, cmH_2O	7.7 ± 1.2	12.3 ± 1.2*	14.3 ± 1.4	17.8 ± 0.9	14.4 ± 1.3	19.1 ± 1.1*
Pga,tidal, cmH_2O	5.2 ± 0.5	9.0 ± 0.8	17.3 ± 1.7	18.0 ± 1.3	19.2 ± 1.6	17.1 ± 1.1
Peak inspiratory Pga, cmH_2O	6.5 ± 1.3	11.7 ± 1.2†	4.3 ± 1.2	14.0 ± 1.0†	4.3 ± 1.1	14.8 ± 0.9†
Peak expiratory Pga, cmH_2O	11.7 ± 1.6	20.7 ± 1.5*	21.6 ± 1.9	32.0 ± 1.7†	23.5 ± 1.9	31.9 ± 1.6*
End-expiratory Pdi, cmH_2O	15.1 ± 0.9	17.3 ± 0.9	19.6 ± 1.3	22.0 ± 1.2	20.4 ± 1.2	24.5 ± 1.3
Pdi,tidal, cmH_2O	9.6 ± 0.9	12.8 ± 1.0	20.4 ± 1.5	36.9 ± 2.3†	21.4 ± 1.5	35.8 ± 2.1†
Peak inspiratory Pdi, cmH_2O	22.9 ± 1.2	28.8 ± 1.6	32.0 ± 1.8	50.2 ± 2.6†	32.4 ± 1.6	49.5 ± 2.5†
Peak expiratory Pdi, cmH_2O	13.4 ± 1.2	16.0 ± 1.1	11.5 ± 1.1	13.4 ± 1.1	11.0 ± 1.2	13.7 ± 1.1
Pdi,tidal:EMGdi,rms, cmH_2O×μV^{-1}	0.44 ± 0.04	0.54 ± 0.06	0.17 ± 0.01	0.33 ± 0.03†	0.17 ± 0.01	0.33 ± 0.03†

Values are means ± SEM. $\dot{V}O_2$ and $\dot{V}CO_2$, rate of oxygen consumption and carbon dioxide output, respectively; CO, cardiac output; HR, heart rate; SV, stroke volume; $\dot{V}_E$, minute ventilation, V_T, tidal volume; f_R, respiratory frequency; IC, inspiratory capacity; IRV, inspiratory reserve volume; EMGdi,rms, root mean square of the crural diaphragm electromyogram; EMGdi%max, root mean square of the crural diaphragm electromyogram expressed as a percentage of the maximal voluntary room mean square of the crural diaphragm electromyogram; Pes, Pga and Pdi, esophageal, gastric, and transdiaphragmatic pressure, respectively; Pes,tidal, tidal esophageal pressure swing; Pga,tidal, tidal gastric pressure swing; Pdi,tidal, tidal transdiaphragmatic pressure swing; Pdi,tidal:EMGdi,rms, tidal transdiaphragmatic swing-to-root mean square of the diaphragmatic electromyogram ratio—an index of neuromuscular efficiency of the diaphragm. $^*p < 0.05$ and $^\dagger p \leq 0.01$ vs. Control.

AB may increase the inflationary action of the diaphragm on the lower rib cage by increasing the zone of apposition and improving the diaphragm's ability to lift and expand the lower rib cage (De Troyer, 1983; Koo et al., 2015). The combination of these mechanically advantageous changes to the shape and configuration of the diaphragm are most likely responsible for the 85–95% increase in neuromuscular efficiency of the diaphragm observed during exercise with vs. without AB.

Although AB increased diaphragmatic contractility/pressure-generating capacity, it had no demonstrable effect on EMGdi,rms, Pes, $\dot{V}_E$, breathing pattern, and dynamic operating lung volume responses to exercise. These findings are similar to those of earlier AB studies by Hussain et al. (1985) in health and by West et al. (2014b) in SCI, and presumably reflect the fact that AB had no untoward effect on expiratory flow generation during exercise (as evidenced by relative preservation of the relationship between exercise-induced increases in peak tidal expiratory Pes and peak expiratory flow) or an effect on exercise-induced increases the rate of CO_2 production, which is the proximate source of increased ventilatory requirements during exercise. It could be argued that the increased intra-abdominal pressures associated with AB may have hindered descent of the diaphragm into the abdomen at rest and particularly during exercise when ventilatory requirements were ∼13-fold higher than at rest. If this was true, then maximal voluntary EMGdi,rms as well as the magnitude of exercise-induced increases in EMGdi,rms should have been consistently higher under AB vs. CTRL conditions. However, this is not what we observed in our study nor what

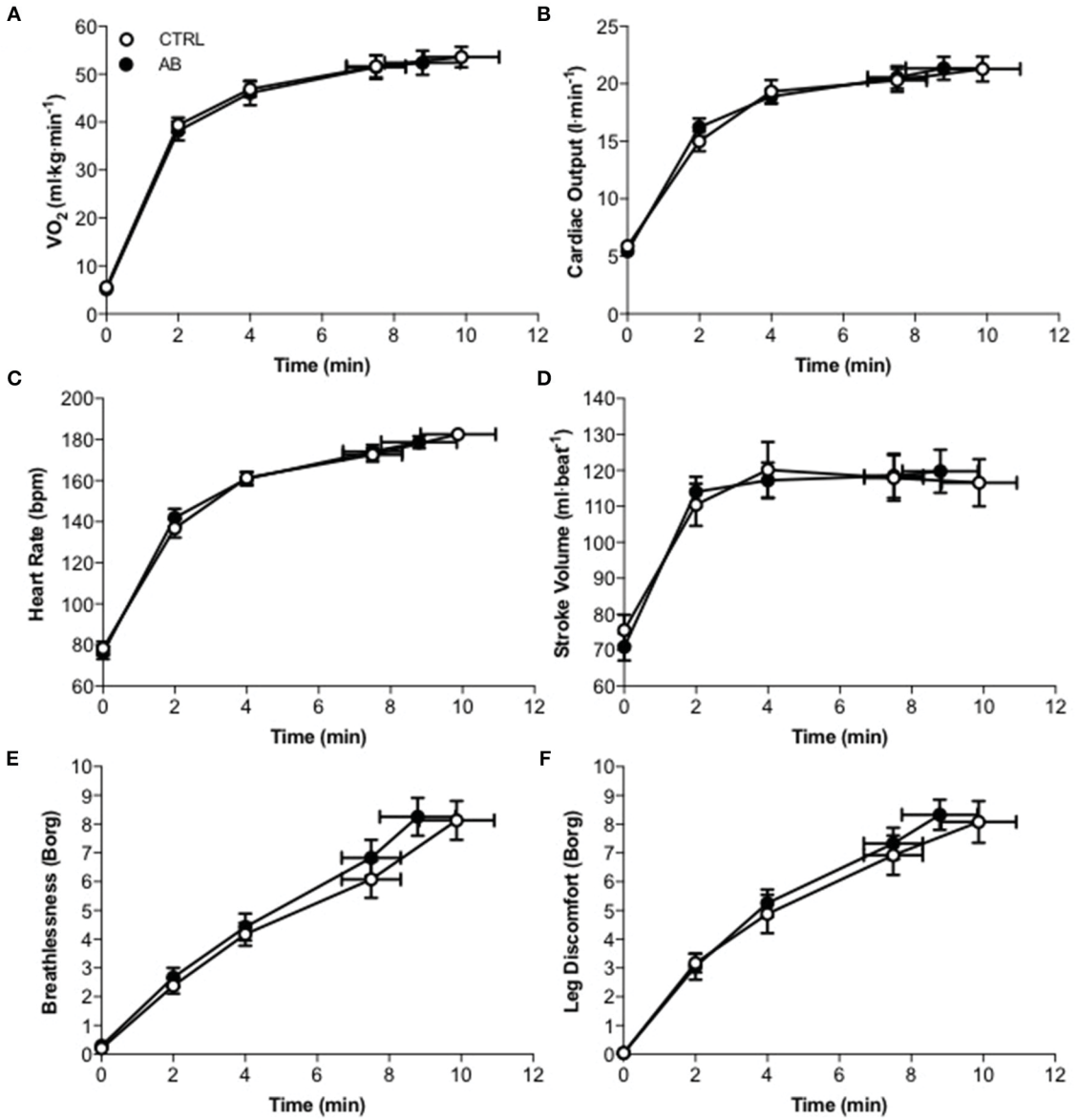

FIGURE 1 | Effect of abdominal binding (AB) vs. control (CTRL) on (A) the rate of oxygen consumption ($\dot{V}O_2$), **(B)** cardiac output **(C)** heart rate, **(D)** stroke volume, **(E)** breathlessness, and **(F)** leg discomfort responses during constant-load cycle endurance exercise testing at 85% of peak incremental power output in healthy men. Values are means ± SEM.

Hussain et al. (1985) reported in their AB study of five healthy men.

In the setting of a relatively preserved EMGdi,rms, $\dot{V}_E$, breathing pattern and dynamic operating lung volume response to exercise with vs. without AB, we speculate that the disparate effect of AB on Pdi and Pes responses to exercise reflected "off-loading" of the inspiratory action(s) of the rib cage muscles. In other words, by increasing Pdi,inspir and thus Pdi,tidal responses to exercise, AB effectively decreased the rib cage muscles' relative contribution to any given level of negative intrathoracic pressure development throughout inspiration during exercise. Additional research with simultaneous measures of accessory inspiratory muscle EMG activity is needed to confirm this postulate.

In the absence of changes in EMGdi,rms, $\dot{V}_E$, breathing pattern, expiratory flow generation, and dynamic operating lung volume responses to exercise, isolated and acute improvements in neuromuscular efficiency of the diaphragm during exercise with vs. without AB had no effect on exercise endurance and/or exertional breathlessness. These findings support the view that, in healthy young adults: (1) respiratory mechanical/muscular factors do not likely contribute to the limits of exercise tolerance;

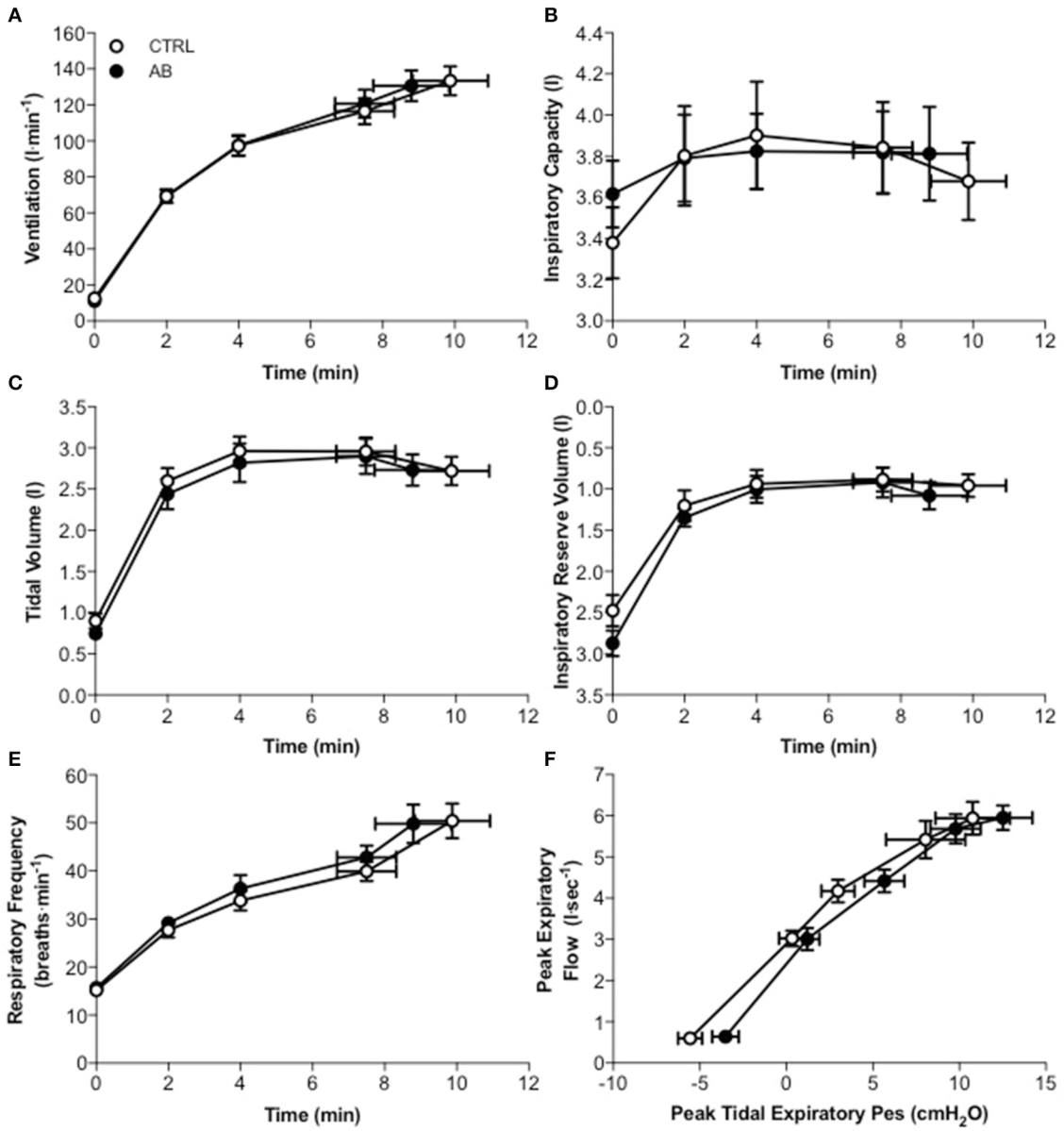

FIGURE 2 | Effect of abdominal binding (AB) vs. control (CTRL) on (A) ventilation, **(B)** inspiratory capacity, **(C)** tidal volume, **(D)** inspiratory reserve volume, **(E)** respiratory frequency, and **(F)** peak expiratory flow vs. peak tidal expiratory esophageal pressure (Pes) responses during constant-load cycle endurance exercise testing at 85% of peak incremental power output in healthy men. Values are means ± SEM.

and (2) progressive neuromuscular uncoupling of the diaphragm is not likely a proximate source of exertional breathlessness. Nevertheless, the results of our study provide a physiological rationale for future examination of AB as a potentially effective non-pharmacological means of improving exercise tolerance in pathophysiological disease states where neuromuscular uncoupling of the diaphragm has been mechanistically linked to a heightened perception of exertional breathlessness, most notably in patients with COPD (Laghi et al., 1998). Interestingly, a case report by Celli et al. (1985) found that AB sufficient to increase Pga,ee from 4 to 12 cmH_2O was associated with objective and potentially clinically meaningful improvements in diaphragmatic function, exercise tolerance, and breathlessness in a symptomatic patient with severe COPD and a large midline hernia of the anterior abdominal wall.

The collective results of studies by Vivier et al. (2006), Aliverti et al. (2009, 2010), and Uva et al. (2015) suggest that AB, by increasing intra-abdominal pressure and/or the abdominal circulatory pump action of the diaphragm and abdominal muscles, has the potential to improve cardiac function at rest and during exercise by increasing central venous return from the splanchnic venous circulation. In our

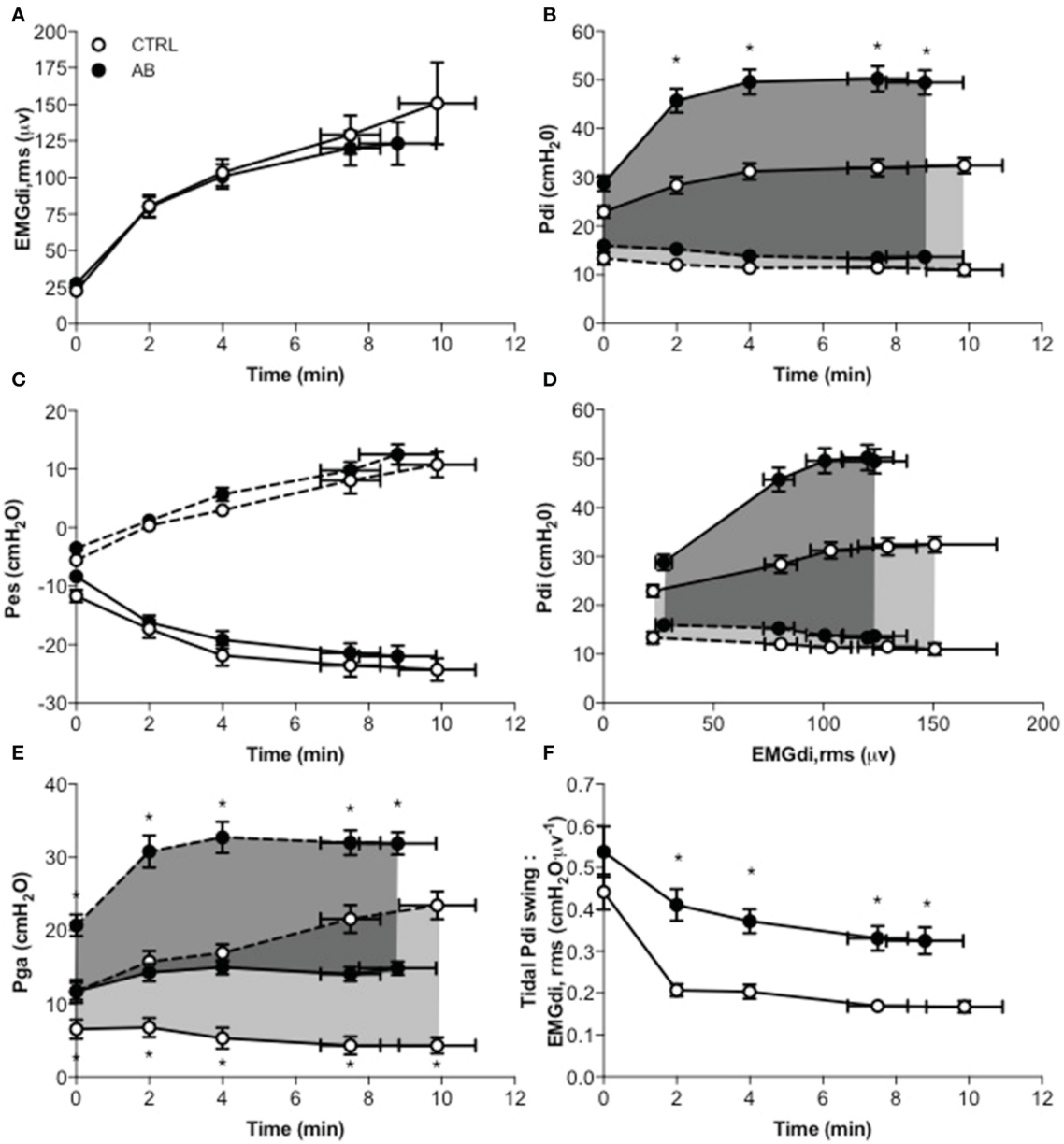

FIGURE 3 | Effect of abdominal binding (AB) vs. control (CTRL) on (A) root mean square of the crural diaphragm electromyogram (EMGdi,rms), **(B)** transdiaphragmatic pressure (Pdi), **(C)** esophageal pressure (Pes), **(D)** Pdi vs. EMGdi,rms, **(E)** gastric pressure (Pga), and **(F)** tidal Pdi swing-to-EMGdi,rms ratio responses during constant-load cycle endurance exercise testing at 85% of peak incremental power output in healthy men. Values are means ± SEM. $^{*}p < 0.05$ vs. CTRL. Dashed lines denote expiratory Pdi, Pes and Pga.

study, however, AB had no demonstrable effect on impedance cardiograph-derived estimates of CO and SV at rest and during exercise, which is in agreement with West et al. (2012a) who reported no effect of AB on echocardiography-derived measures of cardiac function at rest (e.g., CO, SV, end-diastolic volume, end-systolic volume, ejection fraction) in eight healthy adults. We speculate that AB-induced increases in intra-abdominal pressure and/or the abdominal circulatory pump action of the diaphragm and abdominal muscles were of insufficient magnitude(s) to shift large enough quantities of blood from the splanchnic to central venous circulation to enhance cardiac function at rest and during exercise in our participants.

In summary, the increased intra-abdominal pressures associated with AB enhanced neuromuscular efficiency of the diaphragm by 85–95% during high-intensity constant-load cycle endurance exercise testing in healthy men. Additional research is recommended to examine potential benefits of

AB on exertional symptoms in clinical populations where diaphragmatic weakness/dysfunction has been implicated as a source of physical activity-related breathlessness and exercise intolerance.

AUTHOR CONTRIBUTIONS

SA, DC, RG, and DJ contributed to the conception of the study as well as to data collection, analysis, and interpretation. CM contributed to data collection and analysis. SA and DJ wrote the manuscript, with critical input from all other authors. All authors read and approved the final version of the manuscript.

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REFERENCES

Aliverti, A., Bovio, D., Fullin, I., Dellaca, R. L., Lo Mauro, A., Pedotti, A., et al. (2009). The abdominal circulatory pump. *PLoS ONE* 4:e5550. doi: 10.1371/journal.pone.0005550

Aliverti, A., Uva, B., Laviola, M., Bovio, D., Lo Mauro, A., Tarperi, C., et al. (2010). Concomitant ventilatory and circulatory functions of the diaphragm and abdominal muscles. *J. Appl. Physiol.* 109, 1432–1440. doi: 10.1152/japplphysiol.00576.2010

Baydur, A. (1991). Respiratory muscle strength and control of ventilation in patients with neuromuscular disease. *Chest* 99, 330–338. doi: 10.1378/chest.99.2.330

Baydur, A., Alsalek, M., Louie, S. G., and Sharma, O. P. (2001). Respiratory muscle strength, lung function, and dyspnea in patients with sarcoidosis. *Chest* 120, 102–108. doi: 10.1378/chest.120.1.102

Berlowitz, D. J., and Tamplin, J. (2013). Respiratory muscle training for cervical spinal cord injury. *Cochrane Database Syst. Rev.* CD008507. doi: 10.1002/14651858.CD008507.pub2

Borg, G. A. (1982). Psychophysical bases of perceived exertion. *Med. Sci. Sports Exerc.* 14, 377–381. doi: 10.1249/00005768-198205000-00012

Brown, R., DiMarco, A. F., Hoit, J. D., and Garshick, E. (2006). Respiratory dysfunction and management in spinal cord injury. *Respir. Care* 51, 853–868. discussion: 869–870.

Budweiser, S., Moertl, M., Jorres, R. A., Windisch, W., Heinemann, F., and Pfeifer, M. (2006). Respiratory muscle training in restrictive thoracic disease: a randomized controlled trial. *Arch. Phys. Med. Rehabil.* 87, 1559–1565. doi: 10.1016/j.apmr.2006.08.340

Celli, B. R., Rassulo, J., Berman, J. S., and Make, B. (1985). Respiratory consequences of abdominal hernia in a patient with severe chronic obstructive pulmonary disease. *Am. Rev. Respir. Dis.* 131, 178–180.

Charloux, A., Lonsdorfer-Wolf, E., Richard, R., Lampert, E., Oswald-Mammosser, M., Mettauer, B., et al. (2000). A new impedance cardiograph device for the non-invasive evaluation of cardiac output at rest and during exercise: comparison with the "direct" Fick method. *Eur. J. Appl. Physiol.* 82, 313–320. doi: 10.1007/s004210000226

De Troyer, A. (1983). Mechanical role of the abdominal muscles in relation to posture. *Respir. Physiol.* 53, 341–353. doi: 10.1016/0034-5687(83)90124-X

Doorduin, J., Sinderby, C. A., Beck, J., Stegeman, D. F., van Hees, H. W., van der Hoeven, J. G., et al. (2012). The calcium sensitizer levosimendan improves human diaphragm function. *Am. J. Respir. Crit. Care Med.* 185, 90–95. doi: 10.1164/rccm.201107-1268OC

Geddes, E. L., O'Brien, K., Reid, W. D., Brooks, D., and Crowe, J. (2008). Inspiratory muscle training in adults with chronic obstructive pulmonary disease: an update of a systematic review. *Respir. Med.* 102, 1715–1729. doi: 10.1016/j.rmed.2008.07.005

Goldman, J. M., Rose, L. S., Williams, S. J., Silver, J. R., and Denison, D. M. (1986). Effect of abdominal binders on breathing in tetraplegic patients. *Thorax* 41, 940–945. doi: 10.1136/thx.41.12.940

Hart, N., Laffont, I., de la Sota, A. P., Lejaille, M., Macadou, G., Polkey, M. I., et al. (2005). Respiratory effects of combined truncal and abdominal support in patients with spinal cord injury. *Arch. Phys. Med. Rehabil.* 86, 1447–1451. doi: 10.1016/j.apmr.2004.12.025

Harty, H. R., Corfield, D. R., Schwartzstein, R. M., and Adams, L. (1999). External thoracic restriction, respiratory sensation, and ventilation during exercise in men. *J. Appl. Physiol.* 86, 1142–1150.

Hussain, S. N., Rabinovitch, B., Macklem, P. T., and Pardy, R. L. (1985). Effects of separate rib cage and abdominal restriction on exercise performance in normal humans. *J. Appl. Physiol.* 58, 2020–2026.

Jones, N. L., Makrides, L., Hitchcock, C., Chypchar, T., and McCartney, N. (1985). Normal standards for an incremental progressive cycle ergometer test. *Am. Rev. Respir. Dis.* 131, 700–708.

Kabitz, H. J., Lang, F., Walterspacher, S., Sorichter, S., Muller-Quernheim, J., and Windisch, W. (2006). Impact of impaired inspiratory muscle strength on dyspnea and walking capacity in sarcoidosis. *Chest* 130, 1496–1502. doi: 10.1378/chest.130.5.1496

Kabitz, H. J., Walterspacher, S., Walker, D., and Windisch, W. (2007). Inspiratory muscle strength in chronic obstructive pulmonary disease depending on disease severity. *Clin. Sci.* 113, 243–249. doi: 10.1042/CS20060362

Koo, P., Gartman, E. J., Sethi, J. M., and McCool, F. D. (2015). Physiology in Medicine: physiological basis of diaphragmatic dysfunction with abdominal hernias-implications for therapy. *J. Appl. Physiol.* 118, 142–147. doi: 10.1152/japplphysiol.00276.2014

Koulouris, N., Mulvey, D. A., Laroche, C. M., Goldstone, J., Moxham, J., and Green, M. (1989). The effect of posture and abdominal binding on respiratory pressures. *Eur. Respir. J.* 2, 961–965.

Laghi, F., and Tobin, M. J. (2003). Disorders of the respiratory muscles. *Am. J. Respir. Crit. Care Med.* 168, 10–48. doi: 10.1164/rccm.2206020

Laghi, F., Jubran, A., Topeli, A., Fahey, P. J., Garrity, E. R. Jr., Arcidi, J. M., et al. (1998). Effect of lung volume reduction surgery on neuromechanical coupling of the diaphragm. *Am. J. Respir. Crit. Care Med.* 157, 475–483. doi: 10.1164/ajrccm.157.2.9705082

Martin-Valero, R., Zamora-Pascual, N., and Armenta-Peinado, J. A. (2014). Training of respiratory muscles in patients with multiple sclerosis: a systematic review. *Respir. Care* 59, 1764–1772. doi: 10.4187/respcare.02881

McCool, F. D., Pichurko, B. M., Slutsky, A. S., Sarkarati, M., Rossier, A., and Brown, R. (1986). Changes in lung volume and rib cage configuration with abdominal binding in quadriplegia. *J. Appl. Physiol.* 60, 1198–1202.

Mendonca, C. T., Schaeffer, M. R., Riley, P., and Jensen, D. (2014). Physiological mechanisms of dyspnea during exercise with external thoracic restriction:

role of increased neural respiratory drive. *J. Appl. Physiol.* 116, 570–581. doi: 10.1152/japplphysiol.00950.2013

Meyer, F. J., Borst, M. M., Zugck, C., Kirschke, A., Schellberg, D., Kubler, W., et al. (2001). Respiratory muscle dysfunction in congestive heart failure: clinical correlation and prognostic significance. *Circulation* 103, 2153–2158. doi: 10.1161/01.CIR.103.17.2153

Miller, J. D., Beck, K. C., Joyner, M. J., Brice, A. G., and Johnson, B. D. (2002). Cardiorespiratory effects of inelastic chest wall restriction. *J. Appl. Physiol.* 92, 2419–2428. doi: 10.1152/japplphysiol.00394.2001

Miller, M. R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., et al. (2005). Standardisation of spirometry. *Eur. Respir. J.* 26, 319–338. doi: 10.1183/09031936.05.00034805

Moodie, L., Reeve, J., and Elkins, M. (2011). Inspiratory muscle training increases inspiratory muscle strength in patients weaning from mechanical ventilation: a systematic review. *J. Physiother.* 57, 213–221. doi: 10.1016/S1836-9553(11)70051-0

Nishimura, Y., Maeda, H., Tanaka, K., Nakamura, H., Hashimoto, Y., and Yokoyama, M. (1994). Respiratory muscle strength and hemodynamics in chronic heart failure. *Chest* 105, 355–359. doi: 10.1378/chest.105.2.355

O'Donnell, D. E., Hong, H. H., and Webb, K. A. (2000). Respiratory sensation during chest wall restriction and dead space loading in exercising men. *J. Appl. Physiol.* 88, 1859–1869.

Parshall, M. B., Schwartzstein, R. M., Adams, L., Banzett, R. B., Manning, H. L., Bourbeau, J., et al. (2012). An official American Thoracic Society statement: update on the mechanisms, assessment, and management of dyspnea. *Am. J. Respir. Crit. Care Med.* 185, 435–452. doi: 10.1164/rccm.201111-2042ST

Petrof, B. J., Jaber, S., and Matecki, S. (2010). Ventilator-induced diaphragmatic dysfunction. *Curr. Opin. Crit. Care* 16, 19–25. doi: 10.1097/MCC.0b013e328334b166

Polkey, M. I., Kyroussis, D., Hamnegard, C. H., Mills, G. H., Green, M., and Moxham, J. (1996). Diaphragm strength in chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 154, 1310–1317. doi: 10.1164/ajrccm.154.5.8912741

Richard, R., Lonsdorfer-Wolf, E., Charloux, A., Doutreleau, S., Buchheit, M., Oswald-Mammosser, M., et al. (2001). Non-invasive cardiac output evaluation during a maximal progressive exercise test, using a new impedance cardiograph device. *Eur. J. Appl. Physiol.* 85, 202–207. doi: 10.1007/s004210100458

Schaeffer, M. R., Mendonca, C. T., Levangie, M. C., Andersen, R. E., Taivassalo, T., and Jensen, D. (2014). Physiological mechanisms of sex differences in exertional dyspnoea: role of neural respiratory motor drive. *Exp. Physiol.* 99, 427–441. doi: 10.1113/expphysiol.2013.074880

Smart, N. A., Giallauria, F., and Dieberg, G. (2013). Efficacy of inspiratory muscle training in chronic heart failure patients: a systematic review and meta-analysis. *Int. J. Cardiol.* 167, 1502–1507. doi: 10.1016/j.ijcard.2012.04.029

Stubbing, D. G., Pengelly, L. D., Morse, J. L., and Jones, N. L. (1980). Pulmonary mechanics during exercise in normal males. *J. Appl. Physiol.* 49, 506–510.

Tan, W. C., Bourbeau, J., Hernandez, P., Chapman, K., Cowie, R., FitzGerald, M. J., et al. (2011). Canadian prediction equations of spirometric lung function for Caucasian adults 20 to 90 years of age: results from the Canadian Obstructive Lung Disease (COLD) study and the Lung Health Canadian Environment (LHCE) study. *Can. Respir. J.* 18, 321–326. doi: 10.1155/2011/540396

Tantucci, C., Massucci, M., Piperno, R., Betti, L., Grassi, V., and Sorbini, C. A. (1994). Control of breathing and respiratory muscle strength in patients with multiple sclerosis. *Chest* 105, 1163–1170. doi: 10.1378/chest.105.4.1163

Uva, B., Aliverti, A., Bovio, D., and Kayser, B. (2015). The "Abdominal Circulatory Pump": an auxiliary heart during exercise? *Front. Physiol.* 6:411. doi: 10.3389/fphys.2015.00411

van Hees, H. W., Dekhuijzen, P. N., and Heunks, L. M. (2009). Levosimendan enhances force generation of diaphragm muscle from patients with chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 179, 41–47. doi: 10.1164/rccm.200805-732OC

Vanmeenen, M. T., Ghesquiere, J., and Demedts, M. (1984). Effects of thoracic or abdominal strapping on exercise performance. *Bull. Eur. Physiopathol. Respir.* 20, 127–132.

Vivier, E., Metton, O., Piriou, V., Lhuillier, F., Cottet-Emard, J. M., Branche, P., et al. (2006). Effects of increased intra-abdominal pressure on central circulation. *Br. J. Anaesth.* 96, 701–707. doi: 10.1093/bja/ael071

West, C. R., Campbell, I. G., Goosey-Tolfrey, V. L., Mason, B. S., and Romer, L. M. (2014a). Effects of abdominal binding on field-based exercise responses in Paralympic athletes with cervical spinal cord injury. *J. Sci. Med. Sport* 17, 351–355. doi: 10.1016/j.jsams.2013.06.001

West, C. R., Campbell, I. G., Shave, R. E., and Romer, L. M. (2012a). Effects of abdominal binding on cardiorespiratory function in cervical spinal cord injury. *Respir. Physiol. Neurobiol.* 180, 275–282. doi: 10.1016/j.resp.2011.12.003

West, C. R., Campbell, I. G., Shave, R. E., and Romer, L. M. (2012b). Resting cardiopulmonary function in Paralympic athletes with cervical spinal cord injury. *Med. Sci. Sports Exerc.* 44, 323–329. doi: 10.1249/MSS.0b013e31822b7441

West, C. R., Goosey-Tolfrey, V. L., Campbell, I. G., and Romer, L. M. (2014b). Effect of abdominal binding on respiratory mechanics during exercise in athletes with cervical spinal cord injury. *J. Appl. Physiol.* 117, 36–45. doi: 10.1152/japplphysiol.00218.2014

Wilson, T. A., and De Troyer, A. (2013). Effects of the insertional and appositional forces of the canine diaphragm on the lower ribs. *J. Physiol.* 591, 3539–3548. doi: 10.1113/jphysiol.2013.253286

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Appendix II

frontiers in Physiology

ORIGINAL RESEARCH
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Effect of Abdominal Binding on Diaphragmatic Neuromuscular Efficiency, Exertional Breathlessness, and Exercise Endurance in Chronic Obstructive Pulmonary Disease

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We tested the hypothesis that abdominal binding (AB) would reduce breathlessness and improve exercise tolerance by enhancing neuromuscular efficiency of the diaphragm during exercise in adults with chronic obstructive pulmonary disease (COPD). In a randomized, controlled, crossover trial, 20 adults with COPD (mean ± SD FEV_1, 60 ± 16% predicted) completed a symptom-limited constant-load cycle endurance exercise test at 75% of their peak incremental power output with concomitant measures of the diaphragm electromyogram (EMGdi) and respiratory pressures without (CTRL) vs. with AB sufficient to increase end-expiratory gastric pressure (Pga,ee) by 6.7 ± 0.3 cmH_2O at rest. Compared to CTRL, AB enhanced diaphragmatic neuromuscular efficiency during exercise ($p < 0.05$), as evidenced by a 25% increase in the quotient of EMGdi to tidal transdiaphragmatic pressure swing. By contrast, AB had no demonstrable effect on exertional breathlessness and exercise tolerance; spirometry and plethysmography-derived pulmonary function test parameters at rest; and cardiac, metabolic, breathing pattern, inspiratory reserve volume and EMGdi responses during exercise (all $p > 0.05$ vs. CTRL). In conclusion, enhanced neuromuscular efficiency of the diaphragm during exercise with AB was not associated with relief of exertional breathlessness and improved exercise tolerance in adults with COPD.

Clinical Trial Registration: ClinicalTrials.gov Identifier: NCT01852006.

Keywords: breathlessness, abdominal binding, diaphragm, neuromuscular efficiency, exercise endurance

INTRODUCTION

In people with chronic obstructive pulmonary disease (COPD), lung hyperinflation shortens the length of the diaphragm, thereby compromising its length-tension relationship and area of apposition to the rib cage (Cassart et al., 1997; Laghi and Tobin, 2003). Collectively, these changes promote diaphragmatic neuromuscular inefficiency by decreasing diaphragm pressure-generating capacity and provoking high levels of diaphragm electrical activation (EMGdi),

particularly during exercise when dynamic lung hyperinflation further shortens and weakens the diaphragm (Sinderby et al., 2001; Finucane and Singh, 2012). Diaphragmatic neuromuscular inefficiency has been mechanistically linked to abnormally high levels of exertional breathlessness and abnormally low levels of exercise tolerance in COPD (Laghi et al., 1998, 2004). It follows that any intervention capable of enhancing diaphragmatic neuromuscular efficiency may decrease exertional breathlessness and improve exercise tolerance in adults with COPD. Indeed, Laghi et al. (1998) reported that improvements in diaphragmatic neuromechanical coupling after lung volume reduction surgery (LVRS) in patients with severe emphysema correlated with relief of breathlessness at rest and improved 6-min walking distance.

In 1934, Alexander and Kontz (1934) and Gordon (1934) reported symptomatic improvement of breathlessness following application of a belt around the abdomen in adults with various pulmonary diseases, including bronchitis, emphysema, and asthma. In keeping with these observations, Celli et al. (1985) reported that abdominal binding (AB) sufficient to increase end-expiratory gastric pressure (Pga,ee) by 8 cmH_2O increased maximal voluntary transdiaphragmatic pressure-generating capacity by 13 cmH_2O (93%), decreased the perception of breathlessness at rest, and increased exercise tolerance in a symptomatic patient with severe COPD and a large midline hernia of the anterior abdominal wall. Presumably, this improvement in diaphragm pressure-generating capacity via AB reflected the combination of reduced abdominal wall compliance, increased intra-abdominal pressure, improved operating length of the diaphragm due to its ascent to a more mechanically advantageous (cephalad) position, increased area of diaphragm apposition to the rib cage, and increased diaphragm-rib cage insertional forces (Koo et al., 2015).

Recently, West et al. (2012) reported improvements in static lung volumes and capacities following AB in people with cervical spinal cord injury (SCI) as well as in healthy adults. For example, AB decreased functional residual capacity by 0.75 l (23%) in SCI and 0.46 l (14%) in health; increased inspiratory capacity (IC) by 0.47 l in SCI (20%) and 0.33 l (11%) in health; and increased inspiratory reserve volume (IRV) by 0.49 l (29%) in SCI and 0.38 l (16%) in health. A subsequent study by West et al. (2014) demonstrated that, compared to the unbound condition, AB shifted tidal breathing to lower and more mechanically advantageous end-expiratory and end-inspiratory lung volumes during submaximal exercise in athletes with cervical SCI.

We recently demonstrated that increasing Pga,ee by 6.6 ± 0.6 cmH_2O (mean $\pm$ SE) at rest via AB markedly improved diaphragmatic neuromuscular efficiency – quantified as the quotient of tidal transdiaphragmatic pressure swing (Pdi,tidal) to the root mean square of the crural diaphragm electromyogram (EMGdi,rms) – by 85–90% during cycle endurance exercise testing in healthy young men (Abdallah et al., 2017). Despite this improvement, AB had no effect on exertional breathlessness and exercise endurance, likely because diaphragmatic neuromuscular inefficiency is not the proximate source of exertional breathlessness and exercise limitation in healthy young adults (Abdallah et al., 2017). Nevertheless, the collective results of Alexander and Kontz (1934), Gordon (1934), Celli et al. (1985), West et al. (2012, 2014) and ourselves (Abdallah et al., 2017) provide a physiological rationale for the use of AB as a potentially effective non-pharmacological means of alleviating exertional breathlessness and improving exercise tolerance in adults with COPD by improving dynamic operating lung volumes and/or enhancing diaphragmatic neuromuscular efficiency.

Therefore, the primary aim of this study was to evaluate the effect of AB on the inter-relationships between diaphragmatic neuromuscular efficiency, exertional breathlessness and exercise endurance in adults with COPD.

MATERIALS AND METHODS

Study Design

This single-center, randomized, controlled, crossover trial was conducted at the McGill University Health Centre in Montreal, QC, Canada (Clinicaltrials.gov identifier: NCT01852006). The study protocol and informed consent form received ethics approval from the Research Institute of the McGill University Health Centre (13-075-BMA) in accordance with the *Declaration of Helsinki*.

After providing written and informed consent, participants completed a screening/familiarization visit followed by two intervention visits randomized to order. All visits were separated by $\geq$48-h. *Visit 1* included: medical history and clinical assessment; evaluation of activity-related breathlessness using the modified Medical Research Council dyspnoea scale (Bestall et al., 1999), the Baseline Dyspnea Index (Mahler et al., 1984) and the Oxygen Cost Diagram (McGavin et al., 1978); evaluation of health status using the COPD Assessment Test (Jones et al., 2009); evaluation of anxiety and depression using the Hospital Anxiety and Depression scale (Zigmond and Snaith, 1983); post-bronchodilator (400 μg salbutamol) spirometry; and a symptom-limited incremental cardiopulmonary cycle exercise test (CPET) to determine peak power output (PPO), defined as the highest power output that the participant was able to sustain for $\geq$30-s. During *Visits 2 and 3,* participants first inhaled 400 μg of salbutamol. The gastro-esophageal electrode-balloon catheter used to record EMGdi,rms and respiratory pressures (*see below*) was then inserted and positioned in accordance with established techniques (Jensen et al., 2011). During the AB visit, the abdominal binder was applied and optimally fitted (*see below*). Once the AB was optimally fitted, the gastro-esophageal electrode-balloon catheter was re-positioned to achieve optimal recordings of EMGdi during resting breathing (i.e., positioned such that the amplitude of EMGdi during inspiration was greatest in electrode pairs 1 and 5, and lowest in electrode pair 3) (Jensen et al., 2011). In this way, the recording electrodes were similarly positioned at the diaphragm's electrically active center under both CTRL and AB conditions. Thereafter, participants completed spirometry and plethysmography followed by a symptom-limited constant-load cycle CPET at 75% PPO. Participants were permitted to use their respiratory medications according to their regular schedule. Participants were randomized in a 1:1 ratio according to a computer-generated block randomization

schedule (Block size = 4) prepared by a third-party statistician not involved in the trial.

Participants

Participants were recruited from the Montreal Chest institute of the McGill University Health Centre, and included men and women aged ≥40 y with Global Initiative for Obstructive Lung Disease (GOLD) stage II or III COPD (Vogelmeier et al., 2017), cigarette smoking history ≥15 pack-years, and no change in medication dosage or frequency of administration with no exacerbation(s) and/or hospitalization(s) in the preceding 6-weeks. Exclusion criteria were: presence of medical conditions other than COPD that could contribute to breathlessness and/or exercise intolerance; use of domiciliary oxygen; exercise-induced oxyhemoglobin desaturation to <80% on room air; and body mass index <18.5 or ≥35.0 kg/m^2.

Intervention

A commercially available binder (493R Universal Back Support; McDavid Inc., Woodridge, IL, United States) that has been described in detail elsewhere (West et al., 2012) was used to bind the abdomen. The binder was fitted with the upper edge below the costal margin so that it interfered minimally with rib-cage movement. The desired degree of abdominal compression – defined as a 5–8 cmH_2O increase in Pga,ee – was achieved by tightening Velcro fasteners at the front of the binder with participants breathing normally while seated at rest. We recently demonstrated that this level of abdominal compression enhanced diaphragmatic neuromuscular efficiency during exercise in healthy young men, as evidenced by an 85–90% increase in the quotient of Pdi,tidal to EMGdi,rms (Abdallah et al., 2017). Furthermore, West et al. (2012) demonstrated that this level of abdominal compression was associated with significantly greater improvements in diaphragm function than increasing Pga,ee by 1.0–3.5 cmH_2O in healthy adults and people with cervical SCI.

Procedures

Pulmonary Function Testing

Spirometry and plethysmography were performed with participants seated using automated equipment (Vmax Encore^{TM} 29C, CareFusion, Yorba Linda, CA, United States; Medisoft Body Box 5500®, Medisoft Belgium, Sorinnes, Belgium) and according to recommended techniques (Macintyre et al., 2005; Miller et al., 2005a,b; Wanger et al., 2005). Measurements were referenced to predicted normal values (Briscoe and Dubois, 1958; Burrows et al., 1961; Crapo et al., 1981; Hankinson et al., 1999).

Cardiopulmonary Exercise Testing

Exercise tests were conducted on an electronically braked cycle ergometer (Lode Corival, Lode BV Medical Technology, Groningen, Netherlands) using a computerized CPET system (Vmax Encore^{TM} 29C). Incremental CPETs consisted of a baseline rest period of ≥6-min, followed by 10 W/min increases in power output to symptom-limitation. Constant-load CPETs consisted of a baseline rest period of ≥6-min, followed by 1-min of unloaded pedaling and then a step increase in power output to 75% PPO maintained to symptom-limitation. Cardiac, metabolic, gas exchange, and breathing pattern parameters were collected breath-by-breath and analyzed as previously described (Abdallah et al., 2017). Inspiratory capacity maneuvers were performed at rest, every 2-min during CPET, and at end-exercise (Guenette et al., 2013). Measurements of PPO, peak oxygen uptake and peak heart rate were referenced to the predicted normal values of Jones et al. (1985).

Published methods were used to analyze breath-by-breath measures of EMGdi,rms and of esophageal (Pes), gastric (Pga) and transdiaphragmatic pressure (Pdi = Pga-Pes) recorded from a gastro-esophageal electrode-balloon catheter (Guangzhou Yinghui Medical Equipment Ltd., Guangzhou, China) (Jensen et al., 2011; Mendonca et al., 2014; Abdallah et al., 2017). Maximum voluntary EMGdi,rms was identified as the largest of all EMGdi,rms values obtained from IC maneuvers performed either at rest or during exercise. Tidal swings in Pes (Pes,tidal), Pga (Pga,tidal), and Pdi (Pdi,tidal) were calculated as the difference between peak tidal inspiratory and peak tidal expiratory Pes, Pga, and Pdi, respectively. The quotient of Pdi,tidal to EMGdi,rms was used as an index of diaphragmatic neuromuscular efficiency (Abdallah et al., 2017).

Using Borg's modified 0–10 category ratio scale (Borg, 1982), participants rated the intensity and unpleasantness of their breathlessness, as well as the intensity of their leg discomfort at rest, every 2-min during CPET, and at end-exercise. At end-exercise, participants were asked to identify their locus of symptom limitation (breathlessness, leg discomfort, combination of breathlessness, and leg discomfort, other); to quantify the percentage contribution of their selection to exercise cessation; and identify qualitative phrases that best described their breathlessness at end-exercise (O'Donnell et al., 2000).

Outcomes

Primary Outcomes

The primary outcome was the difference in breathlessness intensity ratings during exercise at isotime under AB vs. CTRL conditions, where isotime was defined as the highest equivalent 2-min interval of exercise completed by a given participant during each of the constant-load CPETs. The co-primary outcome was the difference in exercise endurance time (EET) under AB vs. CTRL conditions, where EET was defined as the duration of loaded pedaling during constant-load CPET.

Secondary Outcomes

Spirometry and plethysmography-derived pulmonary function test parameters; physiological and perceptual parameters measured at rest, at standardized submaximal times during constant-load CPETs, and at peak-exercise (defined as the average of the last 30-s of loaded pedaling); reasons for stopping exercise; percentage contribution of breathlessness and leg discomfort to exercise cessation; and qualitative descriptors of breathlessness at end-exercise.

Statistical Methods

Using a two-tailed paired subject formula with $\alpha = 0.05$, $\beta = 0.90$ and an expected effect size of 0.80 (Faul et al., 2009), we estimated

that at least 19 participants were needed to detect a minimal clinically important difference of ±1 Borg unit in breathlessness intensity ratings (Ries, 2005) at isotime and of ±101-s in EET (Puente-Maestu et al., 2009) under AB vs. CTRL conditions.

Participants who completed both AB and CTRL arms of the trial were included in the analysis. Linear mixed-model regression with random intercepts was used to analyze differences in EET as well as in all physiological and perceptual responses to constant-load CPET under AB and CTRL conditions. Two-tailed paired *t*-tests were used to compare the effects of AB vs. CTRL on: spirometry and plethysmography-derived pulmonary function test parameters; maximal voluntary EMGdi,rms; and the percentage contribution of breathlessness and leg discomfort to exercise cessation. Fisher's exact test was used to compare the effect of AB vs. CTRL on the selection frequencies of reasons for stopping exercise as well as the descriptors of breathlessness at end-exercise. Data were analyzed using SAS statistical package, version 9.4 (SAS Institute Inc., Cary, NC, United States) and SigmaStat, version 3.5 (Systat Software Inc., San Jose, CA, United States). Statistical significance was set at $p < 0.05$ and values are reported as mean ± SEM unless stated otherwise.

RESULTS

Twenty-four participants were randomized into the trial. Four of these participants dropped out during follow-up for non-study related reasons (**Figure 1**). Baseline characteristics of the 20 participants (13 men) who completed the trial are presented in **Table 1**. By design, AB increased Pga,ee by 6.7 ± 0.3 cmH_2O above its baseline value during the AB visit.

Primary Outcomes

Compared to CTRL, AB had no effect on breathlessness intensity ratings at isotime (AB, 3.2 ± 0.4 Borg units vs. CTRL, 3.0 ± 0.4 Borg units; $p = 0.454$) or on EET (AB, 6.7 ± 1.1 min vs. CTRL, 6.9 ± 1.1 min; $p = 0.853$) (**Figure 2**). To assess for a possible confounding order effect on our primary outcomes, breathlessness intensity ratings at isotime and EET were compared between *Visits 2 and 3*. There was no statistically significant effect of visit order on breathlessness intensity ratings at isotime (*Visit 2*, 3.2 ± 0.5 Borg units vs. *Visit 3*, 3.1 ± 0.4 Borg units; $p = 0.873$) or on EET (*Visit 2*, 7.3 ± 1.3 min vs. *Visit 3*, 6.4 ± 1.0 min; $p = 0.079$).

Secondary Outcomes

Pulmonary Function

Compared to CTRL, AB had no effect on spirometry and plethysmography-derived pulmonary function test parameters at rest (**Table 2**).

Physiological and Perceptual Responses to Exercise

Except for small and isolated decreases in IC at isotime (AB, 1.96 ± 0.12 l vs. CTRL, 2.07 ± 0.13 l; $p = 0.043$) and at peak exercise (AB, 1.86 ± 0.14 l vs. CTRL, 1.98 ± 0.14 l; $p = 0.024$), AB had no demonstrable effect on cardiac, metabolic, ventilatory,

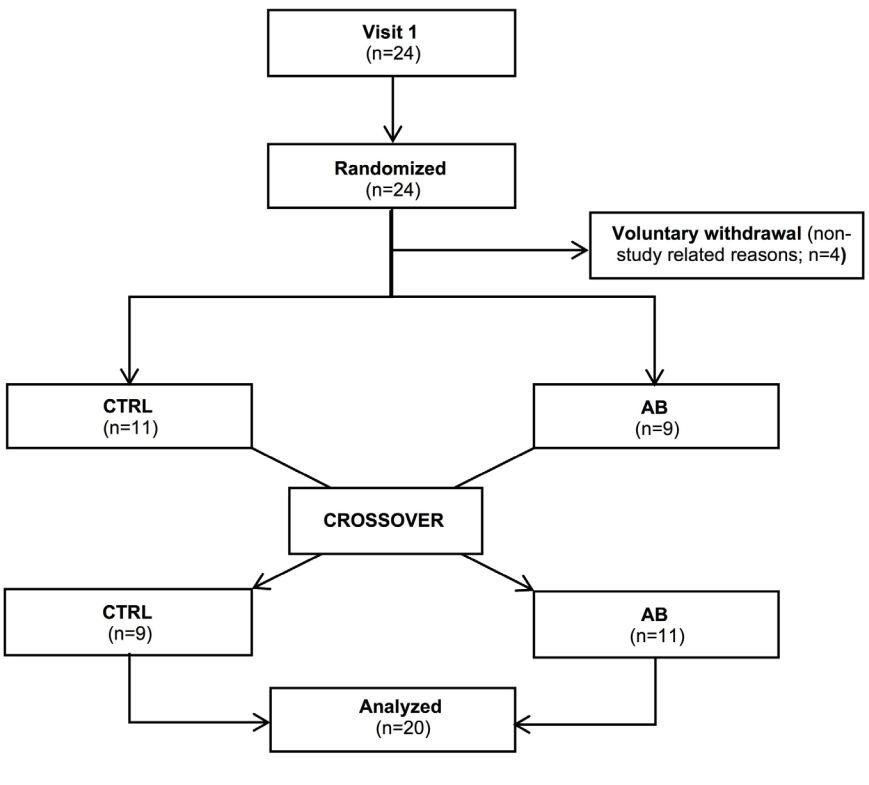

FIGURE 1 | Consort diagram of the study population.

TABLE 1 | Baseline participant characteristics (n = 20).

Parameter	Value
Male:Female, *n*	13:7
Age, years	69.8 ± 8.7
Height, cm	170.1 ± 9.9
Body mass, kg	78.4 ± 15.5
Body mass index, $kg \cdot m^{-2}$	27.1 ± 1.1
Smoking history, pack-years	56.1 ± 30.1
Post-bronchodilator spirometry	
FEV_1, L (% predicted)	1.56 ± 0.57 (60 ± 16)
FEV_1/FVC, %	46.3 ± 12.3
$FEF_{25-75\%}$, $L \cdot s^{-1}$ (% predicted)	0.57 ± 0.31 (23 ± 11)
PEF, $L \cdot s^{-1}$ (% predicted)	4.55 ± 1.98 (59 ± 18)
Breathlessness and health status	
mMRC score, 0–4	1.8 ± 0.9
BDI focal score, out of 12	6.0 ± 2.0
Oxygen cost diagram, % full scale	51 ± 12
CAT score, out of 40	17.0 ± 7.8
HADS score, out of 42	9.8 ± 4.9

Values are mean ± SD. FEV_1, forced expiratory volume in 1-s; FEV_1/FVC, FEV_1 to forced vital capacity ratio; $FEF_{25-75\%}$, forced expiratory flow between 25 and 75% of the FVC maneuver; PEF, peak expiratory flow; mMRC, modified Medical Research Council dyspnoea scale; BDI, Baseline Dyspnoea Index; CAT, COPD Assessment Test; HADS, Hospital Anxiety and Depression Scale.

TABLE 2 | Effect of abdominal binding (AB) on spirometry and plethysmography-derived pulmonary function test parameters in adults with chronic obstructive pulmonary disease (n = 20).

Parameter	Control	AB
FEV_1, L	1.53 ± 0.56	1.54 ± 0.64
FEV_1/FVC, %	45.9 ± 12.7	45.1 ± 13.1
$FEF_{25-75\%}$, $L \cdot s^{-1}$	0.56 ± 0.29	0.52 ± 0.24
PEF, $L \cdot s^{-1}$	4.23 ± 1.63	4.16 ± 1.57
TLC, L (% predicted)	7.14 ± 1.39 (117 ± 19)	6.89 ± 1.45
RV, L (% predicted)	3.37 ± 0.88 (150 ± 45)	3.35 ± 0.99
FRC, L (% predicted)	4.61 ± 1.12 (140 ± 32)	4.28 ± 1.12
IC, L (% predicted)	2.55 ± 0.70 (89 ± 15)	2.67 ± 0.75
sRaw, $cmH_2O \cdot L \cdot s^{-1}$ (% predicted)	17.1 ± 11.2 (406 ± 261)	20.6 ± 15.5

Values are mean ± SD. FEV_1, forced expiratory volume in 1-s; FEV_1/FVC, FEV_1 to forced vital capacity ratio; $FEF_{25-75\%}$, forced expiratory flow between 25 and 75% of the FVC maneuver; PEF, peak expiratory flow; TLC, total lung capacity; RV, residual volume; FRC, functional residual capacity; IC, inspiratory capacity; sRaw, specific airway resistance.

breathing pattern and IRV parameters at rest or during exercise (**Figures 3**, **4**).

Compared to CTRL, AB had no significant effect on maximal voluntary EMGdi,rms (AB, 162 ± 10 μV vs. CTRL, 160 ± 10 μV; p = 0.737). Peak inspiratory Pes values recorded during serial IC maneuvers did not change significantly from rest (AB, −24.6 ± 2.1 cmH_2O vs. CTRL, −24.9 ± 1.5 cmH_2O; p = 0.847) and throughout exercise (e.g., AB, −23.1 ± 1.4 cmH_2O vs. CTRL, −22.8 ± 1.8 cmH_2O at end-exercise; p = 0.816). Peak inspiratory Pdi values recorded during serial IC maneuvers performed at rest and throughout exercise were significantly increased by 4.4–8.3 cmH_2O (10–22%) under AB vs. CTRL conditions [e.g., AB, 50.9 ± 2.2 cmH_2O vs. CTRL, 46.4 ± 2.5 cmH_2O at rest (p = 0.014); and AB, 46.5 ± 2.2 cmH_2O vs. CTRL, 38.3 ± 2.2 cmH_2O at end-exercise (p = 0.001)].

EMGdi,rms (**Figure 5A**) and Pes (**Figure 5C**) responses during exercise were significantly different under AB vs. CTRL conditions. Peak tidal inspiratory Pga and peak tidal expiratory Pga were consistently higher at rest and during exercise with vs. without AB (**Figure 5E**). Similarly, peak tidal inspiratory Pdi and Pdi,tidal were significantly higher at rest and during exercise under AB vs. CTRL conditions (**Figure 5B**). Finally, enhanced neuromuscular efficiency of the diaphragm with vs. without AB was evidenced by the consistently higher Pdi,tidal at any given EMGdi,rms during exercise (**Figure 5D**). Indeed, the quotient of Pdi,tidal to EMGdi,rms increased by an average of ∼25% at each measurement time during exercise under AB vs. CTRL conditions (**Figure 5F**).

Compared to CTRL, AB had no effect on the locus of symptom-limitation (Breathlessness: AB, n = 7 vs. CTR, n = 6; Leg discomfort: AB, n = 6 vs. CTRL, n = 6; and Combination of breathlessness and leg discomfort: AB, n = 7 vs. CTRL, n = 7). The relative contributions of breathlessness (AB, 44 ± 7% vs. CTRL, 47 ± 8%; p = 0.731) and leg discomfort (AB, 48 ± 7% vs. CTRL, 52 ± 8%; p = 0.531) to exercise cessation were not different under AB vs. CTRL conditions. Similarly, the selection frequency

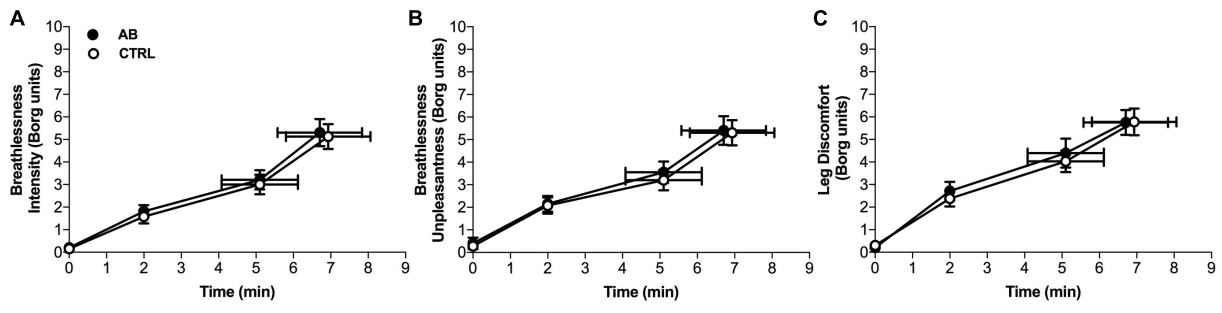

FIGURE 2 | Effects of abdominal binding (AB) vs. control (CTRL) on **(A)** breathlessness intensity, **(B)** breathlessness unpleasantness and **(C)** leg discomfort during constant-load cycle endurance exercise testing at 75% of peak incremental power output in adults with chronic obstructive pulmonary disease (n = 20). Data points are mean ± SEM values at rest, at standardized submaximal times during exercise (including isotime of 5.1 ± 1.0 min), and at peak exercise.

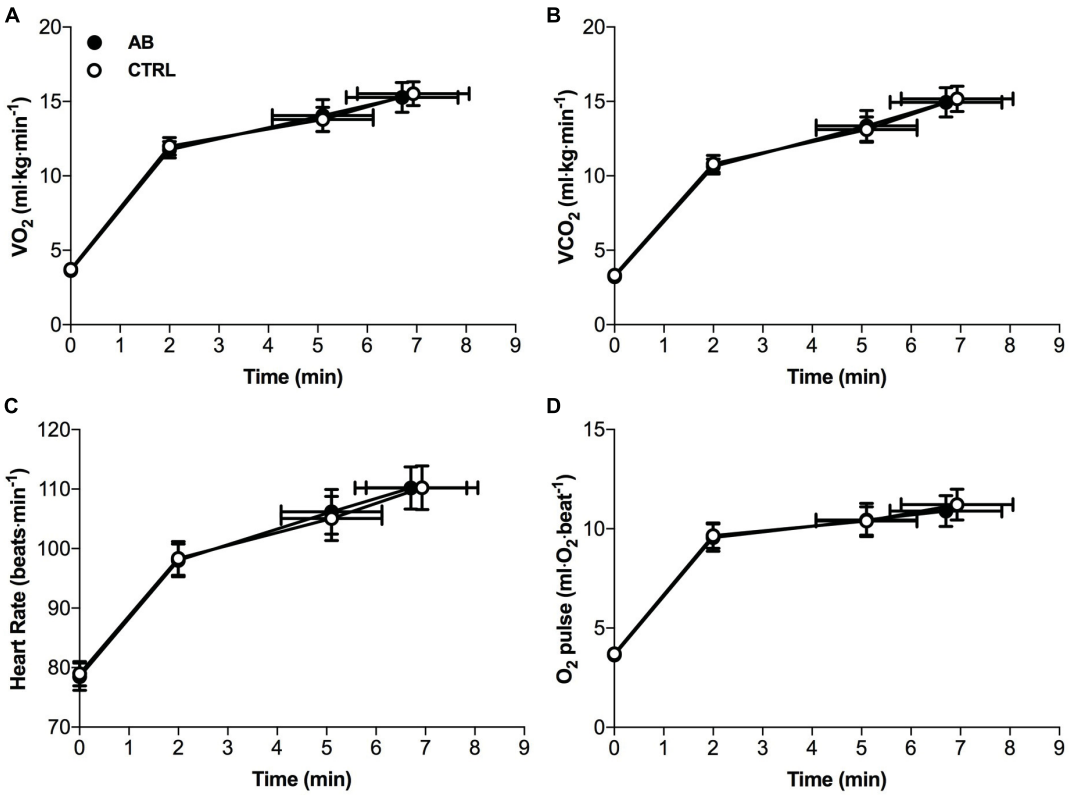

FIGURE 3 | Effects of abdominal binding (AB) vs. control (CTRL) on **(A)** the rate of oxygen consumption (VO_2), **(B)** the rate of carbon dioxide production (VCO_2), **(C)** heart rate and **(D)** oxygen pulse (O_2 pulse) during constant-load cycle endurance exercise testing at 75% of peak incremental power output in adults with chronic obstructive pulmonary disease (n = 20). Data points are mean ± SEM values at rest, at standardized submaximal times during exercise (including isotime of 5.1 ± 1.0 min), and at peak exercise.

of breathlessness descriptors at end-exercise was not significantly different in AB vs. CTRL (data not shown).

DISCUSSION

The main finding of this randomized controlled trial was that AB enhanced neuromuscular efficiency of the diaphragm during exercise but had no effect on exertional breathlessness and exercise endurance in adults with COPD.

In keeping with the results of earlier studies in health (Hussain et al., 1985; Abdallah et al., 2017), SCI (West et al., 2012, 2014) and COPD (Alexander and Kontz, 1934; Gordon, 1934; Celli et al., 1985; Dodd et al., 1985), AB significantly enhanced pressure-generating capacity of the diaphragm at rest and throughout exercise. Presumably, by increasing intra-abdominal pressure, AB functionally "strengthened" the diaphragm and enhanced its pressure-generating capacity by improving its length-tension relationship, thus enabling the diaphragm to initiate its inspiratory contraction at a more favorable length (Koo et al., 2015). Furthermore, cephalad displacement of the diaphragm with AB likely increased the area of diaphragmatic apposition to the rib cage with attendant increases in the inflationary action of the diaphragm on the lower rib cage (Koo et al., 2015). AB presumably also minimized caudal shift of the diaphragm by reducing abdominal wall compliance, thus decreasing the velocity of diaphragm shortening (Koo et al., 2015). Collectively, these mechanically advantageous adaptations are likely responsible for the ∼25% improvement in diaphragmatic neuromuscular efficiency during exercise with vs. without AB in adults with COPD.

Despite enhanced diaphragmatic neuromuscular efficiency, AB had no effect on exertional breathlessness and EET. This is in contrast to the results of LVRS studies in COPD, wherein enhanced diaphragmatic neuromuscular efficiency correlated with relief of exertional breathlessness and increased exercise capacity (Laghi et al., 1998, 2004; Lahrmann et al., 1999; Gorman et al., 2005). Enhanced diaphragmatic neuromuscular efficiency following LVRS is secondary to enhanced respiratory mechanics, as evidenced by reduced static and dynamic lung hyperinflation and improved breathing pattern (Laghi et al., 1998, 2004; Lahrmann et al., 1999; Gorman et al., 2005). By increasing the area of diaphragmatic apposition to the rib cage and improving the operating length of the diaphragm due to its cephalad displacement, these improvements in breathing mechanics following LVRS effectively decrease the load on the diaphragm, increase diaphragm pressure-generating capacity, and reduce the level of diaphragm activation needed to support

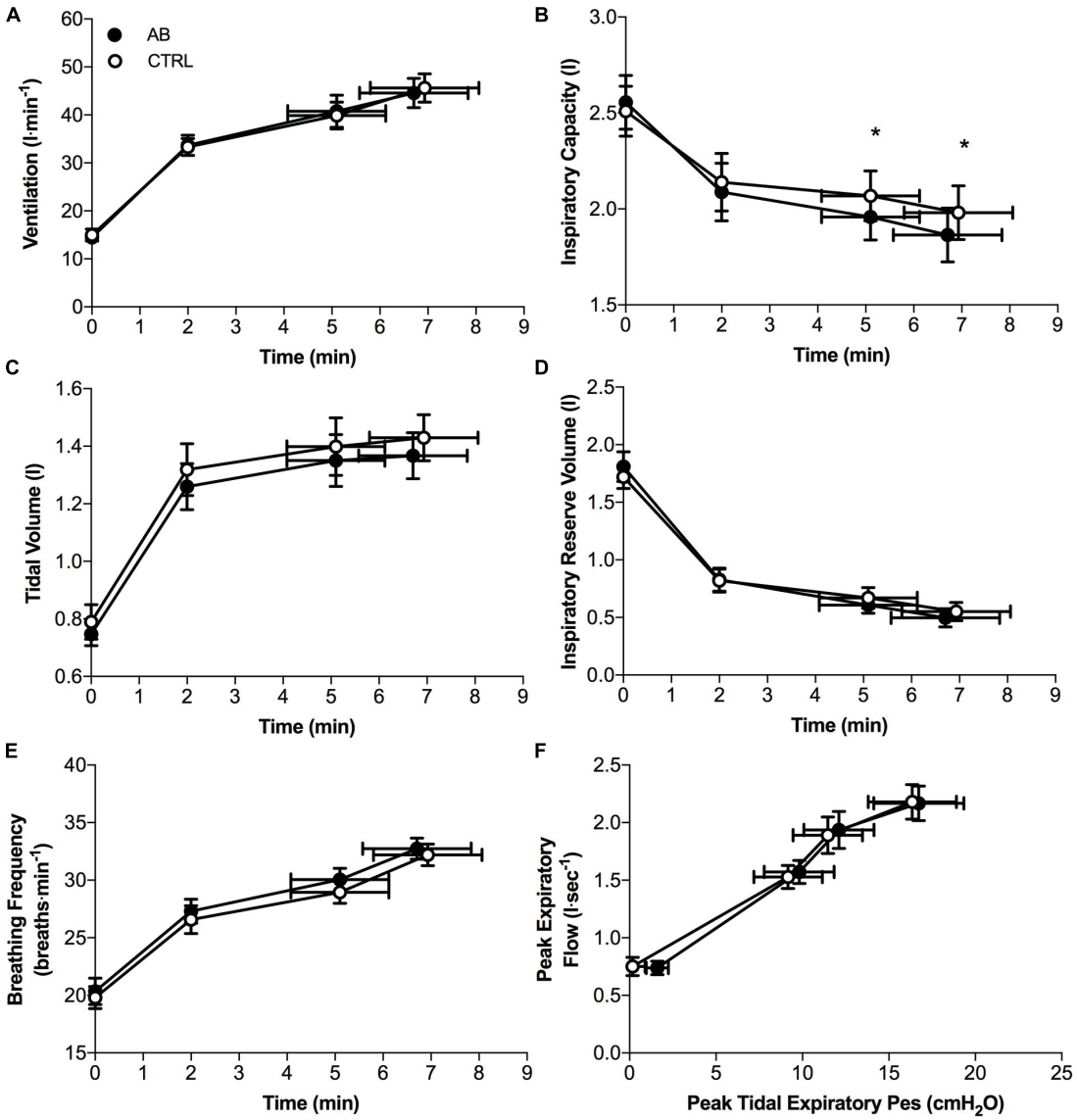

FIGURE 4 | Effects of abdominal binding (AB) vs. control (CTRL) on **(A)** ventilation, **(B)** inspiratory capacity, **(C)** tidal volume, **(D)** inspiratory reserve volume, **(E)** breathing frequency, and **(F)** peak expiratory flow during constant-load cycle endurance exercise testing at 75% of peak incremental power output in adults with chronic obstructive pulmonary disease (n = 20). Pes, esophageal pressure. Data points are mean ± SEM values at rest, at standardized submaximal times during exercise (including isotime of 5.1 ± 1.0 min), and at peak exercise. *$p < 0.05$ vs. CTRL.

a given level of ventilation (Laghi et al., 1998; Lahrmann et al., 1999; Laghi and Tobin, 2003; Gorman et al., 2005). Therefore, in contrast to AB, enhanced diaphragmatic neuromuscular efficiency following LVRS is due to the combination of increased diaphragm pressure-generating capacity and reduced inspiratory neural drive. Consequently, in the absence of improvements in expiratory flow-generating capacity, static and dynamic breathing mechanics, breathing pattern and EMGdi,rms, isolated and acute improvements in diaphragmatic neuromuscular efficiency during exercise with vs. without AB did not translate into relief of exertional breathlessness and/or improved exercise tolerance in our participants with COPD.

Our findings substantiate the mechanistic role of pathophysiological abnormalities in breathing mechanics and inspiratory neural drive (and deemphasize the mechanistic role of diaphragmatic neuromechanical inefficiency) to the etiology of exertional breathlessness and exercise intolerance in COPD. That is, despite improving pressure-generating capacity and neuromuscular efficiency of the diaphragm, AB had no effect on the inter-relationships between exercise-induced changes in ratings of perceived breathlessness, IRV, breathing pattern and EMGdi,rms. Our findings are consistent with those of Ciavaglia et al. (2014) and Faisal et al. (2016) who, respectively, reported that differences in the activity and recruitment of the diaphragm,

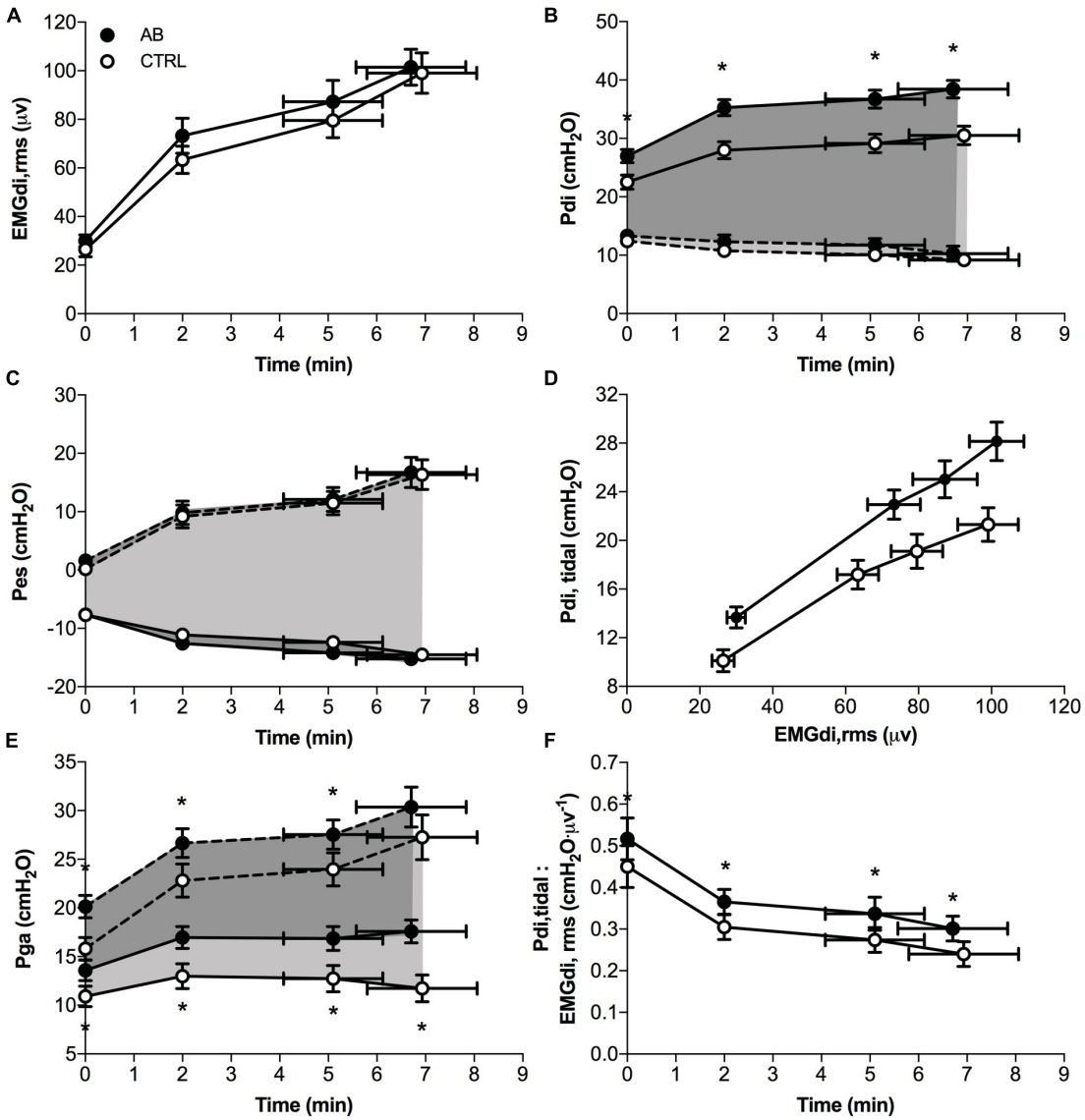

FIGURE 5 | Effects of abdominal binding (AB) vs. control (CTRL) on **(A)** the root mean square of the crural diaphragm electromyogram (EMGdi,rms), **(B)** transdiaphragmatic pressure (Pdi), **(C)** esophageal pressure (Pes), **(D)** tidal Pdi swing (Pdi,tidal) vs. EMGdi,rms, **(E)** gastric pressure (Pga) and **(F)** the quotient of Pdi,tidal to EMGdi,rms (an index of diaphragmatic neuromuscular efficiency) responses during constant-load cycle endurance exercise testing at 75% of peak incremental power output in adults with chronic obstructive pulmonary disease (n = 20). Dashed lines in panels **B,C,E** denote peak tidal expiratory Pdi, Pes, and Pga, respectively. Data points are mean ± SEM values at rest, at standardized submaximal times during exercise (including isotime of 5.1 ± 1.0 min), and at peak exercise. *$p < 0.05$ vs. CTRL.

accessory inspiratory muscles, and expiratory muscles during walking vs. cycling in obese adults with COPD and during symptom-limited incremental cycle CPET in adults with COPD vs. interstitial lung disease did not influence the relationship between exercise-induced changes in ratings of perceived breathlessness and each of the tidal volume-to-IC ratio (the inverse of IRV), breathing pattern and EMGdi,rms. Collectively, the results add to a growing body of evidence emphasizing the importance of increased inspiratory neural drive in the pathogenesis of exertional breathlessness in COPD (Guenette et al., 2014; Jolley et al., 2015; Elbehairy et al., 2016; Langer et al., 2018; O'Donnell et al., 2018), while simultaneously questioning the role of alterations in the activity of mechanosensitive afferents (i.e., Golgi tendon organs and muscle spindles) emanating from the diaphragm as well as from the chest wall and abdominal muscles in the perception of activity-related breathlessness in COPD.

Compared to CTRL, AB was associated with modest but significant decreases in IC at isotime and peak exercise by ∼110 mL, which may have offset the potentially beneficial effects of enhanced diaphragmatic neuromuscular efficiency on exertional breathlessness and EET. However, this is unlikely,

particularly in view of the results of Guenette et al. (2012) who demonstrated that the perception of breathlessness during symptom-limited constant-load CPET in adults with COPD is associated with progressive mechanical constraints on tidal volume expansion as IRV approaches its minimal value, independent of the behavior of dynamic IC. In as much as AB did not affect the behavior of dynamic IRV during exercise, we contend that the small and isolated decreases in IC during exercise with vs. without AB were unlikely to offset the potentially beneficial effects of enhanced diaphragmatic neuromuscular efficiency on exertional breathlessness and EET.

Methodological Considerations

We evaluated the effects of AB sufficient to increase intra-abdominal pressures by 6.7 ± 0.3 cmH_2O on the inter-relationships between diaphragmatic neuromuscular efficiency, exertional breathlessness and exercise endurance in adults with COPD. While this level of abdominal compression effectively enhanced diaphragmatic neuromuscular efficiency in the present study as well as in our earlier AB study of healthy younger men (Abdallah et al., 2017), we cannot rule out the possibility that different degrees of abdominal compression may yield different results on diaphragmatic neuromuscular efficiency, exertional breathlessness, and exercise capacity. While the observed changes in diaphragm pressure-generating capacity for a given level of diaphragm electrical activation with AB are consistent with improved length-tension relationship of the diaphragm due to its ascent to a more mechanically advantageous position, we cannot rule out the possibility that cephalad displacement of the diaphragm with AB increased pressure-generating capacity of the diaphragm by decreasing its radius of curvature, even without a change in force generation. Without radiographic evidence of cephalad displacement of the diaphragm with vs. without AB, we can only speculate on the determinants of improved diaphragm pressure-generating capacity and enhanced diaphragmatic neuromuscular efficiency with AB in our participants with COPD. We cannot comment on the effects of AB on cardiac function since measurements of stroke volume and cardiac output were not obtained; however, we have previously demonstrated that AB sufficient to increase intra-abdominal pressures by 6.6 ± 0.6 cmH_2O had no demonstrable effect on stroke volume and cardiac output responses during constant-load CPET in healthy younger men (Abdallah et al., 2017). As the experimental conditions of this study could not be blinded to the participants and investigators, we cannot rule out the possibility that participant and/or investigator bias may have influenced our results.

CONCLUSION

In the absence of improved static and dynamic lung function, expiratory flow-generating capacity, ventilation, breathing pattern, and inspiratory reserve drive, isolated and acute improvements in diaphragmatic neuromuscular efficiency during exercise with AB were not associated with relief of exertional breathlessness and/or improved exercise endurance in adults with COPD.

AUTHOR CONTRIBUTIONS

SA, BS, JB, and DJ contributed to the conception of the study, and the collection, analysis and interpretation of data. CW-M contributed to data collection and analysis. PL contributed to data analysis. SA and DJ wrote the manuscript with critical input from all authors. All authors read and approved the final version of the manuscript.

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REFERENCES

Abdallah, S. J., Chan, D. S., Glicksman, R., Mendonca, C. T., Luo, Y., Bourbeau, J., et al. (2017). Abdominal binding improves neuromuscular efficiency of the human diaphragm during exercise. *Front. Physiol.* 8:345. doi: 10.3389/fphys.2017.00345

Alexander, H., and Kontz, W. (1934). Symptomatic relief of emphysema by an abdominal belt. *Am. J. Med. Sci.* 187, 687–691. doi: 10.1097/00000441-193405000-00015

Bestall, J. C., Paul, E. A., Garrod, R., Garnham, R., Jones, P. W., and Wedzicha, J. A. (1999). Usefulness of the Medical Research Council (MRC) dyspnoea scale as a measure of disability in patients with chronic obstructive pulmonary disease. *Thorax* 54, 581–586. doi: 10.1136/thx.54.7.581

Borg, G. A. (1982). Psychophysical bases of perceived exertion. *Med. Sci. Sports Exerc.* 14, 377–381. doi: 10.1249/00005768-198205000-00012

Briscoe, W. A., and Dubois, A. B. (1958). The relationship between airway resistance, airway conductance and lung volume in subjects of different age and body size. *J. Clin. Invest.* 37, 1279–1285. doi: 10.1172/JCI103715

Burrows, B., Kasik, J. E., Niden, A. H., and Barclay, W. R. (1961). Clinical usefulness of the single-breath pulmonucy diffusing capacity test. *Am. Rev. Respir. Dis.* 84, 789–806.

Cassart, M., Pettiaux, N., Gevenois, P. A., Paiva, M., and Estenne, M. (1997). Effect of chronic hyperinflation on diaphragm length and surface area. *Am. J. Respir. Crit. Care Med.* 156, 504–508. doi: 10.1164/ajrccm.156.2.9612089

Celli, B. R., Rassulo, J., Berman, J. S., and Make, B. (1985). Respiratory consequences of abdominal hernia in a patient with severe chronic obstructive pulmonary disease. *Am. Rev. Respir. Dis.* 131, 178–180.

Ciavaglia, C. E., Guenette, J. A., Langer, D., Webb, K. A., Alberto Neder, J., and O'Donnell, D. E. (2014). Differences in respiratory muscle activity during cycling and walking do not influence dyspnea perception in obese patients with COPD. *J. Appl. Physiol.* 117, 1292–1301. doi: 10.1152/japplphysiol.00502.2014

Crapo, R. O., Morris, A. H., and Gardner, R. M. (1981). Reference spirometric values using techniques and equipment that meet ats recommendations. *Am. Rev. Respir. Dis.* 123, 659–664.

Dodd, D. S., Brancatisano, T. P., and Engel, L. A. (1985). Effect of abdominal strapping on chest wall mechanics during exercise in patients with severe chronic air-flow obstruction. *Am. Rev. Respir. Dis.* 131, 816–821.

Elbehairy, A. F., Guenette, J. A., Faisal, A., Ciavaglia, C. E., Webb, K. A., Jensen, D., et al. (2016). Mechanisms of exertional dyspnoea in symptomatic smokers without COPD. *Eur. Respir. J.* 48, 694–705. doi: 10.1183/13993003.00077-2016

Faisal, A., Alghamdi, B. J., Ciavaglia, C. E., Elbehairy, A. F., Webb, K. A., Ora, J., et al. (2016). Common mechanisms of dyspnea in chronic interstitial and obstructive lung disorders. *Am. J. Respir. Crit. Care Med.* 193, 299–309. doi: 10.1164/rccm.201504-0841OC

Faul, F., Erdfelder, E., Buchner, A., and Lang, A. G. (2009). Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behav. Res. Methods* 41, 1149–1160. doi: 10.3758/BRM.41.4.1149

Finucane, K. E., and Singh, B. (2012). Diaphragm efficiency estimated as power output relative to activation in chronic obstructive pulmonary disease. *J. Appl. Physiol.* 113, 1567–1575. doi: 10.1152/japplphysiol.01453.2011

Gordon, B. (1934). The mechanism and use of abdominal supports and the treatment of pulmonary diseases. *Am. J. Med. Sci.* 187, 692–699. doi: 10.1097/00000441-193405000-00016

Gorman, R. B., Mckenzie, D. K., Butler, J. E., Tolman, J. F., and Gandevia, S. C. (2005). Diaphragm length and neural drive after lung volume reduction surgery. *Am. J. Respir. Crit. Care Med.* 172, 1259–1266. doi: 10.1164/rccm.200412-1695OC

Guenette, J. A., Chin, R. C., Cheng, S., Dominelli, P. B., Raghavan, N., Webb, K. A., et al. (2014). Mechanisms of exercise intolerance in global initiative for chronic obstructive lung disease grade 1 COPD. *Eur. Respir. J.* 44, 1177–1187. doi: 10.1183/09031936.00034714

Guenette, J. A., Chin, R. C., Cory, J. M., Webb, K. A., and O'Donnell, D. E. (2013). Inspiratory capacity during exercise: measurement. Analysis, and Interpretation. *Pulm. Med.* 2013:956081. doi: 10.1155/2013/956081

Guenette, J. A., Webb, K. A., and O'Donnell, D. E. (2012). Does dynamic hyperinflation contribute to dyspnoea during exercise in patients with COPD? *Eur. Respir. J.* 40, 322–329. doi: 10.1183/09031936.00157711

Hankinson, J. L., Odencrantz, J. R., and Fedan, K. B. (1999). Spirometric reference values from a sample of the general U.S. population. *Am. J. Respir. Crit. Care Med.* 159, 179–187. doi: 10.1164/ajrccm.159.1.9712108

Hussain, S. N., Rabinovitch, B., Macklem, P. T., and Pardy, R. L. (1985). Effects of separate rib cage and abdominal restriction on exercise performance in normal humans. *J. Appl. Physiol.* 58, 2020–2026. doi: 10.1152/jappl.1985.58.6.2020

Jensen, D., O'Donnell, D. E., Li, R., and Luo, Y. M. (2011). Effects of dead space loading on neuro-muscular and neuro-ventilatory coupling of the respiratory system during exercise in healthy adults: implications for dyspnea and exercise tolerance. *Respir. Physiol. Neurobiol.* 179, 219–226. doi: 10.1016/j.resp.2011.08.009

Jolley, C. J., Luo, Y. M., Steier, J., Rafferty, G. F., Polkey, M. I., and Moxham, J. (2015). Neural respiratory drive and breathlessness in COPD. *Eur. Respir. J.* 45, 355–364. doi: 10.1183/09031936.00063014

Jones, N. L., Makrides, L., Hitchcock, C., Chypchar, T., and Mccartney, N. (1985). Normal standards for an incremental progressive cycle ergometer test. *Am. Rev. Respir. Dis.* 131, 700–708.

Jones, P. W., Harding, G., Berry, P., Wiklund, I., Chen, W. H., and Kline Leidy, N. (2009). Development and first validation of the COPD assessment test. *Eur. Respir. J.* 34, 648–654. doi: 10.1183/09031936.00102509

Koo, P., Gartman, E. J., Sethi, J. M., and Mccool, F. D. (2015). Physiology in Medicine: physiological basis of diaphragmatic dysfunction with abdominal hernias-implications for therapy. *J. Appl. Physiol.* 118, 142–147. doi: 10.1152/japplphysiol.00276.2014

Laghi, F., Jubran, A., Topeli, A., Fahey, P. J., Garrity, E. R. Jr., and de Pinto, D. J. (2004). Effect of lung volume reduction surgery on diaphragmatic neuromechanical coupling at 2 years. *Chest* 125, 2188–2195. doi: 10.1378/chest.125.6.2188

Laghi, F., Jubran, A., Topeli, A., Fahey, P. J., Garrity, E. R. Jr., Arcidi, J. M., et al. (1998). Effect of lung volume reduction surgery on neuromechanical coupling of the diaphragm. *Am. J. Respir. Crit. Care Med.* 157, 475–483. doi: 10.1164/ajrccm.157.2.9705082

Laghi, F., and Tobin, M. J. (2003). Disorders of the respiratory muscles. *Am. J. Respir. Crit. Care Med.* 168, 10–48. doi: 10.1164/rccm.2206020

Lahrmann, H., Wild, M., Wanke, T., Tschernko, E., Wisser, W., Klepetko, W., et al. (1999). Neural drive to the diaphragm after lung volume reduction surgery. *Chest* 116, 1593–1600. doi: 10.1378/chest.116.6.1593

Langer, D., Ciavaglia, C. E., Faisal, A., Webb, K. A., Neder, J. A., Gosselink, R., et al. (2018). Inspiratory muscle training reduces diaphragm activation and dyspnea during exercise in COPD. *J. Appl. Physiol.* 125, 381–392. doi: 10.1152/japplphysiol.01078.2017

Macintyre, N., Crapo, R. O., Viegi, G., Johnson, D. C., Van Der Grinten, C. P., Brusasco, V., et al. (2005). Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur. Respir. J.* 26, 720–735. doi: 10.1183/09031936.05.00034905

Mahler, D. A., Weinberg, D. H., Wells, C. K., and Feinstein, A. R. (1984). The measurement of dyspnea. Contents, interobserver agreement, and physiologic correlates of two new clinical indexes. *Chest* 85, 751–758. doi: 10.1378/chest.85.6.751

McGavin, C. R., Artvinli, M., Naoe, H., and Mchardy, G. J. (1978). Dyspnoea, disability, and distance walked: comparison of estimates of exercise performance in respiratory disease. *Br. Med. J.* 2, 241–243. doi: 10.1136/bmj.2.6132.241

Mendonca, C. T., Schaeffer, M. R., Riley, P., and Jensen, D. (2014). Physiological mechanisms of dyspnea during exercise with external thoracic restriction: role of increased neural respiratory drive. *J. Appl. Physiol.* 116, 570–581. doi: 10.1152/japplphysiol.00950.2013

Miller, M. R., Crapo, R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., et al. (2005a). General considerations for lung function testing. *Eur. Respir. J.* 26, 153–161. doi: 10.1183/09031936.05.00034505

Miller, M. R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., et al. (2005b). Standardisation of spirometry. *Eur. Respir. J.* 26, 319–338. doi: 10.1183/09031936.05.00034805

O'Donnell, D. E., Elbehairy, A. F., Faisal, A., Neder, J. A., Webb, K. A., and Canadian Respiratory Research (Crrn). (2018). Sensory-mechanical effects of a dual bronchodilator and its anticholinergic component in COPD. *Respir. Physiol. Neurobiol.* 247, 116–125. doi: 10.1016/j.resp.2017.10.001

O'Donnell, D. E., Hong, H. H., and Webb, K. A. (2000). Respiratory sensation during chest wall restriction and dead space loading in exercising men. *J. Appl. Physiol.* 88, 1859–1869. doi: 10.1152/jappl.2000.88.5.1859

Puente-Maestu, L., Villar, F., De Miguel, J., Stringer, W. W., Sanz, P., Sanz, M. L., et al. (2009). Clinical relevance of constant power exercise duration changes in COPD. *Eur. Respir. J.* 34, 340–345. doi: 10.1183/09031936.00078308

Ries, A. L. (2005). Minimally clinically important difference for the UCSD shortness of breath questionnaire, borg scale, and visual analog scale. *COPD* 2, 105–110. doi: 10.1081/COPD-200050655

Sinderby, C., Spahija, J., Beck, J., Kaminski, D., Yan, S., Comtois, N., et al. (2001). Diaphragm activation during exercise in chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 163, 1637–1641. doi: 10.1164/ajrccm.163.7.2007033

Vogelmeier, C. F., Criner, G. J., Martinez, F. J., Anzueto, A., Barnes, P. J., Bourbeau, J., et al. (2017). Global strategy for the diagnosis, management, and

prevention of chronic obstructive lung disease 2017 report. GOLD executive summary. *Am. J. Respir. Crit. Care Med.* 195, 557–582. doi: 10.1164/rccm.201701-0218PP

Wanger, J., Clausen, J. L., Coates, A., Pedersen, O. F., Brusasco, V., Burgos, F., et al. (2005). Standardisation of the measurement of lung volumes. *Eur. Respir. J.* 26, 511–522. doi: 10.1183/09031936.05.00035005

West, C. R., Campbell, I. G., Shave, R. E., and Romer, L. M. (2012). Effects of abdominal binding on cardiorespiratory function in cervical spinal cord injury. *Respir. Physiol. Neurobiol.* 180, 275–282. doi: 10.1016/j.resp.2011.12.003

West, C. R., Goosey-Tolfrey, V. L., Campbell, I. G., and Romer, L. M. (2014). Effect of abdominal binding on respiratory mechanics during exercise in athletes with cervical spinal cord injury. *J. Appl. Physiol.* 117, 36–45. doi: 10.1152/japplphysiol.00218.2014

Zigmond, A. S., and Snaith, R. P. (1983). The hospital anxiety and depression scale. *Acta Psychiatr. Scand.* 67, 361–370. doi: 10.1111/j.1600-0447.1983.tb09716.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix III

Effect of morphine on breathlessness and exercise endurance in advanced COPD: a randomised crossover trial

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ABSTRACT The objective of the present study was to evaluate the effect of morphine on exertional breathlessness and exercise endurance in advanced chronic obstructive pulmonary disease (COPD).

In a randomised crossover trial, we compared the acute effect of immediate-release oral morphine *versus* placebo on physiological and perceptual responses during constant-load cardiopulmonary cycle exercise testing (CPET) in 20 adults with advanced COPD and chronic breathlessness syndrome.

Compared with placebo, morphine reduced exertional breathlessness at isotime by 1.2±0.4 Borg units and increased exercise endurance time by 2.5±0.9 min (both $p \leqslant 0.014$). During exercise at isotime, morphine decreased ventilation by 1.3±0.5 L·min^{-1} and breathing frequency by 2.0±0.9 breaths·min^{-1} (both $p \leqslant 0.041$). Compared with placebo, morphine decreased exertional breathlessness at isotime by ⩾1 Borg unit in 11 participants (responders) and by <1 Borg unit in nine participants (non-responders). Baseline participant characteristics, including pulmonary function and cardiorespiratory fitness, were similar between responders and non-responders. A higher percentage of responders *versus* non-responders stopped incremental CPET due to intolerable breathlessness: 82 *versus* 33% (p=0.028).

Immediate-release oral morphine improved exertional breathlessness and exercise endurance in some, but not all, adults with advanced COPD. The locus of symptom-limitation on laboratory-based CPET may help to identify patients most likely to benefit from morphine.

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Introduction

Breathlessness and exercise intolerance are independently associated with increased morbidity and mortality in chronic obstructive pulmonary disease (COPD) [1]. Despite optimal treatment of their underlying disease with bronchodilators, corticosteroids and/or phosphodiesterase inhibitors, 46–91% of patients with severe-to-very-severe COPD suffer from chronic and disabling breathlessness at rest and on minimal exertion [2–4]; *i.e.*, chronic breathlessness syndrome [5]. Therefore, symptom-specific therapies that alleviate refractory breathlessness and improve exercise capacity are needed to enhance health outcomes in advanced COPD.

A systematic review and meta-analysis by Ekstrom *et al.* [6] recently concluded that systemic low-dose opioids are safe and effective for decreasing refractory breathlessness but do not improve exercise capacity in advanced COPD. Importantly, published studies have provided little insight into the mechanism(s) mediating opioid-induced relief of breathlessness in COPD, although reductions in ventilation (V'_E) *via* reduced central neural respiratory drive and/or a blunted central perception of breathlessness have been proposed [7–10]. A better understanding of the physiological mechanism(s) of action of systemic opioids on breathlessness is essential to optimising symptom control in advanced COPD.

Although Canadian, American, European and international clinical practice guidelines support the use of systemic low-dose opioids for decreasing refractory breathlessness in advanced COPD [11–14], many physicians do not prescribe opioids for breathlessness [15] due to fear of adverse side-effects (*e.g.* respiratory depression), insufficient scientific evidence supporting a benefit of opioids on refractory breathlessness and an inability to predict which patients will respond to opioids [16, 17].

The primary objective of this randomised crossover trial was to evaluate the acute effect of oral morphine on exertional breathlessness and exercise endurance in advanced COPD. Our secondary objective was to elucidate the physiological mechanism(s) of action of oral morphine on exertional breathlessness and exercise endurance in advanced COPD. We compared detailed physiological and perceptual responses to cycle endurance exercise testing after single-dose administration of immediate-release oral morphine and placebo in participants with advanced COPD and chronic breathlessness syndrome. We hypothesised that oral morphine *versus* placebo would be associated with clinically meaningful improvements in exertional breathlessness and exercise endurance, independent of opioid-related side effects, CO_2 retention and concurrent improvements in the physiological response to exercise.

Materials and methods

Participants

Participants included men and women aged ⩾40 years with Global Initiative for Obstructive Lung Disease stage 3 or 4 COPD [14] and chronic breathlessness syndrome [5], defined as a modified Medical Research Council dyspnoea score of ⩾3 [18], a baseline dyspnoea index focal score of ⩽6 [19] and/or an oxygen cost diagram rating of ⩽50% full scale [20] despite optimal treatment with bronchodilators, corticosteroids and/or phosphodiesterase inhibitors [14]. See the online data supplement for more information on eligibility criteria.

Study design

This single-centre, randomised, double-blind, placebo-controlled, crossover trial consisted of two intervention periods separated by a washout period of ⩾48 h (figure 1). Participants were randomised in a 1:1 ratio to receive immediate-release oral morphine sulphate (0.1 mg·kg^{-1} body mass to a maximum dose of 10 mg (Statex; Paladin Labs Inc., Montreal, QC, Canada)) or diluted simple syrup (placebo) prepared in 250 mL of orange juice. The study received ethical approval from Health Canada and the Research Institute of the McGill University Health Centre.

After providing written informed consent, participants completed a screening/familiarisation visit followed by two randomly assigned treatment visits. Visit 1 included: evaluation of participant-reported breathlessness [18–20], health status [21] and anxiety/depression [22]; measurement of arterialised capillary carbon dioxide tension (P_{acCO_2}) at rest; post-bronchodilator (400 µg salbutamol) pulmonary function testing; and a symptom-limited incremental (5-W·min^{-1}) cardiopulmonary cycle exercise test (CPET) to determine peak power output (PPO). At the start of visits 2 and 3, participants inhaled 400 µg of salbutamol to standardise the time since last bronchodilator administration. 15 min thereafter, participants completed the opioid-related symptom distress scale (ORSDS) [23, 24] followed by blood sampling for measurement of P_{acCO_2} and of plasma concentrations of morphine ([MOR]), morphine-3-glucuronide ([M3G]) and morphine-6-glucuronide ([M6G]). Participants were then administered oral morphine or placebo. 30 min thereafter, participants completed the ORSDS and blood for measurement of P_{acCO_2}, [MOR], [M3G] and [M6G] was collected. Participants then completed a symptom-limited constant-load cycle CPET at 75% PPO.

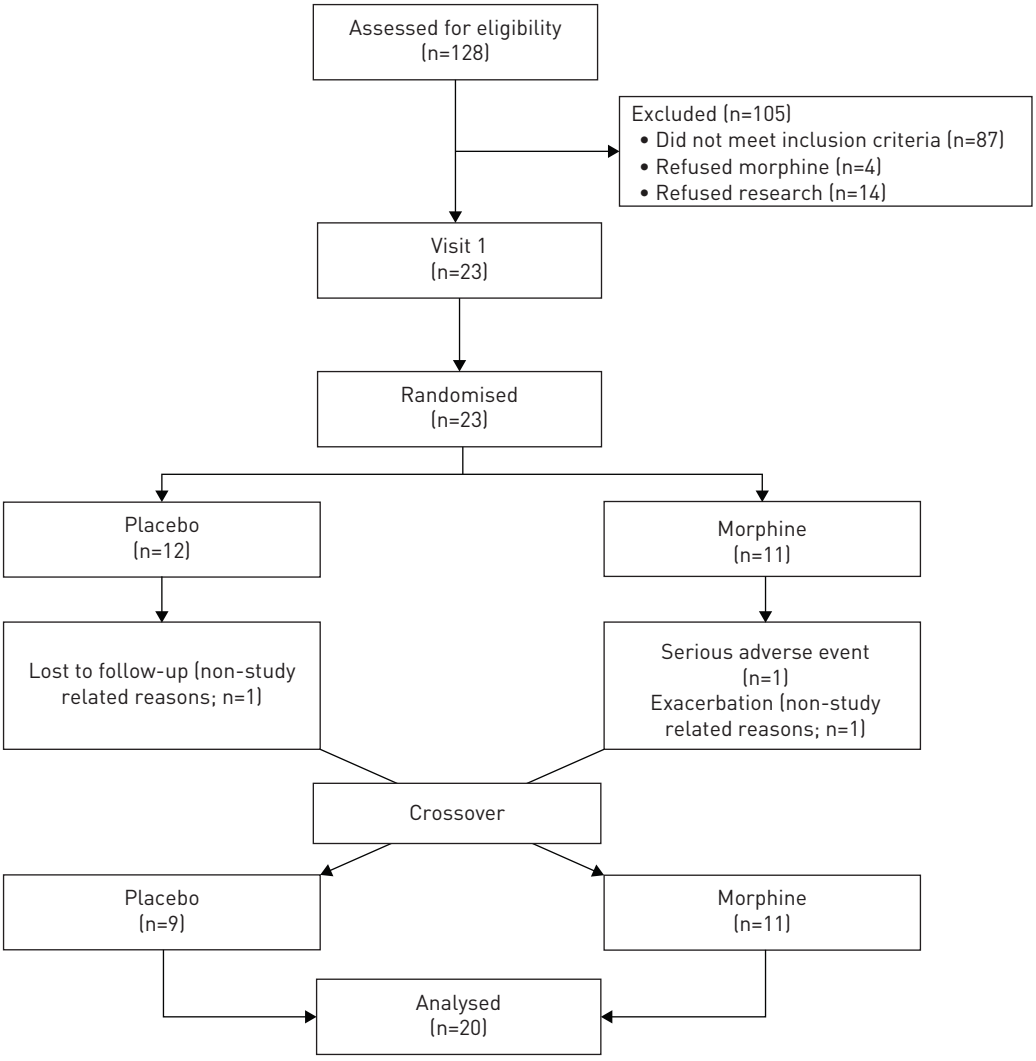

FIGURE 1 CONSORT diagram of the study population.

Procedures

Spirometry, plethysmography and single-breath diffusion capacity of the lung for carbon monoxide were performed using automated equipment and recommended techniques [25–28]. Exercise tests were conducted on an electronically braked cycle ergometer using a computerised CPET system. Cardiac, metabolic, breathing pattern and gas exchange parameters were collected and analysed as previously described [29]. Inspiratory capacity manoeuvres were performed at rest, every 2 min during CPET and at end-exercise [30]. Using Borg's modified 0–10 category ratio scale [31], participants rated the intensity and unpleasantness of their breathlessness, as well as the intensity of their leg discomfort at rest, every 2 min during CPET, and at end-exercise [29, 32]. In a subgroup of seven consenting participants, breath-by-breath measures of the crural diaphragm electromyogram (EMGdi) were recorded from a multi-pair oesophageal electrode catheter and analysed using published methods [32, 33]. Participants verbalised their main reason(s) for stopping exercise, quantified the percentage contribution of breathlessness and leg discomfort to exercise cessation, and identified qualitative phrases that best described their breathlessness at end-exercise [34]. Each participant's blinded treatment preference was assessed at the end of visit 3. See the online data supplement for more information on experimental procedures.

Outcome variables

The primary outcome was the post-dose difference in breathlessness intensity ratings during exercise at isotime, defined as the highest equivalent 2-min interval of exercise completed by a given participant during each of the constant-load CPETs. The co-primary outcome was the post-dose difference in exercise endurance time (EET), defined as the duration of loaded pedalling during constant-load CPET. See online data supplement for details on secondary outcome variables.

Statistical analyses

Using a two-tailed paired subject formula with α=0.05, β=0.90 and an expected effect size of 0.80 [35], we estimated that 20 participants were needed to detect a minimal clinically important difference (MCID) of 1 Borg unit in breathlessness intensity during exercise at isotime [36] and of 101 s in EET [37] after taking morphine *versus* placebo.

All participants who completed both morphine and placebo arms of the trial were included in the analysis. Linear mixed-models regression with random intercepts was used to analyse post-dose differences in EET as well as in all physiological and perceptual responses to constant-load CPET, accounting for period and sequence effects. A secondary analysis was conducted after examination of the data showed that 11 participants had a morphine-induced decrease in breathlessness intensity at isotime by the MCID of $\geqslant$1 Borg unit (responders) compared with the remaining nine participants who did not (non-responders). Statistical significance was set at $p<0.05$. See the online data supplement for additional information on the statistical analyses performed.

Results

23 out of 128 participants assessed for eligibility were randomised (figure 1). Two of these 23 participants were lost during follow-up for non-study-related reasons, while one was lost following a serious adverse event. Baseline characteristics of the 20 participants who completed the trial are presented in tables 1 and 2.

TABLE 1 Baseline participant characteristics

Parameter	Value
Male:female n	15:5
Age years	63.6±7.1
Height cm	169.0±8.3
Body mass kg	71.6±14.4
Body mass index kg·m^{-2}	25.3±5.2
P_{acCO_2} mmHg (range)[#]	37.7±3.4 (32–45)
Smoking history pack-years	59.3±22.8
GOLD stage 3:4 n	12:8
Post-bronchodilator pulmonary function	
FEV_1 L (% predicted)	0.93±0.21 (35±9)
FEV_1/FVC %	36.3±10.3
TLC L (% predicted)	7.79±1.70 (126±17)
RV L (% predicted)	4.70±1.40 (217±57)
FRC L (% predicted)	5.68±1.57 (174±38)
IC L (% predicted)	2.08±0.61(72±17)
D_{LCO} mL·min·mmHg^{-1} (% predicted)[¶]	12.9±6.2 (52±30)
sR_{aw} cmH_2O·L·s^{-1} (% predicted)[#]	46.4 ±35.8 (949±821)
Breathlessness and health status	
mMRC score 0–4	3.0±0.6
BDI focal score out of 12	3.9±1.9
Oxygen cost diagram % full scale	39±16
CAT score out of 40	21.3±6.4
CAT breathlessness item out of 5	4.1±0.9
CAT activity limitation item out of 5	3.3±1.5
HADS score out of 42	12.2±6.1
COPD medication summary	
LABA+LAMA n	4
LABA+LAMA+ICS n	14
LABA+LAMA+PI n	1
LABA+LAMA+ICS+PI n	1

Data are presented as mean±SD, unless otherwise stated. [#]: n=18. [¶]: n=17. P_{acCO_2}: partial pressure of carbon dioxide in arterialised capillary blood; GOLD: Global Initiative for Obstructive Lung Disease; FEV_1: forced expiratory volume in 1 s; FEV_1/FVC, forced expiratory volume in 1s to forced vital capacity ratio; TLC: total lung capacity; RV: residual volume; FRC: functional residual capacity; IC: inspiratory capacity; D_{LCO}: diffusing capacity of the lung for carbon monoxide; sR_{aw}: specific airway resistance; mMRC: modified Medical Research Council Dyspnoea Scale; BDI: Baseline Dyspnoea Index; CAT: COPD Assessment Test; HADS: Hospital Anxiety and Depression Scale; LABA: long-acting β_2 agonist; LAMA: long-acting muscarinic antagonist; ICS: inhaled corticosteroid; PI: phosphodiesterase inhibitor.

Primary outcomes

There was no statistically significant sequence or period effect of treatment. Compared with placebo, morphine (mean±SEM dose, 7.2±3.2 mg (range: 4.4–9.2 mg)) decreased breathlessness intensity ratings at isotime by 1.2±0.4 Borg units (p=0.011) (figure 2a, d and g, table 3) and increased EET by 2.5±0.9 min (148±52 s) or 41±13% (p=0.014) (figure 2f and i, table 3).

Secondary outcomes

Perceptual responses

Compared with placebo, morphine decreased breathlessness unpleasantness ratings by 1.4±0.4 Borg units at isotime (p=0.003) (figure 2b, e and h, table 3), but had no effect on intensity ratings of leg discomfort at rest or during exercise (figure 2c, table 3). Despite differences in EET, breathlessness intensity and unpleasantness ratings were similar between treatments at end-exercise (figure 2a and b, table 3).

Compared with placebo, morphine had no effect on the locus of symptom limitation (table 3), the selection frequency of breathlessness descriptors at end-exercise (see figure E1 in the online data supplement), and the relative contributions of breathlessness (morphine, 61±8% *versus* placebo, 66±8%, p=0.260) and leg discomfort (morphine, 19±6% *versus* placebo, 23±6%, p=0.305) to exercise cessation.

Blood biochemistry

Oral morphine increased plasma [MOR], [M3G] and [M6G] (figure 3a). Morphine-induced changes in breathlessness intensity ratings at isotime and in EET were unrelated to plasma [MOR], [M3G] and [M6G] (Pearson $r \leqslant 0.43$, $p \geqslant 0.09$ for all). There was no treatment, time or treatment×time interaction effect for P_{acCO_2} (figure 3b). There was also no evidence of CO_2 retention at rest: all pre- and post-dose measurements of P_{acCO_2} were <50 mmHg (figure 3b).

Physiological responses

With the exception of a small but significant increase in the end-tidal partial pressure of CO_2 (P_{ETCO_2}) by just 0.9±0.3 mmHg (p=0.002), morphine had no effect on physiological variables at rest (figure 4, table 3). During exercise at isotime after taking morphine *versus* placebo, there were small but significant decreases

TABLE 2 Physiological and perceptual responses at the symptom-limited peak of incremental cycle exercise testing in adults with advanced chronic obstructive pulmonary disease and chronic breathlessness syndrome

Parameter	Value
Cycle exercise time min	6.5±2.7
Power output Watt (% predicted)	37.8±17.7 (27±10)
V'_{O_2} mL·kg·min^{-1} (% predicted)	12.7±2.6(53±16)
Heart rate beats·min^{-1} (% predicted)	113±22 (67±13)
Breathlessness intensity Borg units	6.1±2.3
Breathlessness unpleasantness Borg units	6.3±2.1
Leg discomfort Borg units	5.6±3.2
V'_E L·min^{-1} (% estimated MVV)	31.3±7.9 (97±17)
V_T L	1.11±0.31
f_R breaths·min^{-1}	29.2±6.9
Δ IC from rest L	−0.94±0.61
IRV L	0.29±0.20
V'_E/V'_{CO_2}	37.9±1.5
P_{ETCO_2} mmHg	36.4±5.0
S_{pO_2} %	93±3
Δ S_{pO_2} from rest %	−2.4±2.8
Reasons for stopping exercise	
Breathlessness n	12
Leg discomfort n	1
Breathlessness and leg discomfort n	7

Data are presented as mean±SD, unless otherwise stated. V'_{O_2}: rate of oxygen uptake; V'_E: minute ventilation; MVV: maximal voluntary ventilation estimated as forced expiratory volume in 1s×35; V_T: tidal volume, f_R: breathing frequency; Δ: exercise-induced change; IC: inspiratory capacity; IRV: inspiratory reserve volume; V'_E/V'_{CO_2}: ventilatory equivalent for carbon dioxide; P_{ETCO_2}: partial pressure of end-tidal carbon dioxide; S_{pO_2}: oxygen saturation by pulse oximetry.

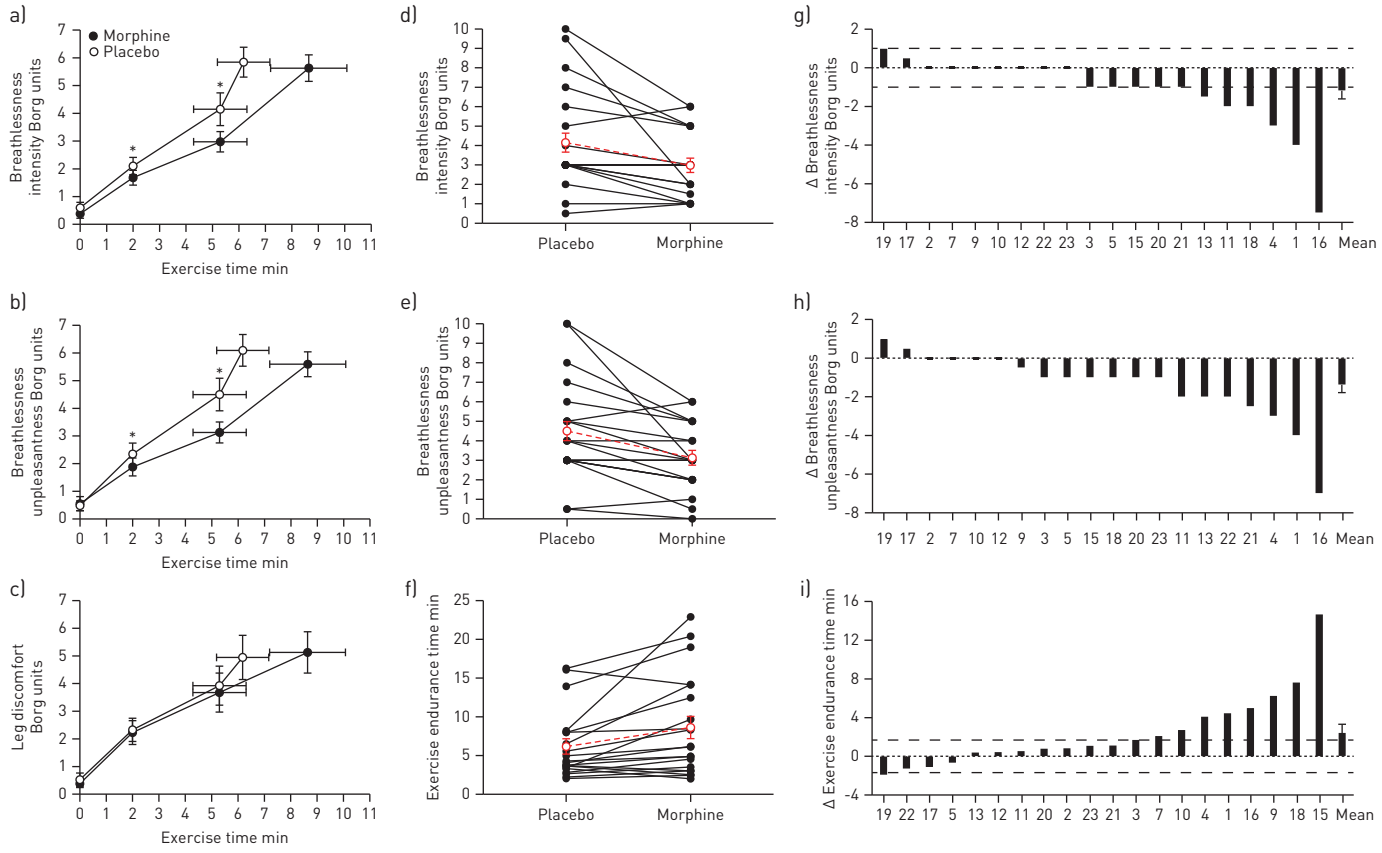

FIGURE 2 Effect of immediate-release oral morphine *versus* placebo on exertional breathlessness and exercise endurance in adults with advanced chronic obstructive pulmonary disease (COPD) and chronic breathlessness syndrome. Mean±SEM a) breathlessness intensity ratings, b) breathlessness unpleasantness ratings and c) leg discomfort ratings at rest and during constant-load cycle exercise testing at 75% of peak incremental power output. Individual participant post-dose values and post-dose differences in d and g) breathlessness intensity ratings during exercise at isotime, e and h) breathlessness unpleasantness ratings during exercise at isotime and f and i) exercise endurance time, where red symbols with dashed horizontal lines in panels d, e and f) denote mean±SEM. Dashed horizontal lines in panels g and i denote minimally clinically important difference for breathlessness intensity [36] and exercise endurance time [37]. Δ: post-dose difference (*i.e.*, morphine *minus* placebo). *: p<0.05 *versus* placebo.

in V'_E (p=0.031) and breathing frequency (f_R; p=0.041) (figure 4, table 3). At peak exercise, f_R decreased after taking morphine *versus* placebo (p=0.041) (figure 4b, table 3). Compared with placebo, morphine had no statistically significant effect on EMG_{di} or the EMG_{di}:V'_E ratio at any measurement time, although EMG_{di} was reduced by ~13% during exercise at isotime after taking morphine *versus* placebo (p=0.061) (table 3).

Opioid-related side effects and adverse events

18 out of 20 participants reported no pre- to post-dose change in ORSDS ratings of headache, nausea, difficulty concentrating, drowsiness, lightheadedness/dizziness, confusion and fatigue after taking either morphine or placebo. One participant with no pre-dose symptoms reported lightheadedness/dizziness and difficulty concentrating 30 min after taking morphine, although the severity and bothersomeness of these symptoms were mild with ratings of <20 mm on a 100-mm visual analogue scale. Another participant with no pre-dose symptoms reported nausea and drowsiness 30 min after taking morphine and placebo, respectively. In both cases, the severity and bothersomeness of these symptoms were moderate, with visual analogue scale ratings of <50 mm. One serious adverse event occurred in a woman with an unreported intolerance to opioids. This participant experienced severe abdominal pain 20 min after taking morphine, was admitted to the emergency department, treated with epinephrine, and discharged 4 h after admission.

Participant's blinded treatment preference

15 out of 20 participants reported a preference for morphine over placebo for exercise: 12 participants volunteered that their breathing was easier during exercise, while three participants volunteered that exercise was less demanding. Three out of 20 participants reported a preference for placebo because they

TABLE 3 Effect of immediate-release oral morphine (0.1 mg·kg^{-1} body mass) *versus* placebo on physiological and perceptual responses at rest, at a standardised submaximal time during constant-load cycle exercise testing (isotime), and at the symptom-limited peak of constant-load cycle exercise testing in adults with advanced COPD and chronic breathlessness syndrome

	Rest		Isotime		Peak	
	Placebo	Morphine	Placebo	Morphine	Placebo	Morphine
Cycle exercise time min			5.3±4.5	5.3±4.5	6.2±4.4	8.6±6.5*
Breathlessness intensity Borg units	0.6±0.9	0.4±0.7	4.2±2.6	3.0±1.6*	5.9±2.4	5.6±2.2
Breathlessness unpleasantness Borg units	0.5±0.8	0.6±1.2	4.5±2.6	3.1±1.7**	6.1±2.5	5.6±2.0
Leg discomfort Borg units	0.5±1.1	0.4±0.7	3.9±3.2	3.7±3.2	4.9±3.6	5.1±3.4
V'_{O_2} mL·kg·min^{-1}	4.5±1.1	4.4±1.1	11.7±2.4	11.3±2.5	12.5±2.3	12.5±2.5
V'_{CO_2} mL·kg·min^{-1}	4.3±1.1	4.1±1.1	11.1±2.6	10.8±2.7	12.0±2.5	12.1±2.5
Heart rate beats·min^{-1}	86±13	87±14	110±21	110±21	112±21	114±21
V'_E L·min^{-1}	14.6±2.3	14.0±1.9	28.9±7.4	27.6±7.5*	30.9±7.2	30.6±7.8
V_T L	0.89±0.24	0.87±0.25	1.12±0.28	1.16±0.28	1.08±0.29	1.14±0.26
f_R breaths·min^{-1}	18.0±6.2	18.0±6.2	26.9±7.2	24.9±7.3*	30.2±7.7	28.1±7.3*
V_T/T_i	0.74±0.16	0.72±0.17	1.48±0.32	1.42±0.31	1.60±0.34	1.58±0.32
T_i/T_{tot}	34.0±5.5	34.3±5.4	32.8±5.0	32.9±4.8	33.1±5.2	32.6±4.6
IC L	2.03±0.51	2.11±0.50	1.43±0.33	1.51±0.31	1.34±0.30	1.38±0.28
Δ IC from rest L			-0.61±0.37	-0.60±0.31	-0.70±0.31	-0.73±0.41
IRV L	1.15±0.43	1.25±0.49	0.31±0.21	0.35±0.19	0.26±0.19	0.25±0.20
V'_E/V'_{CO_2}	49.3±5.6	49.6±6.6	37.7±6.7	36.9±5.6	37.3±6.7	38.2±5.5
P_{ETCO_2} mmHg	32.6±2.9	33.5±3.2**	37.5±5.7	37.9±5.2	37.8±6.0	38.2±5.5
S_{pO_2} %	96±2	96±2	93±4	93±4	93±4	93±4
Gastro-oesophageal balloon-electrode catheter-derived parameters (n=7)						
$EMG_{di,rms}$ μV	59.5±37.1	47.1±26.3	134.2±50.3	117.2±50.9	161.2±63.8	149.8±65.8
$EMG_{di,rms}/V'_E$ μV·L·min^{-1}	4.3±2.3	3.5±2.0	5.2±2.9	4.8±2.7	5.6±2.9	5.4±3.2
$EMG_{di,rms\%max}$	28±11	23±9	64±12	59±17	76±15	74±17
Reasons for stopping exercise						
Breathlessness n					11	9
Leg discomfort n					2	4
Breathlessness and leg discomfort n					5	2
Other n					2	5

Data are presented as mean±SD, unless otherwise stated. V'_{O_2}: rate of oxygen uptake; V'_{CO_2}: rate of carbon dioxide production; HR: heart rate; V'_E: minute ventilation; V_T: tidal volume; f_R: breathing frequency; V_T/T_i: mean tidal inspiratory flow, where T_i represents inspiratory time; T_i/T_{tot}: inspiratory duty cycle, where T_{tot} represents total breath duration; IC: inspiratory capacity; Δ: exercise-induced change; IRV: inspiratory reserve volume; V'_E/V'_{CO_2}: ventilatory equivalent for carbon dioxide; P_{ETCO_2}: partial pressure of end-tidal carbon dioxide; S_{pO_2}: oxygen saturation by pulse oximetry; $EMG_{di,rms}$: root mean square of the crural diaphragm electromyogram; $EMG_{di,rms\%max}$: $EMG_{di,rms}$ expressed as a percentage of maximum voluntary $EMG_{di,rms}$. *: p<0.05 and **: p<0.01 *versus* placebo.

felt more prepared for the study visit (*i.e.* they received placebo at visit 3), while the remaining two participants reported no treatment preference.

Secondary analysis

Baseline characteristics were similar between responders and non-responders (see table E1 in the online data supplement), with the exception of forced expiratory volume in 1 s (FEV_1) expressed as a percentage of predicted which tended to be lower in responders *versus* non-responders (p=0.050).

Intensity and unpleasantness ratings of breathlessness were higher in responders *versus* non-responders at the symptom-limited peak of incremental CPET (intensity: 6.9±0.7 *versus* 5.1±0.8 Borg units (p=0.077); unpleasantness: 7.0±0.7 *versus* 5.5±0.5 Borg units (p=0.119)) and constant-load CPET during the placebo treatment period (intensity: 6.8±0.8 *versus* 4.7±0.6 Borg units (p=0.046); unpleasantness: 7.1±0.8 *versus* 4.9±0.6 Borg units (p=0.050)), even though PPO, EET, peak V'_E and peak rate of oxygen uptake (V'_{O_2}) were not significantly different between groups. A greater percentage of participants within the responders *versus* the non-responders subgroup identified intolerable breathlessness as their primary reason for stopping incremental CPET (82% (n=9 out of 11) *versus* 33% (n=3 out of 9), p=0.028) and constant-load CPET during the placebo treatment period (73% (n=8 out of 11) *versus* 33% (n=3 out of 9), p=0.078). The relative contribution of intolerable breathlessness to the cessation of incremental CPET (76±6 *versus* 51±11%, p=0.042) and constant-load CPET during the placebo treatment period (76±9 *versus* 52±13%, p=0.139) was also higher in responders *versus* non-responders.

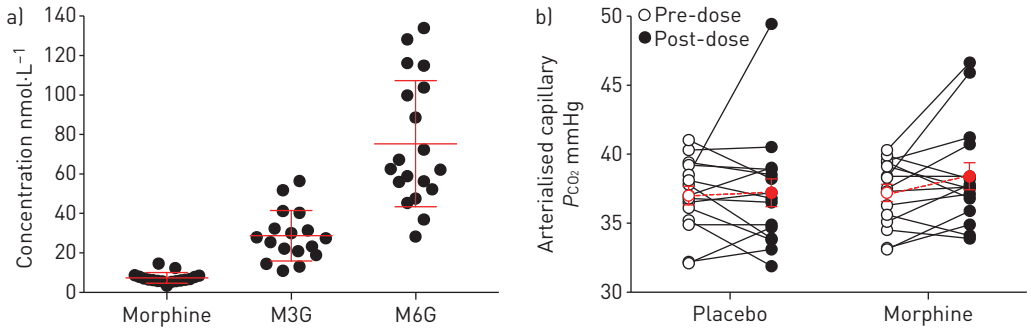

FIGURE 3 Effect of immediate-release oral morphine on blood biochemistry parameters in adults with advanced chronic obstructive pulmonary disease (COPD) and chronic breathlessness syndrome. a) Individual participant (closed circles; n=19) and mean±SEM (red lines) plasma morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) concentrations measured 30-min after taking oral morphine. b) Arterialised capillary CO_2 (P_{CO_2}) tension measurements made at rest before and 30 min after taking oral morphine and placebo among individual participants (n=14), where red symbols with dashed horizontal lines denote mean±SEM.

Plasma [MOR], [M3G] and [M6G] were not different between responders and non-responders, while post-dose measures of P_{acCO_2} were similar within- and between-groups (see figure E2 in the online data supplement). The effect of oral morphine *versus* placebo on breathlessness and selected ventilatory responses to constant-load CPET within responders and non-responders is shown in figure 5. After taking morphine *versus* placebo within the responder subgroup, EET increased by 3.6±1.3 min (p=0.005), while breathlessness intensity and unpleasantness ratings during exercise at the highest equivalent isotime of 6.0±1.5 min decreased by 2.3±0.6 Borg units (p<0.001) and 2.3±0.6 Borg units (p=0.001), respectively. Although the differences were not statistically significant, f_R decreased by 3.2±1.4 breaths·min^{-1} and tidal volume (V_T) increased by 0.08±0.04 L at isotime (with no corresponding change in V'_E) after taking morphine *versus* placebo within responders. By contrast, morphine had no effect on EET or an effect on ventilatory and breathlessness responses to exercise within non-responders.

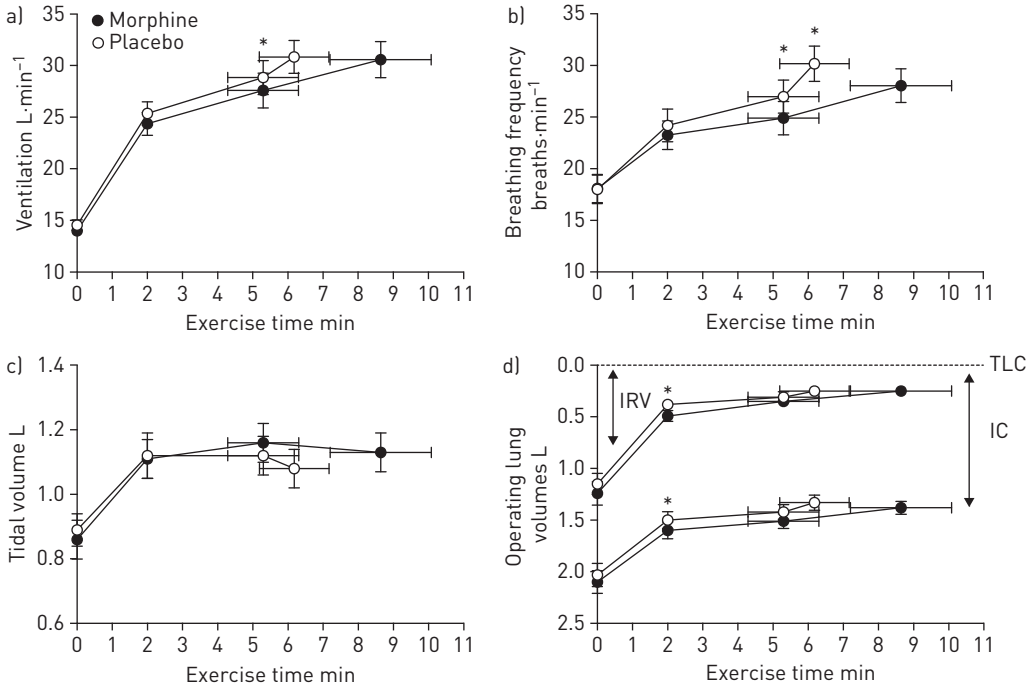

FIGURE 4 Effect of immediate-release oral morphine *versus* placebo on a) minute ventilation, b) breathing frequency, c) tidal volume and d) dynamic operating lung volume responses during constant-load cycle exercise testing at 75% of peak incremental power output in adults with advanced chronic obstructive pulmonary disease and chronic breathlessness syndrome. Data are presented as mean±SEM. IRV: inspiratory reserve volume; IC: inspiratory capacity; TLC: total lung capacity. *: p<0.05 *versus* placebo.

Compared with placebo, morphine had no effect on either the locus of symptom limitation, the selection frequency of breathlessness descriptors at end-exercise or the percentage contributions of breathlessness and leg discomfort to exercise cessation within either responders or non-responders (data not shown).

Discussion

The main findings of this randomised crossover trial are as follows: 1) single-dose administration of immediate-release oral morphine *versus* placebo improved exertional breathlessness and exercise endurance among participants with advanced COPD and chronic breathlessness syndrome; 2) morphine-induced improvements in exertional breathlessness and exercise endurance were accompanied by small but statistically significant decreases in V'_E and f_R during exercise at isotime, without significant opioid-related side effects and/or gas exchange impairment at rest and during exercise; and 3) the locus of symptom-limitation on laboratory-based CPET may help to identify adults with advanced COPD and chronic breathlessness syndrome most likely to respond to morphine.

Compared with placebo, morphine decreased breathlessness intensity ratings during exercise at isotime by 1.2 Borg units and increased EET by 2.5 min (148 s) or 41%. The magnitudes of these improvements exceeded their respective MCIDs [36, 37] and were thus clinically meaningful. Our results are comparable to those of Woodcock *et al.* [10] and Light *et al.* [8] who respectively reported on the acute effect of single-dose oral dyhydrocodeine (1 mg·kg^{-1} body mass) and oral morphine (0.8 mg·kg^{-1} body mass) on exertional breathlessness and exercise tolerance in advanced COPD.

It is noteworthy that improvements in exertional breathlessness and EET after taking morphine *versus* placebo occurred following apparent maximal or near maximal bronchodilatation with 400 µg of inhaled salbutamol. Indeed, morphine-induced improvements in exertional breathlessness and EET could not be easily explained by improved dynamic respiratory mechanics.

In keeping with the results of Woodcock *et al.* [10] and Light *et al.* [8], single-dose administration of oral morphine *versus* placebo was associated with modest but statistically significant reductions in V'_E and f_R during exercise at isotime, which were accompanied by concomitant reductions in neural inspiratory drive to the crural diaphragm (*i.e.*, EMGdi). These results are consistent with the known effect of systemic opioids on central and peripheral chemoreflex drives to breathe [7]. Importantly, the observed changes in V'_E, f_R and EMGdi occurred in the absence of any untoward effect of morphine on cardiac, metabolic and/or gas exchange parameters at rest (*e.g.* P_{acCO_2}) and during exercise (*e.g.* P_{ETCO_2}, oxygen saturation). In view of the observed reductions in V'_E, f_R, EMGdi and the relatively preserved EMGdi:V'_E ratio during exercise at isotime after taking morphine *versus* placebo, we speculate that morphine-induced improvements in exertional breathlessness and EET reflected, at least in part, the awareness of reduced central neural respiratory drive as sensed by reduced central corollary discharge from brainstem respiratory centres to various cortical and sub-cortical regions implicated in the neurophysiology of breathlessness [38]. These regions, all of which express high densities of opioid receptors, include the prefrontal, insular and motor cortices, operculum, anterior and posterior cingulate cortices, amygdala and periaqueductal grey matter [38–42].

It is unlikely that awareness of reduced central neural respiratory drive was the only mechanism responsible for relief of exertional breathlessness and improved EET after taking morphine *versus* placebo, particularly in view of 1) the small reductions in V'_E, f_R and EMGdi at isotime relative to the large improvements in exertional breathlessness and EET and 2) our finding that exertional breathlessness was reduced and EET increased after taking morphine *versus* placebo within responders despite no statistically significant decreases in V'_E and f_R. It is possible that morphine relieved breathlessness and improved EET by suppressing activity of the cortico-limbic regions implicated in the perception of breathlessness, independent of, or in conjunction with, its effect on central neural respiratory drive [9, 38–40, 42].

Although one serious adverse event occurred in a participant with an unreported intolerance to opioids, no meaningful pre-to-post dose changes in any of the symptoms evaluated using the ORSDS were observed following the administration of immediate-release oral morphine in our participants with advanced COPD. These findings are consistent with the results of earlier studies that informally assessed opioid-related side effects in COPD [8, 10].

Secondary exploratory analysis

With the exception of FEV_1 % pred, which tended to be lower in responders *versus* non-responders, baseline participant characteristics, resting P_{acCO_2}, and plasma [MOR], [M3G] and [M6G] were similar between-groups (see table E1 in the online data supplement). Compared with non-responders, the responders subgroup 1) reported higher intensity and unpleasantness ratings of breathlessness at the symptom-limited peak of CPET and 2) were more likely to identify intolerable breathlessness as the

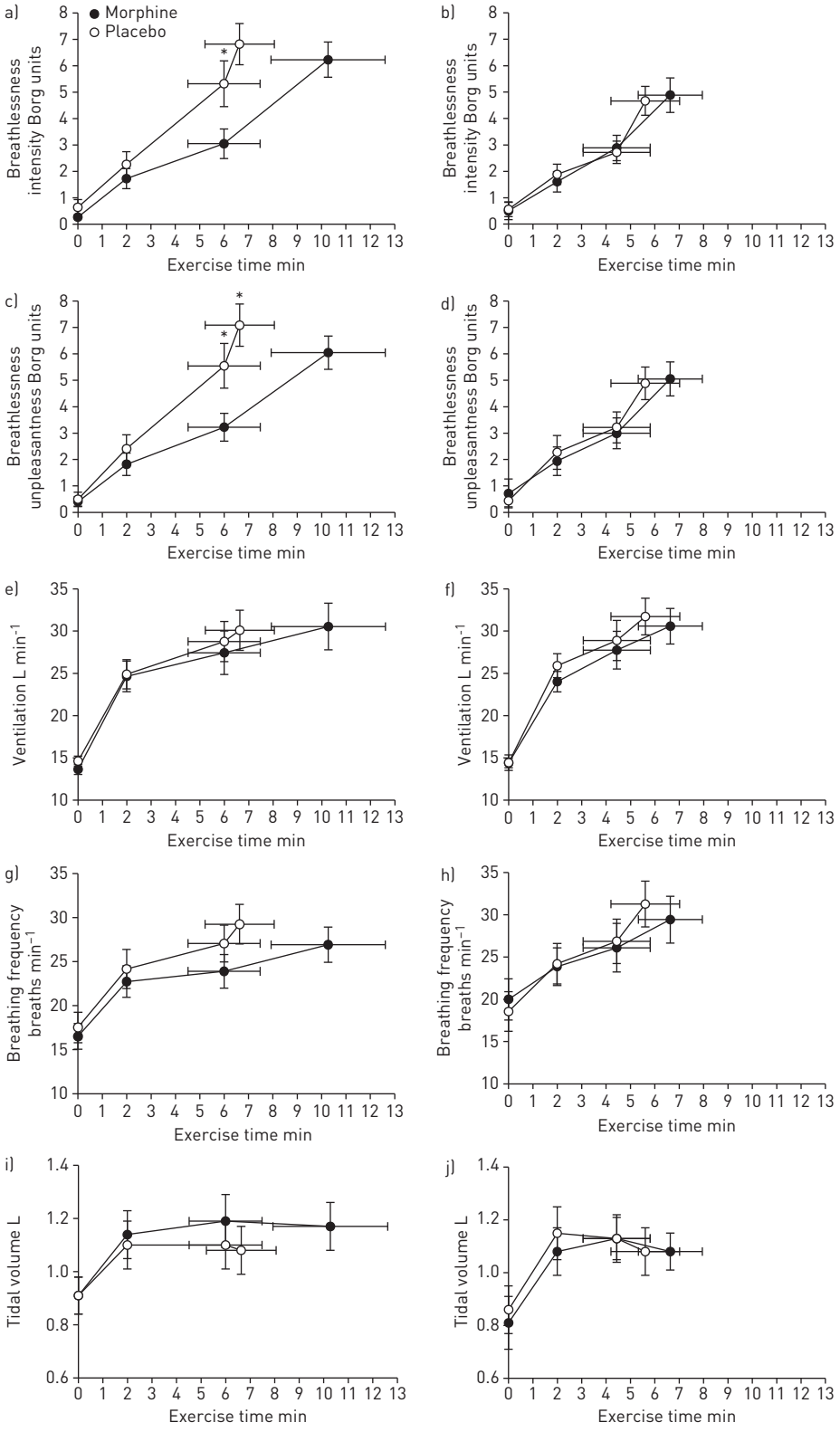

FIGURE 5 Effect of immediate-release oral morphine *versus* placebo on a and b) breathlessness intensity, c and d) breathlessness unpleasantness, e and f) minute ventilation, g and h) breathing frequency and i and j) tidal volume responses during constant-load cycle exercise testing at 75% of peak incremental power output in adults with advanced chronic obstructive pulmonary disease (COPD) and chronic breathlessness syndrome that did (responders (n=11); panels a,c,e,g and i) and did not (non-responders (n=9); panels b,d,f,h and j) report a decrease in breathlessness intensity of ⩾1 Borg unit during exercise at isotime after taking oral morphine *versus* placebo. Data are presented as mean±SEM. *: p<0.05 *versus* placebo.

primary reason for stopping CPET, despite exercising to a similar PPO, EET, peak V'_{O_2} and peak V'_E. Factors contributing to these between-group differences are unclear, particularly in the absence of notable differences in the physiological response to exercise. Collectively, these results suggest that adults with advanced COPD and chronic breathlessness syndrome who achieve relatively high ratings of breathlessness at the symptom-limited peak of exercise and/or who report intolerable breathlessness as their main exercise-limiting symptom may be the most responsive to immediate-release oral morphine. Although we were unable to elucidate the mechanism(s) responsible for the contrasting effect of oral morphine *versus* placebo on exertional breathlessness and EET in responders *versus* non-responders, we speculate that any one or combination of the following factors may be at least partly responsible: relatively greater morphine-induced suppression of central neural respiratory drive in responders *versus* non-responders, as evidenced by the 3.2 breath·min^{-1} decrease in f_R at isotime after taking morphine *versus* placebo within responders alone; unmeasured between-group differences in genetic variability (*e.g.* single nucleotide polymorphisms in opioid receptors) [43]; and unmeasured between-group differences in conditioned anticipatory/associative learning responses to breathlessness [42].

Methodological considerations

The generalizability of our results may be restricted to a relatively small and homogenous group of clinically stable, normocapnic, non-oxygen dependent and opioid-naïve adults with severe-to-very severe COPD and chronic breathlessness syndrome. The 0.1 mg·kg^{-1} body mass dose of immediate-release oral morphine used in this trial may be considered relatively high, particularly in comparison to current recommendations on the use of opioids for managing refractory breathlessness in advanced COPD [12]. Dose-ranging studies are needed to identify the lowest effective dose of immediate-release oral morphine required to achieve clinically meaningful improvements in exertional breathlessness and EET in adults with advanced COPD and chronic breathlessness syndrome. We caution against the extrapolation of our results concerning the acute effect of single-dose immediate-release oral morphine on exertional symptoms in advanced COPD to other modes (*e.g.* inhaled, sublingual), types (*e.g.* fentanyl) and regimens of opioid administration (*e.g.* repeat-dose, sustained-release) in this patient population. Safety aspects of this trial should be interpreted cautiously as it was not powered to detect differences in safety outcomes. Although the results of our exploratory analysis may be limited by a small sample size (*i.e.* susceptible to a type 2 error), we nevertheless identified factors related to the locus of symptom-limitation on laboratory-based cycle CPET as being potentially helpful in identifying which patients will likely respond to a single-dose of immediate-release oral morphine, as previously demonstrated by Deschenes *et al.* [44] for bronchodilator therapy. In moving forward, it will be important to prospectively validate our *post hoc* classification of responders and non-responders by observing the effects of chronic dosing of morphine on breathlessness and exercise endurance in adults with advanced COPD and chronic breathlessness syndrome.

Conclusions

Single-dose administration of immediate-release oral morphine (0.1 mg·kg^{-1} body mass) was associated with statistically significant and clinically meaningful improvements in exertional breathlessness and exercise endurance in adults with advanced COPD and chronic breathlessness syndrome. The observed changes in breathlessness and exercise endurance after taking oral morphine *versus* placebo could not be explained by concurrent changes in cardiac, metabolic, gas exchange and/or dynamic operating lung volume responses to exercise but were associated with reductions in ventilation, breathing frequency and the diaphragm electromyogram during exercise at isotime. Although additional research is necessary, the locus of symptom-limitation on laboratory-based CPET has the potential to help healthcare providers better predict which patients with advanced COPD and chronic breathlessness syndrome are most likely to achieve clinically meaningful improvements in exertional breathlessness and exercise endurance in response to morphine.

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References

1 Agusti A, Edwards LD, Celli B, *et al.* Characteristics, stability and outcomes of the 2011 GOLD COPD groups in the eclipse cohort. *Eur Respir J* 2013; 42: 636–646.
2 Mullerova H, Lu C, Li H, *et al.* Prevalence and burden of breathlessness in patients with chronic obstructive pulmonary disease managed in primary care. *Plos One* 2014; 9: e85540.
3 Small M, Holbrook T, Wood R, *et al.* Prevalence and burden of dyspnoea among COPD patients in japan. *Int J Clin Pract* 2016; 70: 676–681.

4 Sundh J, Ekstrom M. Persistent disabling breathlessness in chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis* 2016; 11: 2805–2812.
5 Johnson MJ, Yorke J, Hansen-Flaschen J, *et al.* Towards an expert consensus to delineate a clinical syndrome of chronic breathlessness. *Eur Respir J* 2017; 49.
6 Ekstrom M, Nilsson F, Abernethy AA, *et al.* Effects of opioids on breathlessness and exercise capacity in chronic obstructive pulmonary disease. A systematic review. *Ann Am Thorac Soc* 2015; 12: 1079–1092.
7 Banzett RB, Adams L, O'Donnell CR, *et al.* Using laboratory models to test treatment morphine reduces dyspnea and hypercapnic ventilatory response. *Am J Respir Crit Care* 2011; 184: 920–927.
8 Light RW, Muro JR, Sato RI, *et al.* Effects of oral morphine on breathlessness and exercise tolerance in patients with chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1989; 139: 126–133.
9 Pattinson KTS, Governo RJ, MacIntosh BJ, *et al.* Opioids depress cortical centers responsible for the volitional control of respiration. *J Neurosci* 2009; 29: 8177–8186.
10 Woodcock AA, Gross ER, Gellert A, *et al.* Effects of dihydrocodeine, alcohol, and caffeine on breathlessness and exercise tolerance in patients with chronic obstructive lung disease and normal blood gases. *N Engl J Med* 1981; 305: 1611–1616.
11 Mahler DA, Selecky PA, Harrod CG, *et al.* American College of Chest Physicians consensus statement on the management of dyspnea in patients with advanced lung or heart disease. *Chest* 2010; 137: 674–691.
12 Marciniuk DD, Goodridge D, Hernandez P, *et al.* Managing dyspnea in patients with advanced chronic obstructive pulmonary disease: a Canadian Thoracic Society clinical practice guideline. *Can Respir J* 2011; 18: 69–78.
13 Qaseem A, Wilt TJ, Weinberger SE, *et al.* Diagnosis and management of stable chronic obstructive pulmonary disease: a clinical practice guideline update from the American College of Physicians, American College of Chest Physicians, American Thoracic Society, and European Respiratory Society. *Ann Intern Med* 2011; 155: 179–191.
14 Vogelmeier CF, Criner GJ, Martinez FJ, *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: 557–582.
15 Ahmadi Z, Bernelid E, Currow DC, *et al.* Prescription of opioids for breathlessness in end-stage COPD: a national population-based study. *Int J Chron Obstruct Pulmon Dis* 2016; 11: 2651–2657.
16 Young J, Donahue M, Farquhar M, *et al.* Using opioids to treat dyspnea in advanced COPD: attitudes and experiences of family physicians and respiratory therapists. *Can Fam Physician* 2012; 58: e401–e407.
17 Janssen DJ, de Hosson SM, bij de Vaate E, *et al.* Attitudes toward opioids for refractory dyspnea in COPD among dutch chest physicians. *Chron Respir Dis* 2015; 12: 85–92.
18 Fletcher CM, Elmes PC, Fairbairn AS, *et al.* The significance of respiratory symptoms and the diagnosis of chronic bronchitis in a working population. *Br Med J* 1959; 2: 257–266.
19 Mahler DA, Weinberg DH, Wells CK, *et al.* The measurement of dyspnea. Contents, interobserver agreement, and physiologic correlates of two new clinical indexes. *Chest* 1984; 85: 751–758.
20 McGavin CR, Artvinli M, Naoe H, *et al.* Dyspnoea, disability, and distance walked: comparison of estimates of exercise performance in respiratory disease. *Br Med J* 1978; 2: 241–243.
21 Jones PW, Harding G, Berry P, *et al.* Development and first validation of the COPD assessment test. *Eur Respir J* 2009; 34: 648–654.
22 Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand* 1983; 67: 361–370.
23 Apfelbaum JL, Gan TJ, Zhao S, *et al.* Reliability and validity of the perioperative opioid-related symptom distress scale. *Anesth Analg* 2004; 99: 699–709.
24 Yadeau JT, Liu SS, Rade MC, *et al.* Performance characteristics and validation of the opioid-related symptom distress scale for evaluation of analgesic side effects after orthopedic surgery. *Anesth Analg* 2011; 113: 369–377.
25 Macintyre N, Crapo RO, Viegi G, *et al.* Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J* 2005; 26: 720–735.
26 Miller MR, Crapo R, Hankinson J, *et al.* General considerations for lung function testing. *Eur Respir J* 2005; 26: 153–161.
27 Miller MR, Hankinson J, Brusasco V, *et al.* Standardisation of spirometry. *Eur Respir J* 2005; 26: 319–338.
28 Wanger J, Clausen JL, Coates A, *et al.* Standardisation of the measurement of lung volumes. *Eur Respir J* 2005; 26: 511–522.
29 Jensen D, Alsuhail A, Viola R, *et al.* Inhaled fentanyl citrate improves exercise endurance during high-intensity constant work rate cycle exercise in chronic obstructive pulmonary disease. *J Pain Symptom Manage* 2012; 43: 706–719.
30 Guenette JA, Chin RC, Cory JM, *et al.* Inspiratory capacity during exercise: measurement, analysis, and interpretation. *Pulm Med* 2013; 2013: 956081.
31 Borg GA. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 1982; 14: 377–381.
32 Mendonca CT, Schaeffer MR, Riley P, *et al.* Physiological mechanisms of dyspnea during exercise with external thoracic restriction: role of increased neural respiratory drive. *J Appl Physiol* 2014; 116: 570–581.
33 Schaeffer MR, Mendonca CT, Levangie MC, *et al.* Physiological mechanisms of sex differences in exertional dyspnoea: role of neural respiratory motor drive. *Exp Physiol* 2014; 99: 427–441.
34 O'Donnell DE, Hong HH, Webb KA. Respiratory sensation during chest wall restriction and dead space loading in exercising men. *J Appl Physiol* 2000; 88: 1859–1869.
35 Faul F, Erdfelder E, Buchner A, *et al.* Statistical power analyses using g*power 3.1: tests for correlation and regression analyses. *Behav Res Methods* 2009; 41: 1149–1160.
36 Ries AL. Minimally clinically important difference for the UCSD shortness of breath questionnaire, Borg scale, and visual analog scale. *Copd* 2005; 2: 105–110.
37 Puente-Maestu L, Villar F, de Miguel J, *et al.* Clinical relevance of constant power exercise duration changes in COPD. *Eur Respir J* 2009; 34: 340–345.
38 von Leupoldt A, Dahme B. Cortical substrates for the perception of dyspnea. *Chest* 2005; 128: 345–354.
39 Herigstad M, Hayen A, Evans E, *et al.* Dyspnea-related cues engage the prefrontal cortex: evidence from functional brain imaging in copd. *Chest* 2015; 148: 953–961.
40 Pattinson KTS. Opioids and the control of respiration. *Br J Anaesth* 2008; 100: 747–758.

41 Baumgartner U, Buchholz HG, Bellosevich A, *et al.* High opiate receptor binding potential in the human lateral pain system. *Neuroimage* 2006; 30: 692–699.
42 Hayen A, Wanigasekera V, Faull OK, *et al.* Opioid suppression of conditioned anticipatory brain responses to breathlessness. *Neuroimage* 2017; 150: 383–394.
43 Currow DC, Quinn S, Ekstrom M, *et al.* Can variability in the effect of opioids on refractory breathlessness be explained by genetic factors? *BMJ Open* 2015; 5: e006818.
44 Deschenes D, Pepin V, Saey D, *et al.* Locus of symptom limitation and exercise response to bronchodilation in chronic obstructive pulmonary disease. *J Cardiopulm Rehabil Prev* 2008; 28: 208–214.

Appendix IV

Effect of Vaporized Cannabis on Exertional Breathlessness and Exercise Endurance in Advanced Chronic Obstructive Pulmonary Disease

A Randomized Controlled Trial

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Abstract

Rationale: A series of studies conducted approximately 40 years ago demonstrated an acute bronchodilator effect of smoked cannabis in healthy adults and adults with asthma. However, the acute effects of vaporized cannabis on airway function in adults with advanced chronic obstructive pulmonary disease (COPD) remain unknown.

Objectives: To test the hypothesis that inhaled vaporized cannabis would alleviate exertional breathlessness and improve exercise endurance by enhancing static and dynamic airway function in COPD.

Methods: In a randomized controlled trial of 16 adults with advanced COPD (forced expiratory volume in 1 second [FEV_1], mean ± SD: 36 ± 11% predicted), we compared the acute effect of 35 mg of inhaled vaporized cannabis (18.2% Δ^9-tetrahydrocannabinol, <0.1% cannabidiol) versus 35 mg of a placebo control cannabis (CTRL; 0.33% Δ^9-tetrahydrocannabinol, <0.99% cannabidiol) on physiological and perceptual responses during cardiopulmonary cycle endurance exercise testing; spirometry and impulse oscillometry at rest; and cognitive function, psychoactivity, and mood.

Results: Compared with CTRL, cannabis had no effect on breathlessness intensity ratings during exercise at isotime (cannabis, 2.7 ± 1.2 Borg units vs. CTRL, 2.6 ± 1.3 Borg units); exercise endurance time (cannabis, 3.8 ± 1.9 min vs. CTRL, 4.2 ± 1.9 min); cardiac, metabolic, gas exchange, ventilatory, breathing pattern, and/or operating lung volume parameters at rest and during exercise; spirometry and impulse oscillometry–derived pulmonary function test parameters at rest; and cognitive function, psychoactivity, and mood.

Conclusions: Single-dose inhalation of vaporized cannabis had no clinically meaningful positive or negative effect on airway function, exertional breathlessness, and exercise endurance in adults with advanced COPD.

Clinical trial registered with www.clinicaltrials.gov (NCT03060993).

Keywords: dyspnea; functional capacity; marijuana; chronic obstructive pulmonary disease

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In adults with chronic obstructive pulmonary disease (COPD), pathophysiological abnormalities in static and dynamic airway function (e.g., hyperinflation) are mechanistically linked to breathlessness and exercise intolerance (1, 2), which are independently associated with increased morbidity and mortality (3, 4). Despite intensive management of their underlying pulmonary pathophysiology with inhaled bronchodilators and antiinflammatory agents, 46–91% of adults with advanced COPD suffer from persistent and disabling breathlessness at rest and on minimal exertion (5–8). Therefore, it is important to identify adjunct therapies to help alleviate breathlessness and improve exercise tolerance in advanced COPD.

Amid widespread changes in the regulatory landscape of recreational and medicinal use of cannabis, there has been growing interest in understanding the therapeutic potential of its main cannabinoid constituent, Δ^9-tetrahydrocannabinol (THC) (9), which provides symptomatic relief of acute and chronic pain across a range of malignant and nonmalignant diagnoses (10).

Mechanistically, THC exerts its effects by binding to cannabinoid type 1 (CB_1) and to a lesser extent type 2 (CB_2) receptors, which are differentially expressed in the central and peripheral nervous systems as well as in some peripheral tissues, including the lungs (11, 12). Grassin-Delyle and colleagues (13) demonstrated that THC induced a concentration-dependent inhibition of cholinergic contraction in human airway smooth cells via activation of prejunctional CB_1 receptors. In keeping with these observations, Vachon and colleagues (14) and Tashkin and colleagues (15–18) demonstrated an acute bronchodilator effect of smoked cannabis (~500 mg of 1–2% THC) in healthy adults and adults with asthma that was comparable in magnitude and duration of effect to the β_2-adrenergic receptor agonist isoproterenol. Although no study has evaluated the bronchodilator and therapeutic potential of inhaled cannabis in COPD, a large cross-sectional study of adults with COPD reported a positive association between cannabis use and forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC), even after adjusting for cigarette smoking history (19). Together, these studies suggest that the endocannabinoid system may represent a novel therapeutic target to enhance static and dynamic airway function, with attendant improvements in exertional breathlessness and exercise tolerance in advanced COPD.

The aim of this randomized controlled trial was to evaluate the acute effect of inhaled vaporized cannabis versus a placebo control (CTRL) on exertional breathlessness

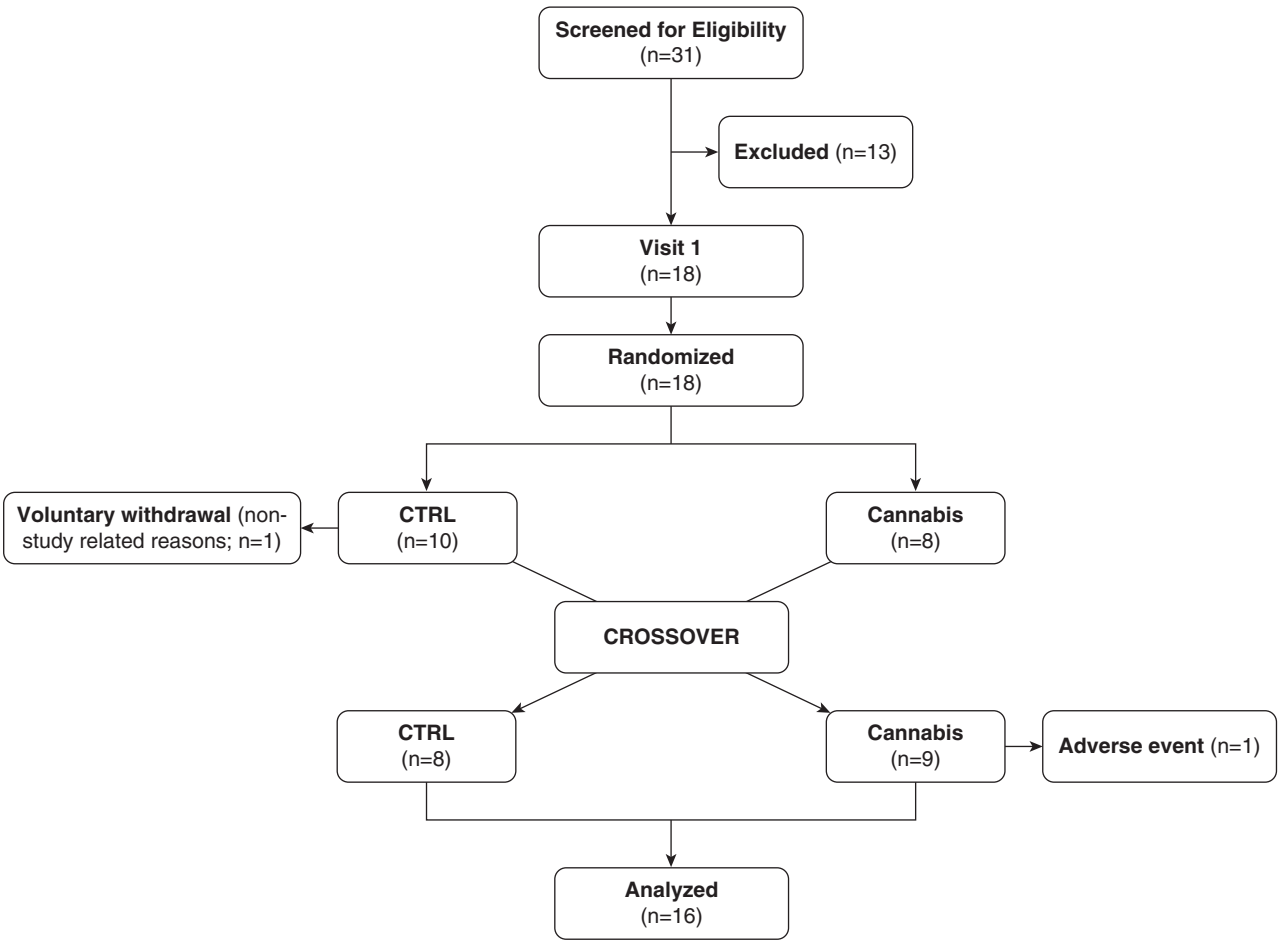

Figure 1. CONSORT diagram of the study population. CONSORT = Consolidated Standards of Reporting Trials; CTRL = placebo control cannabis.

and exercise endurance in symptomatic adults with advanced COPD. We hypothesized that single-dose inhalation of vaporized cannabis versus CTRL would alleviate exertional breathlessness and improve exercise endurance by enhancing static and dynamic airway function.

Methods

Study Design

This single-center, randomized, double-blind, crossover trial (ClinicalTrials.gov; NCT03060993) consisted of two intervention periods separated by a washout period of at least 5 days. The study protocol and informed consent form received regulatory approval from Health Canada (Control No. 202091) and ethics approval from the Research Institute of the McGill University Health Centre (COPD-THC/2017-2614). The study took place at the McConnell Centre for Innovative Medicine of the McGill University Health Centre, and participants were recruited from the Montreal Chest Institute (Montreal, QC, Canada).

After providing written and informed consent, participants completed a screening/familiarization visit followed by two randomly assigned treatment visits. Visit 1 included: evaluation of participant-reported breathlessness (20, 21), health status (22), and anxiety/depression (23); post-bronchodilator pulmonary function testing; and a symptom-limited incremental cardiopulmonary cycle exercise test (CPET) to determine peak power output, defined as the highest power output that the participant was able to sustain for at least 30 seconds. Before the administration of cannabis or CTRL at Visits 2 and 3, a urine sample was collected for toxicology screening of THC; cognitive function (24), psychoactivity, and mood (25) were assessed; and spirometry and impulse oscillometry (iOS) were performed. Participants then inhaled vaporized cannabis or CTRL. Two minutes thereafter, participants completed tests of cognitive function (24), psychoactivity, and mood (25) followed immediately by spirometry, iOS, and a symptom-limited constant-load cycle CPET at 75% of peak power output. Intravenous blood samples for measurement of plasma concentrations of THC and its metabolites and of cannabidiol (CBD) were obtained before and 2, 30, 75, and 180 minutes after inhalation of cannabis and CTRL. *See* the online supplement for details on study design.

Participants

Participants included men and women (age, ⩾40 yr) with Global Initiative for Obstructive Lung Disease stage 3 or 4 COPD (26). *See* the online supplement for details on eligibility criteria.

Intervention

Participants received 35 mg of cannabis (Tilray House Blend-active [THC, 18.2%; CBD, <0.1%]; Tilray) or 35 mg of CTRL (Tilray House Blend-control [THC, 0.33%; CBD, 0.99%]) administered with a Volcano Digit vaporizer (Storz & Bickel America, Inc.).

Procedures

Dried plant cannabis and CTRL material were dispensed into the Volcano Digit filling chamber by the McGill University Health Centre's research pharmacist. The filling chamber was placed in the vaporizer at a heating temperature and filling time of 190°C and 30 seconds, respectively. Approximately 5.5 L of the vaporized compounds was collected in a balloon fitted with a mouthpiece and a one-way valve (Storz & Bickel America, Inc.), allowing the vapor to remain in the balloon until inhalation. Participants inhaled the entire contents of the balloon using the Foltin puff procedure (27). Briefly, participants were instructed to "hold the balloon with one hand and put the mouthpiece in

Table 1. Baseline participant characteristics

Parameter	Value*
Male:Female, No.	10:6
Age, yr	65.4 ± 7.7 (66; 47 to 77)
Height, cm	165.6 ± 7.3 (168; 150 to 175)
Body mass, kg	70.9 ± 11.7 (72; 50 to 89)
Body mass index, $kg \cdot m^{-2}$	25.8 ± 11.8 (26.7; 18.6 to 33.5)
Cigarette smoking history, pack-years	63 ± 28 (60; 21 to 127)
Cannabis smoking history, joint-years	34 ± 99 (0; 0 to 392)
Post-bronchodilator pulmonary function	
FEV_1, L (% predicted)	0.88 ± 0.28 (36 ± 11) (0.98; 0.51 to 1.53)
FEV_1/FVC, %	31 ± 7 (31; 20 to 47)
TLC, L (% predicted)	8.10 ± 2.08 (143 ± 42) (7.86; 5.81 to 13.56)
RV, L (% predicted)	5.04 ± 2.51 (242 ± 123) (4.41; 2.31 to 11.64)
FRC, L (% predicted)	6.40 ± 2.17 (210 ± 78) (5.85; 4.24 to 12.32)
IC, L (% predicted)	1.70 ± 0.43 (64 ± 13) (1.79; 0.92 to 2.24)
D_{LCO}, $ml \cdot min^{-1} \cdot mm\ Hg^{-2}$ (% predicted)	11.9 ± 3.9 (62 ± 4) (11.7; 4.0 to 18.8)
sRaw, $cm\ H_2O \cdot L^{-1} \cdot s^{-2}$ (% predicted)	40.4 ± 17.3 (900 ± 478) (34.9; 20.5 to 78.7)
Impulse oscillometry	
R_5, $kPa \cdot L^{-1} \cdot s$	0.51 ± 0.13 (0.49; 0.27 to 0.82)
R_{20}, $kPa \cdot L^{-1} \cdot s$	0.32 ± 0.07 (0.32; 0.24 to 0.50)
X_5, $kPa \cdot L^{-1} \cdot s$	−0.28 ± 0.12 (−0.27; −0.55 to −0.76)
F_{res}, $1 \cdot s^{-1}$	22.83 ± 3.49 (22.43; 17.73 to 28.06)
A_X, $kPa \cdot L^{-1}$	2.29 ± 1.11 (2.14; 1.1 to 4.8)
Breathlessness and health status	
mMRC score, 0–4	2.8 ± 0.5 (3; 2 to 3)
BDI focal score, out of 12	4.1 ± 1.8 (3; 1 to 7)
Oxygen cost diagram, % full scale	44 ± 17 (38; 23 to 81)
CAT score, out of 40	15.7 ± 7.8 (16; 4 to 28)
HADS score, out of 42	12.3 ± 8.1 (13; 0 to 31)
COPD medication summary	
LABA + LAMA, No.	7
LABA + LAMA + ICS, No.	9

Definition of abbreviations: A_X = area of reactance; BDI = Baseline Dyspnea Index; CAT = Chronic Obstructive Pulmonary Disease Assessment Test; COPD = chronic obstructive pulmonary disease; D_{LCO} = diffusing capacity of the lung for carbon monoxide; F_{res} = resonant frequency; FEV_1 = forced expiratory volume in 1 second; FRC = functional residual capacity; FVC = forced vital capacity; HADS = Hospital Anxiety and Depression Scale; IC = inspiratory capacity; ICS = inhaled corticosteroid; LABA = long-acting β_2-agonist; LAMA = long-acting muscarinic antagonist; mMRC = modified Medical Research Council Dyspnoea Scale; R_5 and R_{20} = resistance at 5 and 20 Hz, respectively; RV = residual volume; sRaw = specific airway resistance; TLC = total lung capacity; X_5 = reactance at 5 Hz.
*Values represent means ± SD (median; range). Cannabis smoking history was calculated as number of joints per day × number of years smoking.

your mouth," "inhale for 5 seconds," "hold vapor in your lungs for 10 seconds," "exhale and wait for 40 seconds before repeating puff cycle." Spirometry and iOS were performed with automated equipment and according to recommended techniques (28–31). Exercise tests were conducted on an electronically braked cycle ergometer, using a computerized CPET system: cardiac, metabolic, gas exchange, breathing pattern, and operating lung volume parameters were collected and analyzed as previously described (32, 33). Using Borg's modified 0–10 category ratio scale (34), participants rated the intensity and unpleasantness of their breathlessness, as well as the intensity of their leg discomfort at rest, every 2 minutes during CPET, and at end-exercise. Each participant's blinded treatment preference was assessed at the end of Visit 3. *See* the online supplement for details on experimental procedures.

Outcome Variables

The primary outcome was the post-treatment difference in breathlessness intensity ratings during exercise at isotime, defined as the highest equivalent 2-minute interval of exercise completed by a given participant during each of the constant-load CPETs. The coprimary outcome was the post-treatment difference in exercise endurance time (EET), defined as the duration of loaded pedaling during constant-load CPET. The constant-load cycle CPET was selected over other exercise test modalities (e.g., endurance shuttle walking test), as it is generally regarded as the most responsive exercise testing modality in the evaluation of interventional efficacy in COPD, particularly as it relates to exertional breathlessness and EET (35). *See* the online supplement for details on secondary outcome variables.

Sample Size

Using a two-tailed paired-subject formula with $\alpha = 0.05$, $\beta = 0.80$, and an expected effect size of 0.80 (36), we estimated that at least 15 participants were needed to detect a minimal clinically important difference (MCID) of ±1 Borg unit in breathlessness intensity during exercise at isotime (37) and of ±101 seconds in EET (38) after inhalation of vaporized cannabis versus CTRL.

Randomization

Participants were randomized at a 1:1 ratio according to a computer-generated block randomization schedule (block size, 4) prepared by a third-party statistician not involved in the trial. Participants and investigators were blinded to the randomization schedule.

Statistical Methods

Participants who completed both cannabis and CTRL arms of the trial were included in the analysis. Linear mixed-models regression with random intercepts was used to analyze post-treatment differences in EET as well as in all physiological and perceptual responses to constant-load CPET, accounting for period and sequence effects. Data were analyzed with the SAS statistical package, version 9.4 (SAS Institute Inc.) and SigmaStat, version 3.5 (Systat Software Inc.). Statistical significance was set at $P < 0.05$, and values are reported as means ± SD unless stated otherwise. *See* the online supplement for additional information on the statistical analyses performed.

Results

Eighteen of 31 participants assessed for eligibility were randomized (Figure 1). One of these 18 participants voluntarily withdrew between Visits 1 and 2, and another participant was excluded after an adverse event (*see below*). Baseline characteristics of the 16 participants who completed the trial are presented in Tables 1 and 2. Twelve of the 16 participants had a self-reported cannabis smoking history of less than one joint in their lifetime. The other four participants had a mean ± SD self-reported cannabis smoking history of 34 ± 99 joint-years (range, 1.4–392). *See* the online supplement for additional information on participant characteristics.

Primary Outcomes

Compared with CTRL, cannabis had no effect on breathlessness intensity ratings at isotime or on EET (Table 3 and Figure 2). There was no period or sequence effect on our primary outcomes. Four participants had a cannabis-induced decrease in breathlessness intensity ratings at isotime by the MCID of at least 1 Borg unit (responders) compared with the remaining 12 participants who did not (nonresponders) (Figures 2D and 2G). Two participants had a cannabis-induced increase in EET by the MCID of at least 101 seconds compared with the remaining 14 participants who did not (Figures 2F and

Table 2. Physiological and perceptual responses at symptom-limited peak of incremental cycle exercise testing in adults with advanced chronic obstructive pulmonary disease

Parameter	Value*
$\dot{V}_{O_2}$, ml · kg · min^{-1} (% predicted)	10.9 ± 2.9 (48 ± 13)
HR, beats · min^{-1} (% predicted)	117 ± 13 (67 ± 13)
Breathlessness intensity, Borg 0–10 units	5.2 ± 2.2
Breathlessness unpleasantness, Borg 0–10 units	5.4 ± 2.6
Leg discomfort, Borg 0–10 units	4.7 ± 1.9
$\dot{V}_E$, L · min^{-1} (% estimated MVV)	29.4 ± 10.5 (96 ± 23)
V_T, L	1.06 ± 0.29
f, breaths · min^{-1}	27.9 ± 7.2
ΔIC from rest, L	−0.67 ± 0.40
IRV, L	0.36 ± 0.20
$\dot{V}_E/\dot{V}_{CO_2}$	38.1 ± 5.7
$P_{ET_{CO_2}}$, mm Hg	41.8 ± 15.9
Sp_{O_2}, %	93 ± 3
ΔSp_{O_2} from rest, %	−2.2 ± 1.4
Reasons for stopping exercise	
Breathlessness, No.	6
Leg discomfort, No.	2
Breathlessness and leg discomfort, No.	7
Other, No.	1

Definition of abbreviations: Δ = exercise-induced change; f = breathing frequency; HR = heart rate; IC = inspiratory capacity; IRV = inspiratory reserve volume; MVV = maximal voluntary ventilation (estimated as $FEV_1 \times 35$); $P_{ET_{CO_2}}$ = partial pressure of end-tidal carbon dioxide; Sp_{O_2} = oxygen saturation by pulse oximetry; $\dot{V}_E$ = minute ventilation; $\dot{V}_E/\dot{V}_{CO_2}$ = ventilatory equivalent for carbon dioxide; $\dot{V}_{O_2}$ = rate of oxygen uptake; V_T = tidal volume.
*Values represent means ± SD.

Table 3. Effect of inhaled vaporized cannabis versus control on physiological and perceptual responses at rest, at a standardized submaximal time (isotime) during constant-load cycle exercise testing, and at symptom-limited peak of constant-load cycle exercise testing in adults with advanced chronic obstructive pulmonary disease*

	Rest		Isotime		Peak	
	Control	Cannabis	Control	Cannabis	Control	Cannabis
Cycle exercise time, min	—	—	2.4 ± 0.8	2.4 ± 0.8	4.2 ± 1.9	3.8 ± 1.9
Breathlessness intensity, Borg 0–10 units	0.4 ± 0.4	0.7 ± 1.1	2.6 ± 1.3	2.7 ± 1.2	5.1 ± 1.8	5.4 ± 2.0
Breathlessness unpleasantness, Borg 0–10 units	0.5 ± 0.8	0.5 ± 1.0	2.6 ± 1.2	2.8 ± 1.8	5.3 ± 2.2	5.1 ± 2.4
Leg discomfort, Borg 0–10 units	0.4 ± 0.6	0.7 ± 1.0	2.4 ± 1.7	2.9 ± 1.9	4.6 ± 2.4	4.4 ± 2.6
$\dot{V}O_2$, ml · kg · min^{-1}	4.0 ± 0.6	4.3 ± 0.8	9.8 ± 2.2	9.8 ± 2.5	11.3 ± 2.1	11.0 ± 2.9
$\dot{V}CO_2$, ml · kg · min^{-1}	3.7 ± 0.5	4.0 ± 0.8	9.5 ± 3.3	9.8 ± 3.5	11.6 ± 3.0	11.3 ± 3.8
HR, beats · min^{-1}	84 ± 12	86 ± 12	104 ± 12	107 ± 14	112 ± 13	114 ± 18
O_2 pulse, ml O_2 · beat^{-1}	3.4 ± 0.5	4.0 ± 2.3	6.7 ± 1.6	7.4 ± 4.5	7.1 ± 1.6	7.7 ± 4.4
$\dot{V}E$, L · min^{-1}	13.4 ± 2.7	14.3 ± 3.4	26.1 ± 10.1	26.4 ± 8.9	29.5 ± 9.6	29.6 ± 10.0
V_T, L	0.81 ± 0.24	0.77 ± 0.16	1.05 ± 0.26	1.07 ± 0.29	1.11 ± 0.28	1.08 ± 0.30
f, breaths · min^{-1}	17.9 ± 6.4	19.6 ± 6.0	25.6 ± 7.4	25.6 ± 7.2	26.8 ± 5.7	27.9 ± 6.7
IC, L	2.08 ± 0.51	2.04 ± 0.60	1.54 ± 0.40	1.48 ± 0.41	1.44 ± 0.44	1.41 ± 0.44
ΔIC from rest, L	—	—	−0.54 ± 0.34	−0.56 ± 0.28	−0.65 ± 0.34	−0.63 ± 0.38
IRV, L	1.27 ± 0.40	1.27 ± 0.46	0.49 ± 0.29	0.41 ± 0.26	0.32 ± 0.24	0.33 ± 0.23
$\dot{V}E/\dot{V}CO_2$	51.7 ± 6.1	51.6 ± 5.7	39.0 ± 5.0	38.9 ± 4.5	36.2 ± 5.3	37.2 ± 5.1
$P_{ET}CO_2$, mm Hg	32.8 ± 3.2	33.1 ± 3.2	37.2 ± 5.0	37.4 ± 4.3	39.0 ± 5.9	38.4 ± 5.3
Sp_{O_2}, %	94 ± 5	96 ± 2	93 ± 3	93 ± 3	92 ± 4	93 ± 3
Reasons for stopping exercise						
Breathlessness, No. (% contribution)	—	—	—	—	8 (62 ± 34)	7 (61 ± 33)
Leg discomfort, No. (% contribution)	—	—	—	—	2 (27 ± 28)	3 (13 ± 29)
Breathlessness and leg discomfort, No.	—	—	—	—	4	4
Other, No.	—	—	—	—	2	2

Definition of abbreviations: Δ = exercise-induced change; f = breathing frequency; HR = heart rate; IC = inspiratory capacity; IRV = inspiratory reserve volume; $P_{ET}CO_2$ = partial pressure of end-tidal carbon dioxide; Sp_{O_2} = oxygen saturation by pulse oximetry; $\dot{V}E$ = minute ventilation; $\dot{V}CO_2$ = rate of carbon dioxide production; $\dot{V}E/\dot{V}CO_2$ = ventilatory equivalent for carbon dioxide; $\dot{V}O_2$ = rate of oxygen uptake; V_T = tidal volume.
*Values represent means ± SD.

2I). A significant negative correlation was observed between cannabis-induced changes in breathlessness intensity ratings at isotime and in EET (Figure 3).

Secondary Outcomes

Pulmonary function. Compared with CTRL, cannabis had no effect on spirometry and iOS-derived pulmonary function parameters at rest (Table 4 and Figure E2).

Physiological and perceptual responses to exercise. Compared with CTRL, cannabis had no effect on cardiac, metabolic, gas exchange, ventilatory, breathing pattern, operating lung volume, breathlessness unpleasantness, and leg discomfort responses at rest or during exercise (Figures 2–5 and Table 3). The locus of symptom limitation (Table 3), the relative contributions of breathlessness and leg discomfort to exercise cessation (Table 3), and the selection frequency of breathlessness descriptors at end-exercise (*see* Figure E1 in the online supplement) were not different after inhalation of cannabis versus CTRL. *See* the online supplement for details on participants' blinded treatment preference.

Blood biochemistry. Plasma THC levels were approximately 17 and 44 times higher after inhalation of cannabis versus CTRL at the 2- and 30-minute post-treatment time periods, respectively. Plasma 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) levels were approximately 16 times higher after inhalation of cannabis versus CTRL at each of the 2-, 30-, 75-, and 180-minute post-treatment time periods (Table 5). Peak plasma THC, *trans*-Δ^9-tetrahydrocannabinol-9-acid A (THCA), and 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC) levels during the cannabis condition, and of THC and CBD during the CTRL condition, were achieved 2 minutes post-treatment. Peak plasma THC-COOH levels were achieved 30 minutes after cannabis and CTRL conditions (Table 5 and Figure 6). Compared with the pretreatment condition, inhaled cannabis increased plasma THCA and 11-OH-THC levels at 2, 30, and 75 minutes post-treatment, whereas inhaled CTRL had no effect (Table 5). Compared with the pretreatment condition, inhaled CTRL increased plasma CBD levels at 2, 30, and 75 minutes post-treatment, whereas inhaled cannabis had no effect (Table 5).

Cannabis-related side effects and adverse events. None of the participants coughed after inhalation of CTRL. By contrast, six participants coughed after inhalation of cannabis, with five of these six participants reporting clinically significant worsening of exertional breathlessness at isotime by at least 1 Borg unit (Figures 2G and 3).

Measures of cognitive function, psychoactivity, and mood were not significantly different after inhalation of vaporized cannabis versus CTRL (Table 6 and Figures E3 and E4). Compared with the pretreatment condition, inhalation of cannabis was associated with modest and statistically significant decreases in ratings of anxiety and increases in ratings of feeling drunk, feeling stoned, feeling high, experiencing good drug effects, experiencing

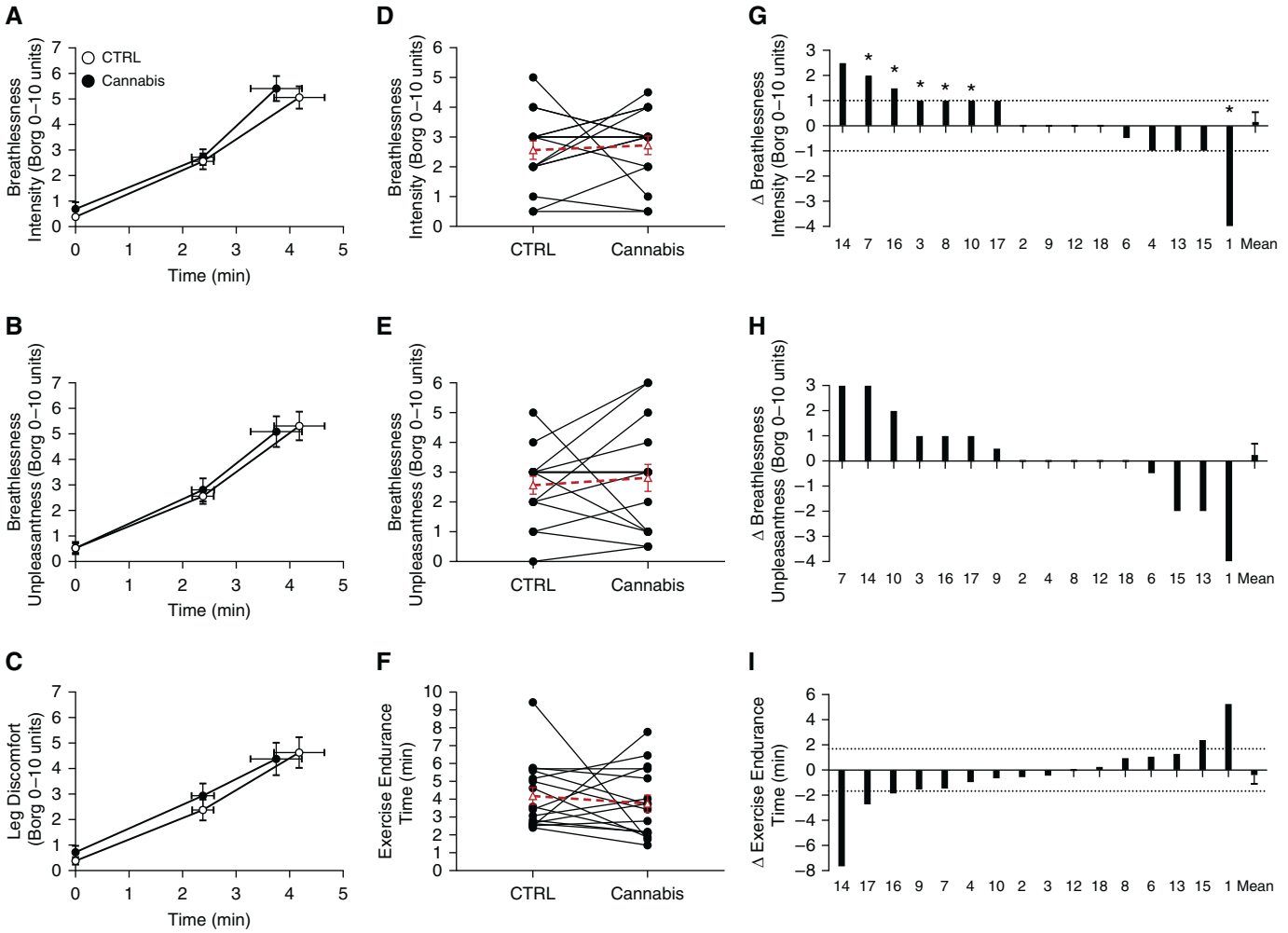

Figure 2. Effect of inhaled vaporized cannabis versus control (CTRL) on exertional breathlessness and exercise endurance in adults with advanced chronic obstructive pulmonary disease. (*A*) Mean ± SEM breathlessness intensity ratings, (*B*) breathlessness unpleasantness ratings, and (*C*) intensity ratings of leg discomfort at rest and during symptom-limited constant-load cycle exercise testing at 75% of peak incremental power output. (*D* and *G*) Individual participant post-treatment values and post-treatment differences in breathlessness intensity ratings during exercise at isotime, (*E* and *H*) breathlessness unpleasantness ratings during exercise at isotime, and (*F* and *I*) exercise endurance time, where red symbols with dashed horizontal lines in panels *D–F* denote means ± SEM. Dashed horizontal lines in panels *G* and *I* denote minimally clinically important differences for breathlessness intensity (37) and exercise endurance time (38). Δ = post-treatment difference (cannabis − CTRL). *Participants who coughed after inhalation of vaporized cannabis.

bad drug effects, and liking the drug effects. In contrast, psychoactivity and mood ratings were not different before versus after inhalation of CTRL.

A participant experienced vasovagal syncope during the 2-minute venous blood-sampling period of the cannabis visit. After a few hours of rest while under medical observation, the participant was permitted to go home. Both the study physician and data safety committee determined that this adverse event was most likely due to the blood-sampling procedure itself and not inhalation of cannabis.

Discussion

This randomized controlled trial is the first to demonstrate that single-dose inhalation of vaporized cannabis versus CTRL had no effect on exertional breathlessness, exercise endurance, and airway function in symptomatic adults with advanced COPD receiving dual- or triple-inhalation therapy for management of their underlying pulmonary pathophysiology.

We administered 35 mg of dried herbal cannabis containing 18.2% THC, a dose comparable to that used in earlier studies by Vachon and colleagues (14) and Tashkin and colleagues (15–18) wherein smoked, aerosolized, and orally administered THC induced bronchodilation in adults with and without asthma. Despite using a similar dose, inhaled vaporized cannabis did not enhance static and dynamic airway function in our participants with advanced COPD.

We offer the following explanations for the lack of effect of inhaled vaporized cannabis versus CTRL on airway function and, by extension, exertional breathlessness and EET in our trial. First, previous

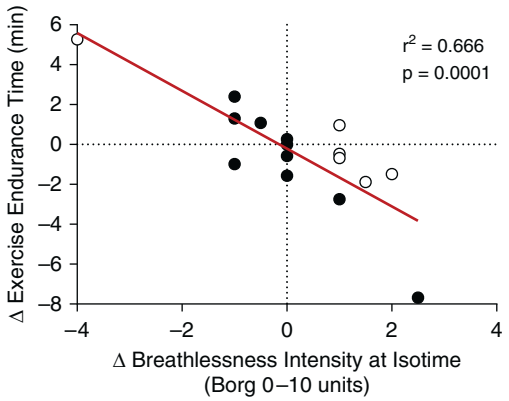

Figure 3. Relationship between post-treatment differences in breathlessness intensity ratings during exercise at isotime and exercise endurance time in adults with advanced chronic obstructive pulmonary disease. Open circles denote participants who coughed after inhalation of vaporized cannabis. Δ = post-treatment difference (cannabis − control).

studies reporting bronchodilation after administration of smoked cannabis used "blended natural marijuana" assayed at 1% or 2% THC (16–18). It is unclear if these cannabis preparations were devoid of other cannabinoids (e.g., CBD, cannabinol) that may have had a direct bronchodilator effect and/or facilitated the bronchodilator effect of THC. However, this is unlikely as large doses (up to 1,200 mg) of orally administered CBD and cannabinol, in the absence of THC, did not induce bronchodilation in healthy men when compared with placebo (39). Second, previous studies that have demonstrated a bronchodilator effect of smoked cannabis used a uniform smoking procedure that consisted of "smoking deeply" over 2–4 seconds followed by a 15-second breathhold (16–18). To standardize drug delivery we utilized the Foltin puff procedure, where participants were instructed to inhale the vaporized cannabis for 5 seconds and to hold the vapor in their lungs for 10 seconds. It is possible that relatively shallower inhalations and shorter breathholding times used in our trial might have diminished the potential positive effects of inhaled THC on static and dynamic airway function in our participants. Third, adults with COPD have abnormal airway geometry and fewer terminal bronchioles compared with their healthy counterparts (40–42). Therefore, limited delivery of vaporized THC into the airways and lungs of our participants may explain our null results. Structural abnormalities of the tracheobronchial tree in our participants may also account for the lower observed peak plasma THC levels of approximately 14 ng/ml versus approximately 45 ng/ml reported by Ware and colleagues (43) in adults with neuropathic pain after single-dose inhalation (smoked) of a comparatively low dose of 25 mg of dried herbal cannabis containing 9.4% THC. Our relatively low peak plasma THC levels may also reflect the vaporization temperature of 190°C used in this trial. Pomahacova and colleagues (44) reported that vaporizing dried herbal cannabis at 230°C versus 185°C produced a vapor with a threefold higher yield of THC. Finally, all of our participants were receiving inhaled dual or triple therapy for management of their COPD, while six participants used their short-acting inhaled β_2-agonist (SABA) bronchodilator 3.5 ± 1.7 and 4.2 ± 1.3 hours before Visits 2 and 3, respectively. It is unlikely that the SABA used by six of our 16 participants significantly altered the effect of inhaled vaporized cannabis airway physiology, breathlessness, and EET, particularly as the duration of efficacy of the SABA is 3–4 hours. Indeed, we found no significant effect of inhaled vaporized cannabis versus CTRL on spirometry and iOS-derived pulmonary function parameters at rest in participants with COPD who used their SABA versus those who did not (data not shown).

We observed a negative correlation between the cannabis-induced change in exertional breathlessness intensity ratings at isotime and EET. We identified four cannabis responders (participants with cannabis-induced relief of exertional breathlessness at isotime by the MCID of ⩾1 Borg unit) and 12 nonresponders.

Table 4. Effect of inhaled vaporized cannabis versus control on spirometry and impulse oscillometry–derived pulmonary function test parameters at rest in adults with advanced chronic obstructive pulmonary disease*

	Control		Cannabis	
	Pretreatment	**Post-treatment**	**Pretreatment**	**Post-treatment**
Spirometry				
FVC, L	2.87 ± 0.91	2.93 ± 0.85	2.94 ± 0.91	2.90 ± 0.90
FEV_1, L	0.89 ± 0.26	0.89 ± 0.26	0.89 ± 0.25	0.89 ± 0.24
FEV_1/FVC, %	32 ± 8	31 ± 7	32 ± 9	32 ± 6
$FEF_{25–75\%}$, $L \cdot s^{-1}$	0.26 ± 0.06	0.26 ± 0.07	0.26 ± 0.07	0.26 ± 0.07
PEF, $L \cdot s^{-1}$	2.81 ± 0.82	2.59 ± 0.87	2.60 ± 0.78	2.62 ± 0.84
Impulse oscillometry				
R_5, $kPa \cdot L^{-1} \cdot s$	0.60 ± 0.18	0.59 ± 0.24	0.60 ± 0.14	0.58 ± 0.17
R_{20}, $kPa \cdot L^{-1} \cdot s$	0.34 ± 0.08	0.34 ± 0.13	0.34 ± 0.05	0.33 ± 0.06
X_5, $kPa \cdot L^{-1} \cdot s$	−0.35 ± 0.15	−0.34 ± 0.16	−0.35 ± 0.15	−0.34 ± 0.16
F_{res}, $1 \cdot s^{-1}$	23.9 ± 4.1	24.0 ± 5.1	22.6 ± 3.7	23.3 ± 4.2
A_X, $kPa \cdot L^{-1}$	3.04 ± 1.76	3.04 ± 1.99	2.85 ± 1.55	2.88 ± 1.81

Definition of abbreviations: A_X = area of reactance; $FEF_{25–75\%}$ = forced expiratory flow at 25–75% of the FVC maneuver; FEV_1 = forced expiratory volume in 1 second; F_{res} = resonant frequency; FVC = forced vital capacity; PEF = peak expiratory flow; R_5 and R_{20} = resistance at 5 and 20 Hz, respectively; X_5 = reactance at 5 Hz.
*Values represent means ± SD.

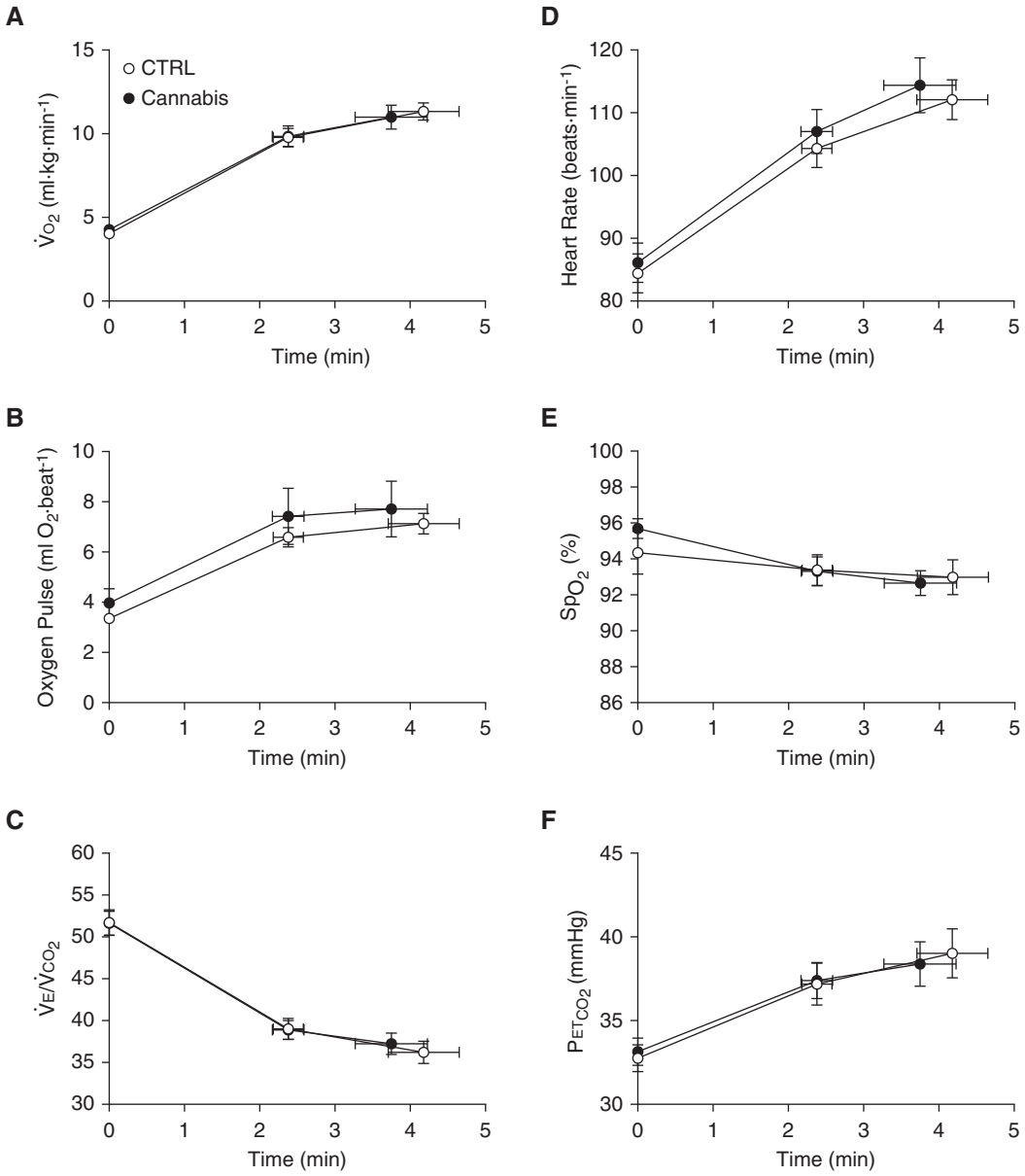

Figure 4. Effect of inhaled vaporized cannabis versus control (CTRL) on (*A*) oxygen consumption, (*B*) oxygen pulse, (*C*) the ventilatory equivalent for carbon dioxide, (*D*) heart rate, (*E*) oxygen saturation, and (*F*) the partial pressure of end-tidal carbon dioxide during symptom-limited constant-load cycle exercise testing at 75% of peak incremental power output in adults with advanced chronic obstructive pulmonary disease. Data are presented as means ± SEM. $P_{ET_{CO_2}}$ = end-tidal (partial) carbon dioxide pressure; Sp_{O_2} = oxygen saturation by pulse oximetry; $\dot{V}_E/\dot{V}_{CO_2}$ = ventilatory equivalent for carbon dioxide; $\dot{V}_{O_2}$ = oxygen consumption.

Importantly, five of the nonresponders coughed after inhalation of vaporized cannabis and reported clinically significant worsening of their exertional breathlessness at isotime after inhalation of cannabis versus CTRL (Figure 2G). Tashkin and colleagues (15) similarly reported that inhalation of 5 and 10 mg of aerosolized THC provoked a cough in four of five patients with asthma, two of whom exhibited THC-induced bronchospasm. Therefore, the cough induced by vaporized cannabis in five of the 12 nonresponders could have masked a potentially positive effect of inhaled vaporized cannabis versus CTRL on airway function, exertional breathlessness, and EET in our participants. The mechanisms mediating the THC-induced cough reflex are not fully understood. Previous studies have demonstrated that CB_1 receptor agonists may inhibit or induce bronchospasm; this dual effect of CB_1 receptor activation on bronchial responsiveness is dependent on cholinergic tone (45). As all of our participants were receiving at least

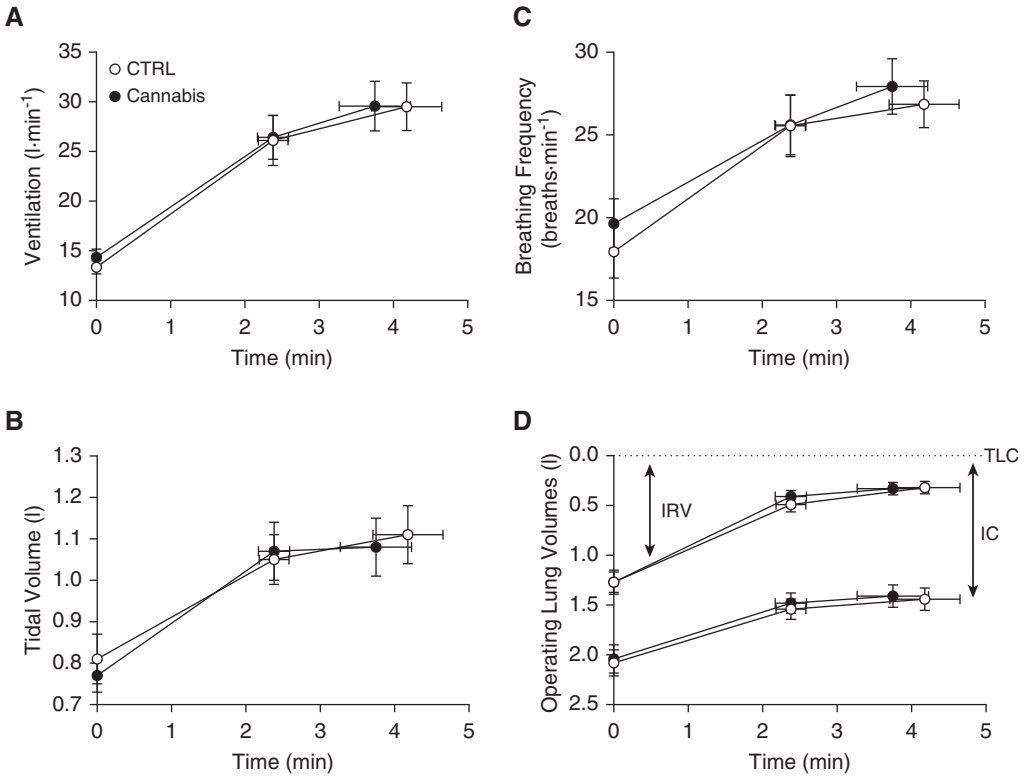

Figure 5. Effect of inhaled vaporized cannabis versus control (CTRL) on (*A*) minute ventilation, (*B*) tidal volume, (*C*) breathing frequency, and (*D*) dynamic operating lung volume responses during symptom-limited constant-load cycle exercise testing at 75% of peak incremental power output in adults with advanced chronic obstructive pulmonary disease. Data are presented as means ± SEM. IC = inspiratory capacity; IRV = inspiratory reserve volume; TLC = total lung capacity.

dual-inhalation therapy for management of their COPD, we cannot rule out the possibility that differences in bronchial smooth muscle tone may have contributed to the observed heterogeneity in the cough reflex elicited by inhalation of vaporized cannabis. Future studies should evaluate the effect of inhaled vaporized cannabis on airway function, exertional breathlessness, and EET in adults with COPD receiving anticholinergic bronchodilator therapy versus those who are not.

Neuroimaging studies evaluating the effect of cannabis on pain have demonstrated altered activity in brain regions (46) associated with negative affect and implicated in the perception of breathlessness (47), particularly its affective (unpleasantness) dimension. To this end, cannabis could alter the central perception of breathlessness and improve EET by reducing negative affect and/or increasing feelings of euphoria. Indeed, earlier studies demonstrating cannabis-induced bronchodilation often reported concomitant psychoactive effects, particularly a feeling of being "high" within minutes of treatment administration (15–18). Importantly, these studies reported a greater degree of intoxication after administration of smoked cannabis (i.e., the degree of "high" was rated ∼6 on a 7-point scale) relative to that observed in our participants after inhalation of vaporized cannabis (i.e., the degree of "high" was rated ∼4.8 on a 100-mm visual analog scale) (17). The low peak plasma THC levels achieved in our study likely account for the relatively modest effects of inhaled vaporized cannabis on psychoactivity. Nevertheless, we observed a modest but significant within-treatment effect (i.e., pre- to post-treatment) of inhaled vaporized cannabis on psychoactivity, including decreased ratings of anxiety and increased ratings of feeling high, drunk, and stoned. It is possible that the potentially positive effects of this altered psychoactive state on exertional breathlessness and EET may have been confounded by the cough reflex and its effect on exertional breathlessness exhibited in some of our participants after inhalation of vaporized cannabis. Moreover, a preliminary study of five adults with mild-to-moderate COPD by Pickering and colleagues (48) reported that sublingual administration of Sativex—a cannabis-based medicinal extract containing both THC and CBD—reduced the selection frequency of respiratory descriptors associated with air hunger, an inherently unpleasant form of breathlessness (49). By contrast, we observed no effect of inhaled vaporized cannabis versus CTRL on unpleasantness ratings of exertional breathlessness and the selection frequency of breathlessness descriptors at end-exercise.

Table 5. Pharmacokinetics of inhaled vaporized cannabis versus control in adults with advanced chronic obstructive pulmonary disease*

Metabolite	Control					Cannabis				
	Pretreatment	2 min	30 min	75 min	180 min	Pretreatment	2 min	30 min	75 min	180 min
THC, ng · ml^{-1}	—	0.82 ± 0.55	0.05 ± 0.10	—	—	—	13.91 ± 6.16†	2.18 ± 0.96‡	0.79 ± 0.44	0.14 ± 0.16
THCA, ng · ml^{-1}	—	—	—	—	—	—	0.70 ± 0.27	0.30 ± 0.15	0.09 ± 0.12	—
11-OH-THC, ng · ml^{-1}	—	—	—	—	—	—	0.87 ± 0.71	0.56 ± 0.41	0.29 ± 0.22	0.06 ± 0.10
THC-COOH, ng · ml^{-1}	—	0.08 ± 0.22	0.21 ± 0.28	0.14 ± 0.23	0.10 ± 0.21	—	1.54 ± 1.38†	3.05 ± 1.95†	2.23 ± 1.57†	1.37 ± 1.04†
CBD, ng · ml^{-1}	—	1.36 ± 0.84	0.19 ± 0.19	0.04 ± 0.10	—	—	—	—	—	—

Definition of abbreviations: 11-OH-THC = 11-hydroxy-Δ^9-tetrahydrocannabinol; CBD = cannabidiol; THC = Δ^9-tetrahydrocannabinol; THCA = *trans*-Δ^9-tetrahydrocannabinol-9-acid A; THC-COOH = 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol.

*Values represent means ± SD.

†$P < 0.0001$ in control versus cannabis.

‡$P < 0.01$ in control versus cannabis.

Earlier studies demonstrating cannabis-induced bronchodilation in healthy adults and adults with asthma often reported a concomitant increase in heart rate that was sustained for approximately 60 minutes after inhalation (15–17). In contrast to these findings, we did not observe a significant effect of inhaled vaporized cannabis versus CTRL on heart rate, presumably due to the relatively low plasma levels of THC.

Methodological Considerations

The generalizability of our results is restricted to a small and relatively homogeneous group of clinically stable and symptomatic adults with advanced COPD. Larger randomized clinical trials with more participants are needed to draw definitive conclusions regarding the effect of inhaled vaporized cannabis on exertional breathlessness, EET, and cardiopulmonary physiological parameters in adults with COPD.

We caution against the extrapolation of our results to other doses, modes (e.g., smoked, oral), types (e.g., various THC:CBD ratios), and regimens (e.g., repeat-dose) of cannabis dispensation in this patient population.

In our study, inhaled vaporized cannabis had a modest but significant within-treatment effect on some measures of psychoactivity. Future studies should utilize existing cannabinoid preparations (e.g., CBD) that do not affect psychoactivity but act on cannabinoid receptors to assess changes in airway function, exertional breathlessness, and EET in COPD.

The dried herbal cannabis material used in the CTRL arm of our trial may not have represented a "true" placebo as it contained trace amounts of CBD (<1%) that were detected in the plasma 2 minutes after vaporization. Furthermore, 12 of the 16 participants correctly identified the visit at which they received cannabis, with four of these 12 participants citing a noticeable difference in taste/smell of the inhaled vapor between cannabis and CTRL visits. Thus, a placebo devoid of THC and CBD and with the same taste and smell as the active cannabis should be identified for use in future trials

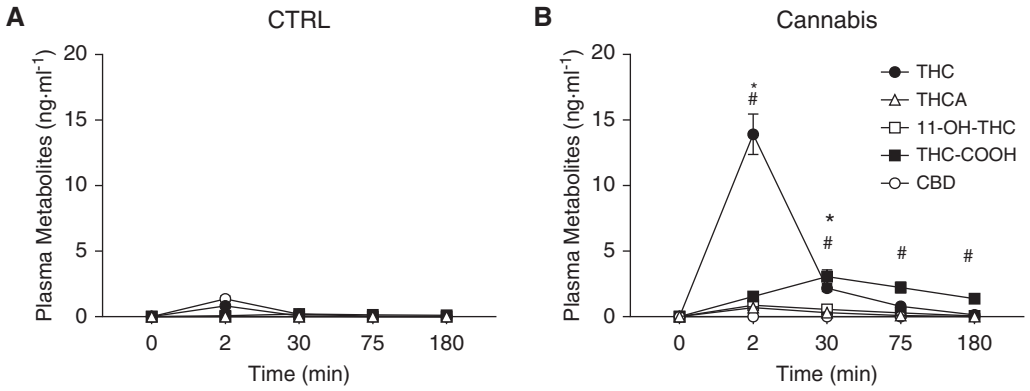

Figure 6. Effect of inhaled vaporized (*A*) control (CTRL) and (*B*) cannabis on blood biochemistry in adults with advanced chronic obstructive pulmonary disease. Data are presented as means ± SEM. 11-OH-THC = 11-hydroxy-Δ^9-tetrahydrocannabinol; CBD = cannabidiol; THC = Δ^9-tetrahydrocannabinol; THCA = *trans*-Δ^9-tetrahydrocannabinol-9-acid A; THC-COOH = 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol. *Significant difference in CTRL versus THC for plasma THC and #significant difference in CTRL versus THC for plasma THC-COOH ($P < 0.05$).

Conclusions

In 2015, the American Thoracic Society Marijuana Workgroup highlighted a need for controlled studies to evaluate the clinical effects of inhaled vaporized cannabis on lung disease, sleep, and critical illness (9). In response to this call for research, our randomized controlled trial is the first to demonstrate that 35 mg of inhaled vaporized cannabis containing 18.2% THC had no clinically meaningful positive or negative effect on exertional breathlessness, exercise endurance, and airway function in symptomatic adults with advanced COPD receiving dual- or triple-inhalation therapy for management of their underlying pulmonary pathophysiology. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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Table 6. Effect of inhaled vaporized cannabis versus control on cognitive function, mood, and psychoactivity in adults with advanced chronic obstructive pulmonary disease*

	Control		Cannabis	
	Pretreatment	**Post-treatment**	**Pretreatment**	**Post-treatment**
Mini-Mental State Examination, out of 30	29.6 ± 0.5	29.7 ± 0.6	29.4 ± 0.9	29.6 ± 0.8
Mood Effects, 100-mm VAS				
Sad/Happy	89.5 ± 13.5	87.9 ± 12.4	89.1 ± 13.9	89.9 ± 13.3
Anxious/Relaxed	83.7 ± 22.6	89.4 ± 10.8	80.5 ± 23.3	84.7 ± 25.3
Jittery/Calm	85.2 ± 17.8	91.5 ± 8.1	81.4 ± 22.9	86.9 ± 21.1
Bad/Good	91.2 ± 10.4	89.9 ± 10.6	90.1 ± 10.2	89.9 ± 12.5
Paranoid/Self-assured	94.0 ± 6.5	92.8 ± 8.0	90.4 ± 12.8	91.6 ± 11.4
Fearful/Unafraid	90.9 ± 15.3	93.1 ± 8.1	91.9 ± 11.4	94.1 ± 6.4
Psychoactive Effects, 100-mm VAS				
Down	13.2 ± 19.1	11.6 ± 19.3	15.0 ± 19.8	12.4 ± 2.5
Anxious	9.8 ± 12.4	9.1 ± 13.8	17.6 ± 18.0	8.2 ± 1.2†
Hungry	13.2 ± 16.9	16.2 ± 18.9	12.6 ± 16.2	11.4 ± 1.5
Sedated	8.6 ± 17.0	8.5 ± 14.3	8.6 ± 18.1	8.4 ± 8.7
Impaired	5.2 ± 7.9	4.4 ± 4.6	5.5 ± 7.9	8.4 ± 1.2
Drunk	2.5 ± 2.8	3.2 ± 3.7	1.6 ± 1.6	4.5 ± 4.3†
Stoned	2.7 ± 3.1	3.7 ± 3.6	1.6 ± 1.5	6.3 ± 5.6†
High	2.8 ± 3.4	3.8 ± 3.9	1.9 ± 2.1	4.8 ± 4.5†
Good drug effects	2.4 ± 3.2	5.3 ± 7.0	1.8 ± 2.1	17.6 ± 27.8†
Bad drug effects	2.1 ± 3.0	3.2 ± 3.3	1.5 ± 1.8	4.0 ± 4.6†
Do you like the drug effects	2.1 ± 3.1	6.9 ± 12.0	2.1 ± 2.8	15.3 ± 27.7†

Definition of abbreviation: VAS = visual analog scale.
*Values represent mean ± SD.
†$P < 0.05$ versus pretreatment within condition.

References

1 O'Donnell DE, Guenette JA, Maltais F, Webb KA. Decline of resting inspiratory capacity in COPD: the impact on breathing pattern, dyspnea, and ventilatory capacity during exercise. *Chest* 2012;141: 753–762.
2 O'Donnell DE, Ora J, Webb KA, Laveneziana P, Jensen D. Mechanisms of activity-related dyspnea in pulmonary diseases. *Respir Physiol Neurobiol* 2009;167:116–132.
3 Nishimura K, Izumi T, Tsukino M, Oga T. Dyspnea is a better predictor of 5-year survival than airway obstruction in patients with COPD. *Chest* 2002;121:1434–1440.
4 Oga T, Nishimura K, Tsukino M, Sato S, Hajiro T. Analysis of the factors related to mortality in chronic obstructive pulmonary disease: role of exercise capacity and health status. *Am J Respir Crit Care Med* 2003; 167:544–549.
5 Müllerová H, Lu C, Li H, Tabberer M. Prevalence and burden of breathlessness in patients with chronic obstructive pulmonary disease managed in primary care. *PLoS One* 2014;9: e85540.
6 Small M, Holbrook T, Wood R, Müllerová H, Naya I, Punekar YS. Prevalence and burden of dyspnoea among COPD patients in Japan. *Int J Clin Pract* 2016;70:676–681.
7 Sundh J, Ekström M. Persistent disabling breathlessness in chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis* 2016;11:2805–2812.
8 Chen S, Small M, Lindner L, Xu X. Symptomatic burden of COPD for patients receiving dual or triple therapy. *Int J Chron Obstruct Pulmon Dis* 2018;13:1365–1376.
9 Douglas IS, Albertson TE, Folan P, Hanania NA, Tashkin DP, Upson DJ, *et al*. Implications of marijuana decriminalization on the practice of pulmonary, critical care, and sleep medicine: a report of the American Thoracic Society Marijuana Workgroup. *Ann Am Thorac Soc* 2015;12: 1700–1710.
10 Lynch ME, Ware MA. Cannabinoids for the treatment of chronic non-cancer pain: an updated systematic review of randomized controlled trials. *J Neuroimmune Pharmacol* 2015;10:293–301.
11 Galiègue S, Mary S, Marchand J, Dussossoy D, Carrière D, Carayon P, *et al*. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur J Biochem* 1995;232:54–61.
12 Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 1993;365:61–65.
13 Grassin-Delyle S, Naline E, Buenestado A, Faisy C, Alvarez JC, Salvator H, *et al*. Cannabinoids inhibit cholinergic contraction in human airways through prejunctional CB1 receptors. *Br J Pharmacol* 2014;171:2767–2777.
14 Vachon L, FitzGerald MX, Solliday NH, Gould IA, Gaensler EA. Single-dose effects of marihuana smoke: bronchial dynamics and respiratory-center sensitivity in normal subjects. *N Engl J Med* 1973;288: 985–989.
15 Tashkin DP, Reiss S, Shapiro BJ, Calvarese B, Olsen JL, Lodge JW. Bronchial effects of aerosolized Δ^9-tetrahydrocannabinol in healthy and asthmatic subjects. *Am Rev Respir Dis* 1977;115:57–65.
16 Tashkin DP, Shapiro BJ, Frank IM. Acute pulmonary physiologic effects of smoked marijuana and oral Δ^9-tetrahydrocannabinol in healthy young men. *N Engl J Med* 1973;289:336–341.
17 Tashkin DP, Shapiro BJ, Frank IM. Acute effects of smoked marijuana and oral Δ^9-tetrahydrocannabinol on specific airway conductance in asthmatic subjects. *Am Rev Respir Dis* 1974;109:420–428.
18 Tashkin DP, Shapiro BJ, Lee YE, Harper CE. Effects of smoked marijuana in experimentally induced asthma. *Am Rev Respir Dis* 1975; 112:377–386.
19 Morris MA, Jacobson SR, Kinney GL, Tashkin DP, Woodruff PG, Hoffman EA, *et al*. Marijuana use associations with pulmonary symptoms and function in tobacco smokers enrolled in the Subpopulations and Intermediate Outcome Measures in COPD Study (SPIROMICS). *Chronic Obstr Pulm Dis* 2018;5:46–56.
20 McGavin CR, Artvinli M, Naoe H, McHardy GJ. Dyspnoea, disability, and distance walked: comparison of estimates of exercise performance in respiratory disease. *BMJ* 1978;2:241–243.
21 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;54:581–586.
22 Jones PW, Harding G, Berry P, Wiklund I, Chen WH, Kline Leidy N. Development and first validation of the COPD Assessment Test. *Eur Respir J* 2009;34:648–654.
23 Zigmond AS, Snaith RP. The Hospital Anxiety and Depression Scale. *Acta Psychiatr Scand* 1983;67:361–370.
24 Kurlowicz L, Wallace M. The Mini Mental State Examination (MMSE). *Director* 1999;7:62.
25 Wilsey B, Marcotte T, Deutsch R, Gouaux B, Sakai S, Donaghe H. Low-dose vaporized cannabis significantly improves neuropathic pain. *J Pain* 2013;14:136–148.
26 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;195:557–582.
27 Chait LD, Corwin RL, Johanson CE. A cumulative dosing procedure for administering marijuana smoke to humans. *Pharmacol Biochem Behav* 1988;29:553–557.
28 Macintyre N, Crapo RO, Viegi G, Johnson DC, van der Grinten CP, Brusasco V, *et al*. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J* 2005;26: 720–735.
29 Miller MR, Crapo R, Hankinson J, Brusasco V, Burgos F, Casaburi R, *et al*.; ATS/ERS Task Force. General considerations for lung function testing. *Eur Respir J* 2005;26:153–161.
30 Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, *et al*.; ATS/ERS Task Force. Standardisation of spirometry. *Eur Respir J* 2005;26:319–338.
31 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;26:511–522.
32 Jensen D, Alsuhail A, Viola R, Dudgeon DJ, Webb KA, O'Donnell DE. Inhaled fentanyl citrate improves exercise endurance during high-intensity constant work rate cycle exercise in chronic obstructive pulmonary disease. *J Pain Symptom Manage* 2012;43:706–719.
33 Abdallah SJ, Wilkinson-Maitland C, Saad N, Li PZ, Smith BM, Bourbeau J, *et al*. Effect of morphine on breathlessness and exercise endurance in advanced COPD: a randomised crossover trial. *Eur Respir J* 2017; 50:1701235.
34 Borg GA. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 1982;14:377–381.
35 Puente-Maestu L, Palange P, Casaburi R, Laveneziana P, Maltais F, Neder JA, *et al*. Use of exercise testing in the evaluation of interventional efficacy: an official ERS statement. *Eur Respir J* 2016; 47:429–460.
36 Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behav Res Methods* 2009;41:1149–1160.
37 Ries AL. Minimally clinically important difference for the UCSD Shortness of Breath Questionnaire, Borg Scale, and Visual Analog Scale. *COPD* 2005;2:105–110.
38 Puente-Maestu L, Villar F, de Miguel J, Stringer WW, Sanz P, Sanz ML, *et al*. Clinical relevance of constant power exercise duration changes in COPD. *Eur Respir J* 2009;34:340–345.
39 Gong H Jr, Tashkin DP, Simmons MS, Calvarese B, Shapiro BJ. Acute and subacute bronchial effects of oral cannabinoids. *Clin Pharmacol Ther* 1984;35:26–32.
40 Kirby M, Tanabe N, Tan WC, Zhou G, Obeidat M, Hague CJ, *et al*.; CanCOLD Collaborative Research Group; Canadian Respiratory Research Network. Total airway count on computed tomography and the risk of chronic obstructive pulmonary disease progression: findings from a population-based study. *Am J Respir Crit Care Med* 2018;197:56–65.
41 McDonough JE, Yuan R, Suzuki M, Seyednejad N, Elliott WM, Sanchez PG, *et al*. Small-airway obstruction and emphysema in chronic obstructive pulmonary disease. *N Engl J Med* 2011;365: 1567–1575.

42 Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, *et al*. The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med* 2004;350:2645–2653.
43 Ware MA, Wang T, Shapiro S, Robinson A, Ducruet T, Huynh T, *et al*. Smoked cannabis for chronic neuropathic pain: a randomized controlled trial. *CMAJ* 2010;182:E694–E701.
44 Pomahacova B, Van der Kooy F, Verpoorte R. Cannabis smoke condensate. III. The cannabinoid content of vaporised *Cannabis sativa*. *Inhal Toxicol* 2009;21:1108–1112.
45 Calignano A, Kátona I, Désarnaud F, Giuffrida A, La Rana G, Mackie K, *et al*. Bidirectional control of airway responsiveness by endogenous cannabinoids. *Nature* 2000;408:96–101.
46 Bhattacharyya S, Morrison PD, Fusar-Poli P, Martin-Santos R, Borgwardt S, Winton-Brown T, *et al*. Opposite effects of Δ^9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. *Neuropsychopharmacology* 2010;35:764–774.
47 Herigstad M, Hayen A, Wiech K, Pattinson KT. Dyspnoea and the brain. *Respir Med* 2011;105:809–817.
48 Pickering EE, Semple SJ, Nazir MS, Murphy K, Snow TM, Cummin AR, *et al*. Cannabinoid effects on ventilation and breathlessness: a pilot study of efficacy and safety. *Chron Respir Dis* 2011;8:109–118.
49 Banzett RB, Pedersen SH, Schwartzstein RM, Lansing RW. The affective dimension of laboratory dyspnea: air hunger is more unpleasant than work/effort. *Am J Respir Crit Care Med* 2008;177:1384–1390.

Appendix V

Opioids for breathlessness: Psychological and neural factors influencing response variability

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"Take home" message: Diminished opioid efficacy in the treatment of breathlessness was related to negative affect and anticipatory brain activity in the anterior cingulate and medial prefrontal cortex.

ABSTRACT

Effective management of distressing bodily symptoms (such as pain and breathlessness) is an important clinical goal. However, extensive variability exists in both symptom perception and treatment response. One theory for understanding variability in bodily perception is the 'Bayesian Brian Hypothesis', whereby symptoms may result from the combination of sensory inputs and prior expectations. In light of this theory, we explored the relationships between opioid responsiveness, behavioural/physiological symptom modulators and brain activity during anticipation of breathlessness.

Methods. We utilised two existing opioid datasets to investigate the relationship between opioid efficacy and physiological/behavioural qualities, employing hierarchical cluster analyses in: 1) a clinical study in chronic obstructive pulmonary disease, and 2) a functional magnetic resonance brain imaging study of breathlessness in healthy volunteers. We also investigated how opioid efficacy relates to anticipatory brain activity using linear regression in the healthy volunteers.

Results. Consistent across both datasets, diminished opioid efficacy was more closely associated with negative affect than with other physiological and behavioural properties. Furthermore, in healthy individuals, brain activity in the anterior cingulate and ventromedial prefrontal cortices during anticipation of breathlessness were inversely correlated with opioid effectiveness.

Conclusion. Diminished opioid efficacy for relief of breathlessness may be associated with high negative affective qualities, and was correlated with the magnitude of brain activity during anticipation of breathlessness.

Clinical implications. Negative affect and symptom expectations may influence perceptual systems to become less responsive to opioid therapy.

INTRODUCTION

Chronic breathlessness is a multidimensional and aversive symptom, which is often poorly explained by underlying pathophysiology (1). For many patients, breathlessness is refractory to maximal medical therapies targeting disease processes (2). However, opioids are thought to be a possible therapeutic avenue to treat symptomology independently of disease (3, 4). Importantly, research in other aversive symptoms (such as chronic pain) has demonstrated that qualities such as anxiety and depression (collectively termed *negative affect* here) can both exacerbate symptoms (5) and reduce opioid efficacy (6, 7). Therefore, it may be pertinent to consider such behavioural factors when contemplating the use of opioids for breathlessness.

According to the Bayesian Brain Hypothesis, perception (e.g., breathlessness) is the result of a delicate balance between the brain's set of expectations and beliefs (collectively known as *priors*), and incoming sensory information (8, 9). An individual's priors are shaped by previous experiences and learned behaviours. For example, if climbing a flight of stairs triggers severe breathlessness, an individual may "expect" to experience severe breathlessness during subsequent stair climbing. Negative affect may act as a moderator within this perceptual system (8-11), altering the balance between priors and sensory inputs to influence and potentially exacerbate symptom perception.

In a recent clinical study, Abdallah et al. (4) demonstrated that 11 of 20 (55%) adults with advanced chronic obstructive pulmonary disease (COPD) reported clinically significant relief (defined as a decrease by $\geq$ 1 Borg units) of exertional breathlessness following single-dose administration of immediate-release oral morphine. While the authors were unable to elucidate the physiological mechanisms underlying opioid response variability, they speculated that unmeasured differences in "*conditioned anticipatory/associative learning*" might play a role.

Therefore, the aim of the present study was to better understand the mechanisms underlying variability in opioid responsiveness for the relief of breathlessness. We have reanalysed data from 1) Abdallah et al. (4) and 2) a behavioural and functional

neuroimaging dataset in healthy volunteers by Hayen et al. (12), where the perception of laboratory-induced breathlessness was manipulated with the opioid remifentanil. As with Abdallah et al. (4), Hayen et al. (12) observed variability in responsiveness to opioid therapy, with 9 of 19 (47%) subjects reporting a remifentanil-induced decrease in experimentally-induced breathlessness by ≥10 mm on a 100-mm visual analogue scale (VAS). This parallel approach allowed us to verify associations observed in a clinical population in an independent sample that were free of the confounds of chronic disease, and to shed light on associated changes in brain activity with opioid efficacy. Furthermore, the healthy volunteer dataset allowed us to test the hypotheses presented in Abdallah et al. (4) regarding how potential differences in conditioned anticipatory/associative learning might influence variability in the effect of opioids upon breathlessness.

METHODS

A brief overview of the study methods is provided here. Full methodological details can be found in the supplementary material found in pages 21 to 43.

COPD exercise study:

Twenty participants with severe COPD (mean±SD forced expiratory volume in 1 sec % predicted (FEV_1%predicted): 35±9) completed two sessions (morphine 0.1 mg/kg or placebo – randomized order and double-blinded), where physiological and perceptual parameters were measured during constant-load cardiopulmonary cycle exercise testing at 75% of peak incremental cycle power output. Intensity and unpleasantness of breathlessness were rated using Borg's modified 0-10 category ratio scale at rest and during exercise (13). Specifically, subjects were asked "How intense is your sensation of breathing overall?" and "How unpleasant or bad does your breathing make you feel?" (see online data supplement for details). Data were analyzed at isotime, defined as the highest equivalent 2-min interval of exercise completed by a given participant during constant-load cardiopulmonary cycle exercise tests performed after treatment with oral morphine and placebo. The change in all scores were calculated as opioid minus placebo. Participants were also characterized using questionnaires listed in **Table 1.**

Table 1. Variables included in the hierarchical cluster analysis.

	COPD dataset (from Abdallah et al. (4))	Healthy dataset (from Hayen et al. (12))
Physiological and subjective measures during the placebo saline conditions	Sex Forced expiratory volume in 1-second Cigarette smoking history (pack years) Isotime tidal volume (placebo) Isotime breathing frequency (placebo) Iostime breathlessness unpleasantness (placebo) Isotime breathlessness intensity (placebo)	Sex Discontentment (saline) Tension (saline) Sedation (saline) Mouth pressure (mild load; saline) Mouth pressure (strong load; saline) Anticipation mouth pressure (mild load; saline) Anticipation mouth pressure (strong load; saline) Breathlessness intensity (mild load; saline) Breathlessness unpleasantness (mild load; saline) Breathlessness intensity (strong load; saline) Breathlessness unpleasantness (strong load;
Breathlessness and psychological affective measures	Oxygen cost diagram Modified medical research council scale COPD assessment test – Dyspnea item COPD assessment test activity – Limitation item Hospital anxiety and depression scale – Depression subscale Hospital anxiety and depression scale – Anxiety subscale	Spielberger state-trait anxiety inventory Anxiety sensitivity index The revised Center for Epidemiological Studies The Positive Affect Negative Affect Schedule – The Positive Affect Negative Affect Schedule – The Thought Control Questionnaire – Worry The Thought Control Questionnaire – Reappraisal The Thought Control Questionnaire – Social Locus of control inventory – Internal control Locus of control inventory – Chance control Locus of control inventory – Others in control The defence style questionnaire – Neuroticism
Physiological and subjective responses to opioid administration	Δ Tidal volume Δ Breathing frequency Δ Isotime breathlessness unpleasantness Δ Isotime breathlessness intensity Plasma morphine Plasma morphine-6-glucuronide	Δ Discontentment Δ Tension Δ Sedation Δ Mouth pressure (mild load) Δ Mouth pressure (strong load) Δ Anticipation mouth pressure (mild load) Δ Anticipation mouth pressure (strong load) Δ Breathlessness intensity (mild load) Δ Breathlessness unpleasantness (mild load) Δ Breathlessness intensity (strong load) Δ Breathlessness unpleasantness (strong load)

FMRI study in healthy volunteers:

In the original study (12), 19 healthy participants underwent two functional magnetic resonance imaging (FMRI) scans (3T Siemens Trio scanner), on two separate visits. An intravenous infusion of either remifentanil or saline placebo was delivered in a counterbalanced, randomised and double-blind fashion. The remifentanil dose was modelled on a concentration of 0.7 ng/ml in the brain, achieved using a target-controlled infusion pump. Experimental breathlessness was induced using inspiratory resistive loading combined with mild hypercapnia. Participants also underwent a delay-conditioning paradigm before the scanning visits, wherein they learned associations between three visual cues (shapes) presented on a screen, and three conditions: mild inspiratory load (approximately -3 cmH_20), strong load (approximately -12 cmH_20) and unloaded breathing. A cued anticipation period of 8 seconds then preceded each loading condition. Participants rated the intensity and unpleasantness of their breathlessness using a VAS (0-100 mm). The change in all scores was calculated as opioid minus placebo. Participants were also characterized using questionnaires listed in **Table 1.**

During the breathlessness protocol, a familiarisation phase of at least 5 minutes was first allowed for each participant to become accustomed to the breathing system. Throughout the experiment, the end-tidal partial pressures of carbon dioxide and oxygen were maintained constant by manual adjustment of inspiratory gasses.

Hierarchical cluster analyses:

Studies in chronic pain have demonstrated an inverse relationship between negative affect and opioid analgesia (6, 7). Therefore, we explored the possible relationships between the magnitude of opioid-induced relief of breathlessness, behavioural measures and physiological traits. A hierarchical cluster analysis (MATLAB: 2013a, MathWorks Inc., Natick, MA, USA) was performed on questionnaires, breathlessness ratings, and physiological measures from each of the two datasets (**Table 1**). Despite differences in the experimental protocols of Abdallah et al. (4) and Hayen et al. (12), the measurement tools used in both studies broadly represented similar domains (see **Table 1)**.

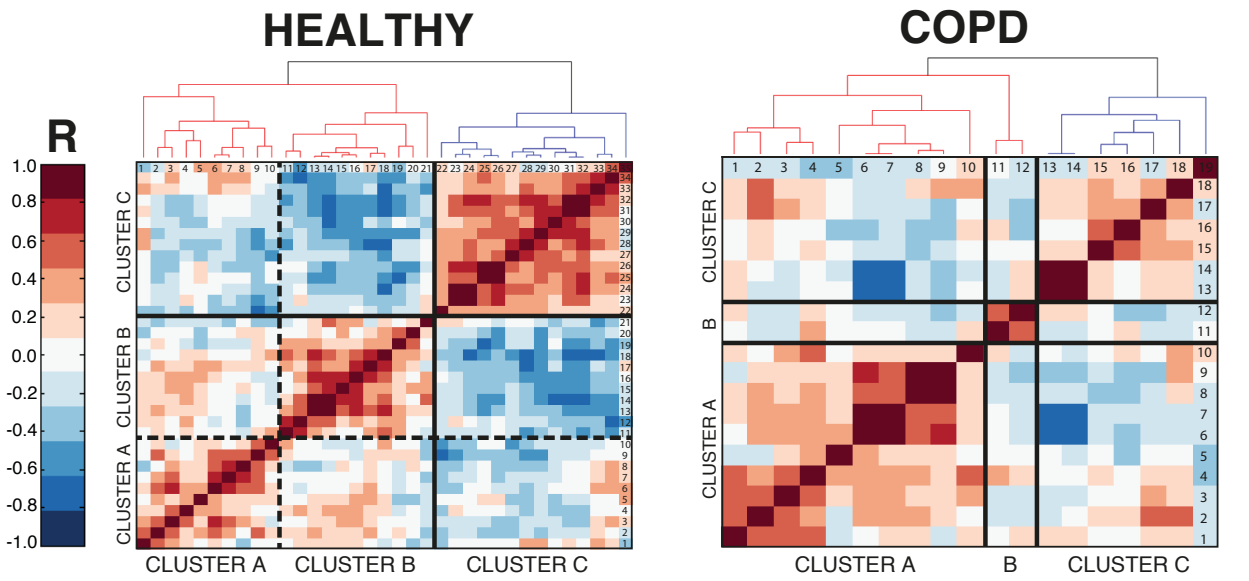
Variables were first aligned such that larger values represented more negative properties. This was achieved through the multiplication of relevant variables by -1 (e.g., FEV_1% predicted was multiplied by -1 as a larger FEV_1%predicted value reflects less severe disease). All measures were then individually normalised via Z-transformation, to allow accurate variable comparisons and distance calculations.

Hierarchical cluster models reorder variables based on their correlation strengths, such that groups of related measures sit closer to each other than non-related measures. This modeling process formalizes the relationship between pairs of variables and the manner by which shared variance can be described as part of larger, related clusters. The clustering algorithm is a form of unsupervised machine learning that initially considers pairs of variables in terms of their similarity or "distance", and will therefore find links between groups of measures regardless of their significance. Linked pairs are incorporated into larger clusters with the goal of minimizing a cost function (distance to be bridged) - a process that can be thought of as minimizing the dissimilarity within clusters. As pairs become clusters, a cluster tree or dendrogram is created. The distance between neighboring branches indicates the relative similarity of two measures. The branching of these levels is an arbitrary distance measure and does not indicate the strength of the correlation in terms of a Pearson's R value.

A 'threshold of relatedness' can then be applied to the hierarchical cluster tree, to formally separate clusters of variables thought to be statistically distinct from one-another. In applying this 'threshold of relatedness', the most mathematically distinct cut point needs to be considered together with the most biologically relevant information, given an a-priori hypothesis. We applied a typical threshold technique of the 'elbow method' to identify the most mathematically distinct clusters, before considering further cluster divisions utilizing a-priori knowledge and visual inspection of the dendrogram structure. This analysis allowed us to explore natural 'groupings' within the recorded measures, and the relationship of these groupings to each other. Clusters were defined by higher-order variable groupings denoted by the hierarchical clustering structure, with a minimum intra-cluster correlation coefficient of 0.3 between the variables.

FMRI analysis of opioid efficacy:

In the present study, the FMRI data from the healthy volunteers was then investigated in consideration of the Bayesian Brain Hypothesis. We explored how brain activity associated with anticipation of breathlessness (during the saline placebo condition) may relate to an individual's 'opioid efficacy' for the treatment of breathlessness. The group of items that formed **Cluster B** within the hierarchical cluster analysis on the healthy volunteers (see **Fig. 1**) were used to define overall 'opioid efficacy' (i.e., items that represented opioid-induced changes in physiological and subjective measures). These items included opioid-induced changes in: discontentment, tension and mouth pressure during anticipation of mild and strong loading; mouth pressure and breathlessness intensity and unpleasantness during mild and strong loading; and sedation during the saline condition. We undertook the data reduction technique of a principal component analysis (PCA; MATLAB 2013a) on this group of variables, and the resulting individual scores were included within a group FMRI analysis of the saline placebo condition only, using a general linear model ($Z > 2.3$, whole brain corrected $p < 0.05$).



Cluster identifier	Variable number	Physiological variable
Cluster A	1	Locus of control inventory – Chance control
	2	Locus of control inventory – Others in control
	3	The positive affect negative affect schedule – Negative affect
	4	Δ Sedation
	5	The thought control questionnaire – Reappraisal
	6	Spielberger state-trait anxiety inventory
	7	The revised center for epidemiological studies depression scale
	8	Locus of control inventory – Internal control
	9	The positive affect negative affect schedule – Positive affect
	10	The thought control questionnaire – Social control
Cluster B	11	Δ Discontentment
	12	Δ Tension
	13	Δ Mouth pressure (mild load)
	14	Δ Anticipation mouth pressure (mild load)
	15	Δ Anticipation mouth pressure (strong load)
	16	Δ Mouth pressure (strong load)
	17	Δ Breathlessness intensity (strong load)
	18	Δ Breathlessness unpleasantness (strong load)
	19	Δ Breathlessness unpleasantness (mild load)
	20	Δ Breathlessness intensity (mild load)
	21	Sedation (saline)
Cluster C	22	Sex
	23	Breathlessness intensity (strong load; saline)
	24	Breathlessness unpleasantness (strong load; saline)
	25	Breathlessness unpleasantness (mild load; saline)
	26	Breathlessness intensity (mild load; saline)
	27	The thought control questionnaire – Worry
	28	Mouth pressure (strong load; saline)
	29	Anticipation mouth pressure (strong load; saline)
	30	The defence style questionnaire – Neuroticism
	31	Anticipation mouth pressure (mild load; saline)
	32	Mouth pressure (mild load; saline)
	33	Discontentment (saline)
	34	Tension (saline)
	35	Anxiety sensitivity index

Cluster identifier	Variable number	Physiological variable
Cluster A	1	COPD assessment test – Breathlessness item
	2	Hospital anxiety and depression scale – Depression subscale
	3	COPD assessment test activity – Activity limitation item
	4	Oxygen cost diagram
	5	Sex
	6	Δ Isotime breathlessness intensity
	7	Δ Isotime breathlessness unpleasantness
	8	Δ breathing frequency
	9	Δ tidal volume
	10	Modified medical research council scale
Cluster B	11	Isotime tidal volume (placebo)
	12	Isotime breathing frequency during the (placebo)
Cluster C	13	Isotime breathlessness unpleasantness (placebo)
	14	Isotime breathlessness intensity (placebo)
	15	Plasma morphine
	16	Forced expiratory volume in 1-second
	17	Hospital anxiety and depression scale – anxiety subscale
	18	Plasma morphine-6-glucuronide
	19	Cigarette smoking history (pack years)

Figure. 1 – Clustergram of physiological and behavioural variables in healthy volunteers and participants with COPD (top panel). Identified hard cluster boundaries (via 'elbow method') are denoted in solid black lines, whilst sub-clusters (via visual inspection) are denoted with dashed lines. Tables identify the physiological and behavioural variables induced in each of the sub-clusters (bottom panel). The change (Δ) in all scores was calculated as: opioid minus placebo. In the COPD dataset, physiological and perceptual responses were evaluated during exercise at isotime - defined as the highest equivalent 2-min interval of exercise completed by each participant after oral morphine and placebo.

RESULTS

The breathlessness stimuli in the COPD (exercise) and healthy volunteer (inspiratory resistive load) datasets both increased perceptions of work-effort and air hunger during the saline condition (see original manuscripts (4, 12) and **Supplementary Fig. 5).** In both groups, there was significant inter-individual variability in the magnitude of opioid-induced relief of breathlessness.

Hierarchical cluster analysis.

The hierarchical cluster analysis of the COPD group supported the existence of three distinct clusters of variables, which were verified by the elbow method (**Fig. 1,** and **Supplementary Fig. 4**).

In the COPD dataset (**Fig. 1**), 4/10 items in **Cluster A** represented physiological and subjective responses to opioid administration, and 5/10 items represented breathlessness and psychological affective measures. This **'response-affect'** cluster included the: COPD assessment test (CAT) breathlessness and activity-limitation items (14); hospital anxiety and depression scale (HADS) - depression subscale (15); oxygen cost diagram (16); modified medical research council dyspnoea scale (mMRC) (17); opioid-induced changes in isotime breathlessness intensity and unpleasantness, and breathing frequency and tidal volume. **Cluster B** consisted of 2 items that represent baseline (placebo condition) physiological measures: isotime tidal volume and breathing frequency. **Cluster C** consisted of 7 items, 4 of which represented baseline physiological and subjective measures at rest and during the placebo condition. This **'baseline'** cluster included: breathlessness intensity and unpleasantness at isotime during the placebo condition; cigarette smoking history; FEV1%predicted; plasma morphine concentrations; plasma morphine-6-glucuronide concentrations; and the HADS anxiety subscale (15).

In the healthy volunteer dataset, the elbow method initially supported the existence of two distinct clusters (**Fig. 1**, solid lines; and **Supplementary Fig. 4**). Upon visual inspection, the larger cluster could clearly split further into two distinct and related clusters (**Fig. 1**, dashed lines). **Cluster A** appeared to be a predominantly **'affective'** cluster, consisting

of 10 items - 9 of which are representative of psychological affective measures. These were the Levenson Multidimensional Locus of Control Inventory– Chance Control, Others in control, and Internal control subscales (18), The thought control questionnaire - Social Control, and Reappraisal subscales (19); Positive and Negative affect schedules (20); the Spielberger State-Trait Anxiety Inventory (21); the revised Center for Epidemiological Studies Depression Scale (22). The remaining item in this cluster is opioid-induced change in sedation (23). **Cluster B** was representative of physiological and subjective responses to opioid administration, as 9/10 items in this cluster were related to opioid-induced changes (see **Fig. 1** – **Cluster B** list of the healthy dataset). The remaining item in this cluster was baseline sedation in the saline placebo condition. **Cluster C** predominantly represented (11/14 items) physiological and subjective measures during the baseline (saline placebo) condition. These items were: breathlessness intensity and unpleasantness in the strong and mild load; mouth pressure during anticipation of breathlessness in both strong and mild loading; discontentment and tension (23) during the saline placebo condition; and sex. The remaining 3 items were related to the affective domain; the Thought Control Questionnaire – Worry subscale (19); the Defence Style Questionnaire – Neuroticism subscale (24); and Anxiety sensitivity index (25).

In both datasets, the predominant 'affect' and 'response' clusters were more closely related to each other than to the 'baseline' cluster. Importantly, the association (smaller distances between respective clusters) between the 'affect' and 'response' clusters indicated that worse affective scores corresponded to a smaller degree of opioid-induced relief of breathlessness (i.e. variables were aligned such that larger values represented more negative properties).

FMRI analysis.

The FMRI analysis revealed significant *anticipatory* brain activity that directly correlated with opioid unresponsiveness (i.e., greater brain activity correlated with a smaller 'response' score from the PCA of **Cluster B** - the sub-cluster representing 'opioid responsiveness') in the anterior cingulate cortex (ACC) and ventromedial prefrontal cortex (vmPFC) during both mild and strong loading; and the caudate nucleus (CN) during

anticipation of mild loading only (**Fig. 2**). That is, the greater the activity in these brain regions during anticipation of breathlessness, the smaller the degree of opioid-induced relief of breathlessness.

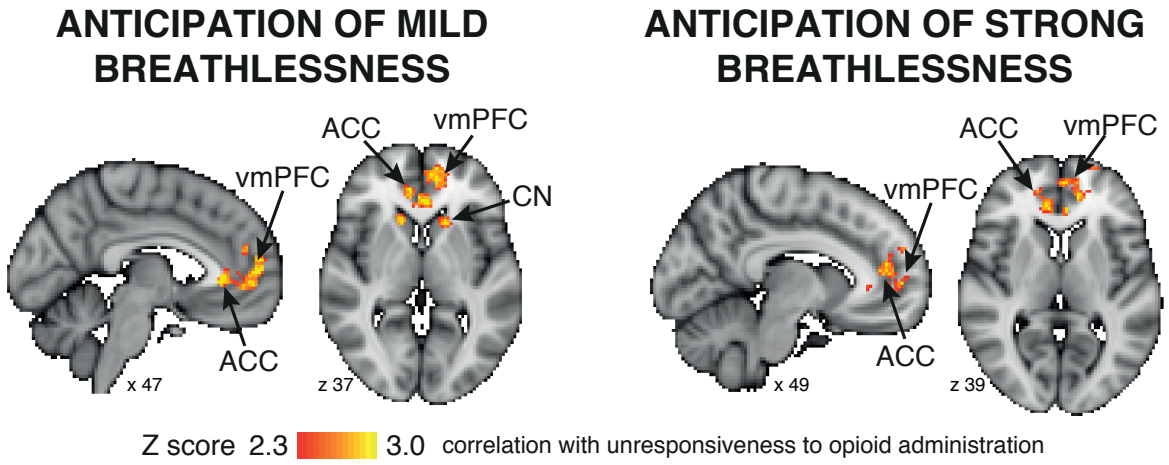


Figure. 2 – Mean BOLD changes identified during anticipation of the mild (left panel) and strong breathlessness (right panel) challenge. The image consists of a colour-rendered statistical map superimposed on a standard (MNI 2x2x2) brain. Significant regions are displayed with a threshold $Z > 2.3$, using a cluster probability threshold of $p < 0.05$. ACC, anterior cingulate cortex; CN, caudate nucleus; vmPFC, ventromedial prefrontal cortex.

DISCUSSION

In this study, we have shown that diminished opioid efficacy for breathlessness was more closely associated with negative affect than other physiological and behavioural properties in both a COPD and a healthy volunteer dataset. Furthermore, functional neuroimaging findings of the healthy volunteer study revealed that the magnitude of opioid-induced relief of breathlessness was inversely correlated with brain activity in the ACC and vmPFC during anticipation of breathlessness, under a saline placebo infusion. These findings suggest that opioid efficacy for breathlessness may be associated with broader negative affective qualities within an individual and may be directly related to brain activity during conditioned responses to breathlessness stimuli.

Our findings from the behavioral data in both studies suggest that opioid responsiveness is inversely associated with the collective co-existing weight of affective moderators (**Fig. 1**). That is, individuals with high vs. low negative affect are less likely to experience opioid-induced relief of breathlessness. These results align with previous work in chronic pain, where it has been found that in addition to less effective analgesia, negative affective qualities are associated with dose escalation (26) and greater difficulty in reducing opioid medication use (27). Interestingly, the hierarchical cluster analysis revealed a 'baseline' cluster in COPD dataset that was not as tightly correlated as the healthy dataset. These results support the view that in COPD, breathlessness is a multifactorial symptom that is likely influenced by variables other than baseline pulmonary function. Notably, we have demonstrated that opioid responsiveness was not related to plasma levels of morphine, as plasma morphine and plasma morphine-6-glucuronide levels in the COPD dataset were more closely related to physiological measures at baseline than to opioid responsiveness. These findings are in keeping with the results of Abdallah et al. (4) who found no correlation between plasma morphine and morphine-6-glucuronide levels and opioid-induced changes in exertional breathlessness.

Interestingly, the cluster structure revealed in the COPD participants was conceptually consistent with that found in the healthy volunteers. Free of the confounds of respiratory disease and chronic breathlessness, the results in these healthy individuals suggest that even subtle variations in affective traits may have measurable effects upon opioid responsiveness. Our results suggest that individual affective traits, and not baseline levels of breathlessness *per se*, may contribute towards the magnitude of opioid responsiveness.

The healthy volunteer dataset also allowed us to examine the relationship between opioid efficacy and anticipatory brain activity in the context of the Bayesian Brain Hypothesis. Opioid-induced changes (quantified by the scores form the 'response' cluster variables) were directly related to *anticipatory* brain activity towards an upcoming breathlessness stimulus under baseline (placebo) conditions. The activated regions - ACC and vmPFC - are contributors to the brain network involved in evaluating the relevance of a stimulus

and its associated value (i.e., reward processing), and are thought to be involved in generating predictions on emotional state and bodily awareness (9, 28). When anticipating breathlessness, individuals with greater brain activity in these regions were less likely to experience meaningful opioid-induced relief of breathlessness, and therefore potentially more 'resistant' to opioid therapy. In particular, associated negative affective properties might influence breathlessness perception by more heavily weighting the brain's perceptual system towards learned associations (or priors) during anticipation of breathlessness (8). For example, in anticipation of climbing a set of stairs, an individual with worse affective traits may have greater breathlessness expectations (e.g., via 'catastrophizing' the severity of breathlessness during previous experiences of stair climbing) relative to an individual with more 'normal' affective traits. In turn, and despite receiving the same sensory afferent inputs when they do climb the stairs, the individual with worse affective traits may be less responsive to opioid therapy as their breathlessness perception is more rigidly attracted towards their breathlessness expectations (i.e., strong priors) relative to the individual with 'normal' affective traits.

Finally, whilst this neuroimaging work was completed in healthy volunteers, previous neuroimaging studies have evaluated the relationship between learned associations and relief of breathlessness in COPD. In a study of pulmonary rehabilitation, and in contrast to the present findings with opioids, Herigstad et al. (29) reported that baseline activity in the brain network responsible for generating predictions (e.g., ACC) correlated positively with changes in breathlessness following pulmonary rehabilitation in COPD. Pulmonary rehabilitation is thought to exert its benefits, in part, by re-shaping associations and modulating negative affect (29-31). The results of these studies suggest that individuals with strong learned associations (priors) and negative affective comorbidities may be more likely to benefit from treatments such as pulmonary rehabilitation than opioids for relief of breathlessness. It is also possible that individuals with these strong associations and negative affective comorbidities may require higher opioid doses to experience adequate relief of breathlessness, as previously demonstrated in pain (6, 26, 27).

Methodological considerations.

Interpretation of the cluster analysis is limited by sample size, which influences model sensitivity and stability. Given more subjects, techniques such as factor analysis could provide additional statistical confidence. Factor analysis allows a formal model to be fitted to the data. This allows investigation of the relationship between variables and establishes whether an underlying shared construct exists, via a description of the full variance of the data and a number of fit statistics. Although we identified several clear clusters that would easily feed into a confirmatory factor analysis, it is generally accepted that for this technique the number of samples should be at least 5 times greater than the number of investigatory variables (32). This would limit us to a model consisting of 4 variables, which we believe does not accurately describe the clusters identified in this dataset. Therefore, we selected the elbow method as a cluster threshold technique, which proposed 2 or 3 clusters for the healthy population and 3 clusters for the COPD population; these clusters were validated and refined visually.

A second important consideration of hierarchical cluster analysis is that omitted variables cannot be inferred upon, and that unrelated variables may randomly cluster together. Despite these limitations, our ability to replicate the behavioural results across two independent datasets increases confidence in our findings. Nevertheless, further replications with larger sample sizes will allow more thorough investigation into the major contributing affective variables. For example, in contrast to the healthy dataset, the COPD dataset measured a smaller number of psychological affective measures. In addition to HADS depression, the “response-affect” cluster in the COPD dataset included the mMRC, oxygen cost diagram and the CAT breathlessness item. This clustering may be indicative of the fact that the mMRC, oxygen cost diagram and CAT are associated with anxiety and depression in COPD (33, 34). Alternatively, this may relate to the fact that in this small sample size, the hierarchical cluster model may be less stable than when compared to a larger sample size.

Conclusions.

In conclusion, the datasets by Abdallah et al. (4) and Hayen et al. (12) have allowed us to explore predictors of opioid responsiveness, and to generate hypotheses based upon potential neurobiological mechanisms of action. Although additional research is necessary, our results are novel and support the hypothesis that opioids may be less effective for the treatment of breathlessness among individuals with higher levels of negative affect comorbidities and strong learned associations. Our results provide clues towards opioid mechanisms of action for relief of breathlessness, which could be tested in future prospective and longitudinal studies, especially as we move towards individualized, safe and targeted symptom management.

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References

1. Hayen A, Herigstad M, Pattinson KT. Understanding dyspnea as a complex individual experience. *Maturitas* 2013; 76: 45-50.
2. Chen S, Small M, Lindner L, Xu X. Symptomatic burden of COPD for patients receiving dual or triple therapy. *Int J Chron Obstruct Pulmon Dis* 2018; 13: 1365-1376.
3. Ekstrom M, Nilsson F, Abernethy AA, Currow DC. Effects of opioids on breathlessness and exercise capacity in chronic obstructive pulmonary disease. A systematic review. *Annals of the American Thoracic Society* 2015; 12: 1079-1092.
4. Abdallah SJ, Wilkinson-Maitland C, Saad N, Li PZ, Smith BM, Bourbeau J, Jensen D. Effect of morphine on breathlessness and exercise endurance in advanced COPD: a randomised crossover trial. *The European respiratory journal* 2017; 50.
5. Wiech K, Tracey I. The influence of negative emotions on pain: behavioral effects and neural mechanisms. *Neuroimage* 2009; 47: 987-994.
6. Wasan AD, Davar G, Jamison R. The association between negative affect and opioid analgesia in patients with discogenic low back pain. *Pain* 2005; 117: 450-461.
7. Wasan AD, Michna E, Edwards RR, Katz JN, Nedeljkovic SS, Dolman AJ, Janfaza D, Isaac Z, Jamison RN. Psychiatric Comorbidity Is Associated Prospectively with Diminished Opioid Analgesia and Increased Opioid Misuse in Patients with Chronic Low Back Pain. *Anesthesiology* 2015; 123: 861-872.
8. Van den Bergh O, Witthoft M, Petersen S, Brown RJ. Symptoms and the body: Taking the inferential leap. *Neurosci Biobehav Rev* 2017; 74: 185-203.
9. Barrett LF, Simmons WK. Interoceptive predictions in the brain. *Nat Rev Neurosci* 2015; 16: 419-429.
10. Bogaerts K, Notebaert K, Van Diest I, Devriese S, De Peuter S, Van den Bergh O. Accuracy of respiratory symptom perception in different affective contexts. *J Psychosom Res* 2005; 58: 537-543.
11. Tang J, Gibson SJ. A psychophysical evaluation of the relationship between trait anxiety, pain perception, and induced state anxiety. *Journal of Pain* 2005; 6: 612-619.

12. Hayen A, Wanigasekera V, Faull OK, Campbell SF, Garry PS, Raby SJM, Robertson J, Webster R, Wise RG, Herigstad M, Pattinson KTS. Opioid suppression of conditioned anticipatory brain responses to breathlessness. *Neuroimage* 2017; 150: 383-394.
13. Borg GAV. Psychophysical Bases of Perceived Exertion. *Medicine and science in sports and exercise* 1982; 14: 377-381.
14. Jones PW, Harding G, Berry P, Wiklund I, Chen WH, Leidy NK. Development and first validation of the COPD Assessment Test. *European Respiratory Journal* 2009; 34: 648-654.
15. Zigmond AS, Snaith RP. The Hospital Anxiety and Depression Scale. *Acta psychiatrica Scandinavica* 1983; 67: 361-370.
16. McGavin CR, Artvinli M, Naoe H, McHardy GJ. Dyspnoea, disability, and distance walked: comparison of estimates of exercise performance in respiratory disease. *Br Med J* 1978; 2: 241-243.
17. 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: 581-586.
18. Levenson H. Multidimensional locus of control in psychiatric patients. *J Consult Clin Psychol* 1973; 41: 397-404.
19. Reynolds M, Wells A. The Thought Control Questionnaire--psychometric properties in a clinical sample, and relationships with PTSD and depression. *Psychological medicine* 1999; 29: 1089-1099.
20. Watson D, Clark LA. The PANAS-X: Manual for the positive and negative affect schedule-expanded form. 1999.
21. Spielberger CD. State-Trait anxiety inventory. Wiley Online Library; 2010.
22. Radloff LS. The CES-D scale: A self-report depression scale for research in the general population. *Applied psychological measurement* 1977; 1: 385-401.
23. Bond A, Lader M. Use of Analog Scales in Rating Subjective Feelings. *Brit J Med Psychol* 1974; 47: 211-218.

24. Muris P, Merckelbach H. The short version of the Defense Style Questionnaire: Factor structure and psychopathological correlates. *Pers Indiv Differ* 1996; 20: 123-126.
25. Reiss S, Peterson RA, Gursky DM, McNally RJ. Anxiety sensitivity, anxiety frequency and the prediction of fearfulness. *Behaviour research and therapy* 1986; 24: 1-8.
26. Edwards RR, Dolman AJ, Michna E, Katz JN, Nedeljkovic SS, Janfaza D, Isaac Z, Martel MO, Jamison RN, Wasan AD. Changes in Pain Sensitivity and Pain Modulation During Oral Opioid Treatment: The Impact of Negative Affect. *Pain medicine* 2016; 17: 1882-1891.
27. Sullivan MD. Why does depression promote long-term opioid use? *Pain* 2016; 157: 2395-2396.
28. Gusnard DA, Akbudak E, Shulman GL, Raichle ME. Medial prefrontal cortex and self-referential mental activity: Relation to a default mode of brain function. *Proceedings of the National Academy of Sciences of the United States of America* 2001; 98: 4259-4264.
29. Herigstad M, Faull OK, Hayen A, Evans E, Hardinge FM, Wiech K, Pattinson KTS. Treating breathlessness via the brain: changes in brain activity over a course of pulmonary rehabilitation. *The European respiratory journal* 2017; 50.
30. Carrieri-Kohlman V, Gormley JM, Eiser S, Demir-Deviren S, Nguyen H, Paul SM, Stulbarg MS. Dyspnea and the affective response during exercise training in obstructive pulmonary disease. *Nursing research* 2001; 50: 136-146.
31. Wadell K, Webb KA, Preston ME, Amornputtisathaporn N, Samis L, Patelli J, Guenette JA, O'Donnell DE. Impact of pulmonary rehabilitation on the major dimensions of dyspnea in COPD. *COPD* 2013; 10: 425-435.
32. Streiner DL. Figuring out factors: the use and misuse of factor analysis. *Can J Psychiatry* 1994; 39: 135-140.
33. Dodd JW, Hogg L, Nolan J, Jefford H, Grant A, Lord VM, Falzon C, Garrod R, Lee C, Polkey MI, Jones PW, Man WD, Hopkinson NS. The COPD assessment test (CAT): response to pulmonary rehabilitation. A multicentre, prospective study. *Thorax* 2011; 66: 425-429.

34. 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. *Respiratory research* 2012; 13: 85.

Opioids for breathlessness: Psychological and neural factors influencing response variability - SUPPLEMENTARY MATERIAL

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INTRODUCTION

The original study by Abdallah et al. (1) investigated 1) the efficacy of oral morphine in reducing exertional breathlessness and improving exercise tolerance in patients with advanced chronic obstructive pulmonary disease (COPD), and 2) the physiological (e.g. minute ventilation) and perceptual (e.g. ratings of breathlessness at peak exercise during incremental cycle exercise testing) factors mediating opioid responsiveness. Meanwhile, the study by Hayen et al. (2) explored functional brain activity during perception of conditioned mild and strong breathlessness stimuli, following either saline placebo or remifentanil infusion in healthy participants.

Abdallah et al. (1) induced breathlessness in adults with COPD using a constant-load cycle exercise test. Hayen et al. (2) induced breathlessness in healthy adults using an inspiratory resistive load combined with mild hypercapnia (details provided below). The stimuli selected in both studies increased breathing intensity and unpleasantness during the saline condition. In both studies, opioid decreased breathlessness intensity and unpleasantness. Importantly, significant inter-individual variability in responsiveness to

opioid therapy was demonstrated in both studies. In Abdallah et al. (1), 11 out of 20 adults with COPD reported a morphine-induced decrease in breathlessness intensity that met or exceeded the minimally clinically important difference (MCID) of 1 Borg unit (3). In Hayen et al. (2), 9 out of 19 healthy adults reported a remifentanil-induced decrease in breathlessness intensity that met or exceeded 10 mm on 100 mm visual analogue scale (VAS) (3, 4). The mechanisms mediating this variability in responsiveness to opioid therapy are currently unknown.

In the current study we have undertaken a reanalysis of behavioural and physiological measures collected by Abdallah et al. (1) and Hayen et al. (2) (including previously unpublished data) to investigate the association between behavioural measures and opioid responsiveness in both health and COPD, and second, to identify brain regions that may contribute to this relationship. These questions regarding inter-individual variability were not the primary aims of the two original studies.

METHODS

COPD study

Participants. Participants included men and women aged ≥40 yrs with clinically stable Global Initiative for Obstructive Lung Disease stage 3 or 4 COPD (5) and chronic breathlessness. This was defined as a modified Medical Research Council dyspnoea score of ≥3 (6), a Baseline Dyspnoea Index focal score of ≤6 (7) and/or an Oxygen Cost Diagram rating of ≤50% full scale (8), despite optimal treatment with bronchodilators, corticosteroids and/or phosphodiesterase inhibitors (5). Exclusion criteria included: smoking history <20 pack-years; change in medication dosage and/or frequency of administration in the preceding 2-weeks; exacerbation and/or hospitalization in preceding 6-weeks; arterialized capillary PCO_2 ($P_{ac}CO_2$) >50 mmHg at rest; presence of other medical condition(s) that could contribute to breathlessness and/or exercise intolerance;

important contraindications to cardiopulmonary exercise testing (CPET); self-reported history of addiction and/or substance abuse; use of anti-seizure drugs or opioids; use of daytime oxygen; and exercise-induced oxyhemoglobin desaturation to <80% on room air.

Study design. This single-center, randomized, double-blind, placebo-controlled, crossover trial (ClinicalTrials.gov identifier NCT01718496) consisted of two intervention periods separated by a washout period of ≥48 hrs. Participants were randomized in a 1:1 ratio to receive immediate-release oral morphine sulphate (0.1 mg/kg body mass; Statex^{TM}, Paladin Labs Inc., Montreal, QC, Canada) or diluted simple syrup (placebo) prepared in 250 ml of orange juice. A computer-generated block randomization schedule was prepared by a third-party not involved in the trial. The study protocol and informed consent form received ethical approval from Health Canada (File No. 9427-M1647-48C) and the Research Ethics Board of the Research Institute of the McGill University Health Centre (MP-CUSM-12-325-T).

After providing written informed consent, participants completed a screening/familiarization visit followed by two randomly assigned treatment visits. During Visit 1 participants completed: ***behavioural questionnaires*** including the Hospital Anxiety and Depression Scale (9), the modified Medical Research Council dyspnoea scale (6), the Oxygen Cost Diagram (8) and the COPD Assessment Test (10); post-bronchodilator (400 μg salbutamol) pulmonary function testing; and a symptom-limited incremental cardiopulmonary cycle exercise test (CPET) to determine peak power output, defined as the highest power output that the participant was able to sustain for 30-sec or longer. At the beginning of Visits 2 and 3, participants inhaled 400 μg of salbutamol to standardize the time since last bronchodilator administration. Participants were then administered oral morphine or placebo. Thirty-minutes thereafter, blood was collected for measurements of plasma concentrations of morphine and its metabolites: morphine-3-glucuronide and morphine-6-glucuronide. Participants then completed a symptom-limited constant-load cycle CPET at 75% peak power output.

Symptom-limited exercise tests were conducted on an electronically braked cycle ergometer (Lode Corival, Lode B.V. Medical Tech., Groningen, The Netherlands) using a computerized CPET system (Vmax Encore^{TM} 29C). Incremental CPETs consisted of a steady-state rest period of at least 6-min, followed by 1-min of unloaded pedalling and then 5 W/min increases in power output. Constant-load CPETs consisted of a steady-state rest period of at least 6-min, followed by 1-min of unloaded pedaling and then a step increase in power output to 75% peak power output. Cardiac, metabolic, breathing pattern and gas exchange parameters were collected and analyzed as previously described (11). Using Borg's modified 0-10 category ratio scale (12), participants rated the intensity and unpleasantness of their breathlessness every 2-min during CPET, and at end-exercise. Subjects were asked "How intense is your sensation of breathing overall" and "How unpleasant or bad does your breathing make you feel". The "radio analogy" was used to distinguish between intensity and unpleasantness of breathlessness (13). Briefly, the radio analogy makes the distinction between the volume of the music emanating from the radio (i.e. intensity) and the unpleasantness of the music being heard. Subjects were instructed that a loud volume (i.e. high intensity) can be pleasant if their favourite song was playing on the radio, and unpleasant if a song they dislike was playing. Therefore, in rating their breathlessness on the Borg's scale, individuals were specifically rating breathlessness intensity and unpleasantness. Physiological and perceptual responses were evaluated during exercise at isotime – defined as the highest equivalent 2-min interval of exercise completed by each participant. The change in tidal volume, breathing frequency, and breathlessness ratings at isotime were calculated as: morphine – saline placebo.

Hierarchical cluster analysis. A full hierarchical cluster analysis (MATLAB: 2013a, MathWorks Inc., Natick, MA, USA) was performed on the: behavioural questionnaires collected during Visit 1; isotime breathing frequency and tidal volume and breathlessness intensity and unpleasantness ratings during the placebo condition; isotime changes in breathing frequency, tidal volume and breathlessness intensity and unpleasantness ratings; and morphine-induced changes in plasma morphine and morphine-6-

glucuronide. Morphine-3-glucuronidel levels were not included in the hierarchical cluster analysis as it is an inactive metabolite. These hierarchical cluster analyses allowed us to explore natural 'groupings' within the recorded measures in each dataset, and the relationship of these groupings to each other. Clusters were defined by higher-order variable groupings denoted by the hierarchical clustering structure, with a minimum intra-cluster correlation coefficient of 0.3 between the variables. See page 21 for further methodological details on the hierarchical cluster analysis.

Healthy volunteer FMRI study

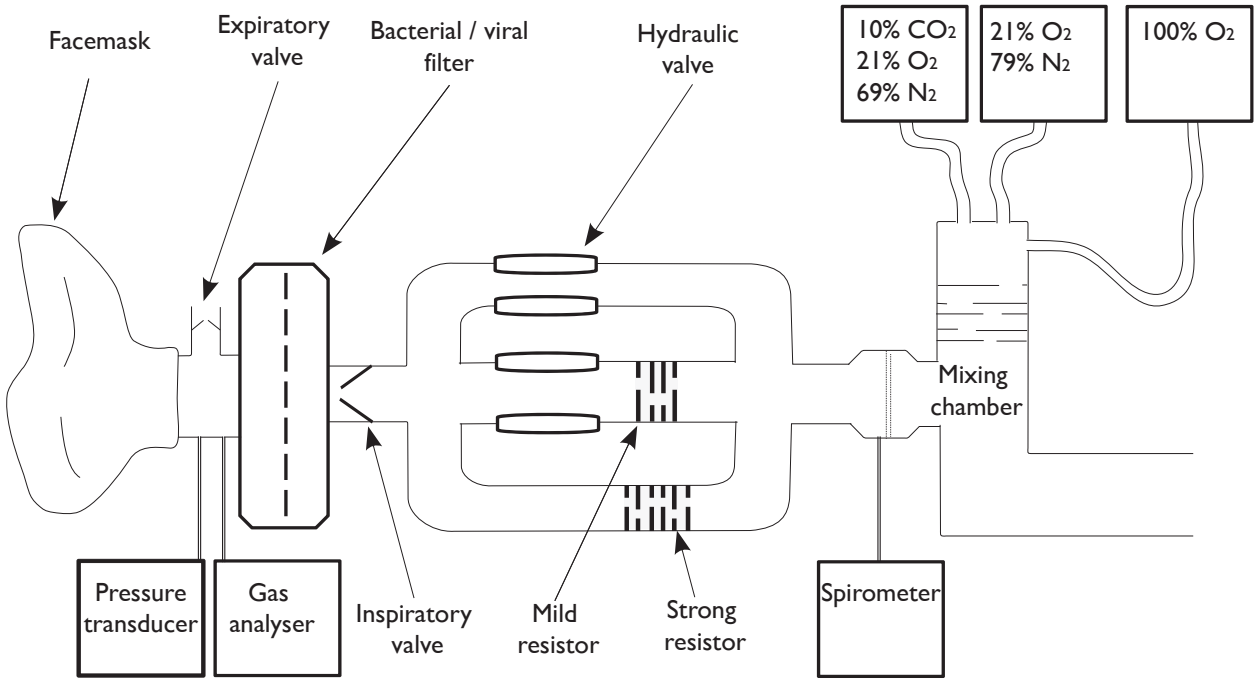
This double-blind, randomized, placebo-controlled mechanistic study investigated the neural correlates of the opioid remifentanil upon the anticipation and perception of breathlessness. An aversive delay-conditioning session was followed by two FMRI sessions (remifentanil or saline placebo, counterbalanced across participants). The sessions were performed on three consecutive days at the same time each day.

Participants. Data from 19 healthy participants (10 females, age 24 (±7 SD) years) was analysed in this study. Written informed consent was obtained in accordance with the Oxfordshire Research Ethics Committee. Although 29 participants originally participated, 10 were excluded for the following reasons: 2 participants exhibited vasovagal syncope during cannulation; 1 participant did not comply with study instructions; 4 participants did not learn the association between visual cues and respiratory stimuli; 3 participants were excluded because of technical difficulties with the MRI equipment. Participants were right-handed non-smokers and had no history of neurological (including painful conditions), pulmonary or cardiovascular disease, were free from acute respiratory infections and were currently not receiving any medication. Participants fasted solids for 6 hours and liquids for 2 hours before every session.

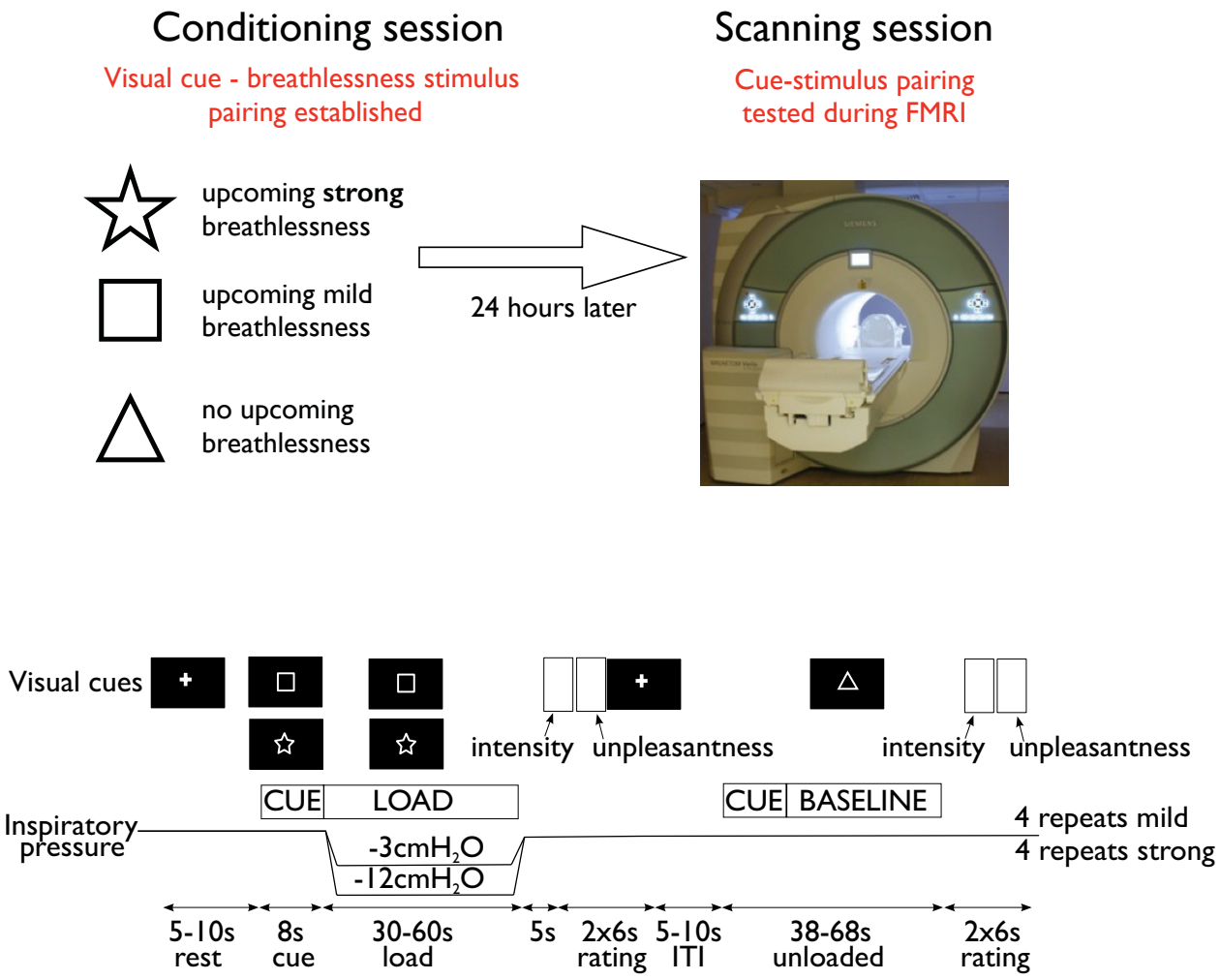
Initial session. The Center for Epidemiologic Studies Depression Scale (CES-D; (14)) was used to identify (and exclude) participants with clinical depression. The trait scale of the Spielberger State-Trait Anxiety Inventory (STAI) (15) was used to characterize general participant anxiety. The following questionnaires were also collected (these were not analysed in the original manuscript of Hayen et al. (2)): The Anxiety Sensitivity Index (ASI; (16)), the Positive Affect Negative Affect Schedule (PANAS; (17)), the Thought Control Questionnaire (TCQ; (18)), the Defence Style Questionnaire (DSQ-40; (19)) , and the Levenson Multidimensional Locus of Control Inventory (LOC; (20)).

Hierarchical cluster analysis. As with the COPD data set, a full hierarchical cluster analysis was performed on the behavioural questionnaires, mouth pressure and subjective breathlessness scores during the placebo condition; and the change in mouth pressure, subjective breathlessness scores, tension-relaxation and discontentment scores for each level of loading. See page 21 for further details pertaining to the cluster analysis techniques.

Breathlessness stimulus. The breathlessness stimulus used in this study was intermittent resistive inspiratory loading for 30 to 60 seconds, administered via the magnetic resonance imaging (MRI) compatible breathing system illustrated in Supplementary **Fig. 1**. Manually operated hydraulic valves diverted inspiratory flow via one of three routes that either did not restrict breathing, or provided a mild or strong resistive load. Expiration was unrestricted via a one-way valve (Hans Rudolph, Shawnee, Kansas, USA). The strong load was induced with a static porous glass disc and the mild load was induced with a static spirometry filter. Using these technique, the loads applied were related to the speed and depth of inspiration, and therefore they adapted to physiological differences between individuals or even between breaths. Throughout the experiment, the actual load produced at the mouth was measured. We found no correlation between the pressure generated and the load reported.



Supplementary Figure 1. Schematic diagram of respiratory circuit. A facemask (Hans Rudolph, Kansas City, MO, USA) connects to a bacterial and viral filter (Vitalograph, Buckingham, UK) from which respiratory gases and respiratory pressure are sampled via polyethylene extension tubing (Vygon SA, Ecouen, France). One sampling line leads to a pressure transducer (MP 45, ± 50 cmH_2O, Validyne Corp., Northridge, CA, USA) connected to an amplifier (Pressure transducer indicator, PK Morgan Ltd, Kent, UK). The second sampling line connects to a gas analyzer that samples O_2 and CO_2 (ADInstrument Ltd, Oxford, UK). A one-way valve allows expired air to escape close to the mouth in order to minimize rebreathing (Hans Rudolf, Kansas City, MO, USA). The breathing system contains three arms. Participants usually breathe through the first, unobstructed arm. This arm can be closed off by inflating a balloon (embolectomy balloon, Microtek Medical B.V., Zutphen, Netherlands) via a hydraulic system. Closure of the arm forces participants to breathe through the second arm, which contains a pediatric respiratory filter (mild resistor, Intersurgical, Wokingham, UK) and a second balloon valve. Closure of both valves forces breathing through a third arm, which contains a porous glass disk (strong resistor). The three arms recombine into a spirometry module that records respiratory flow (ADInstruments Ltd, Oxford, UK) connected to a custom-made mixing chamber in which medical air, oxygen and 10% CO_2 in air are combined.

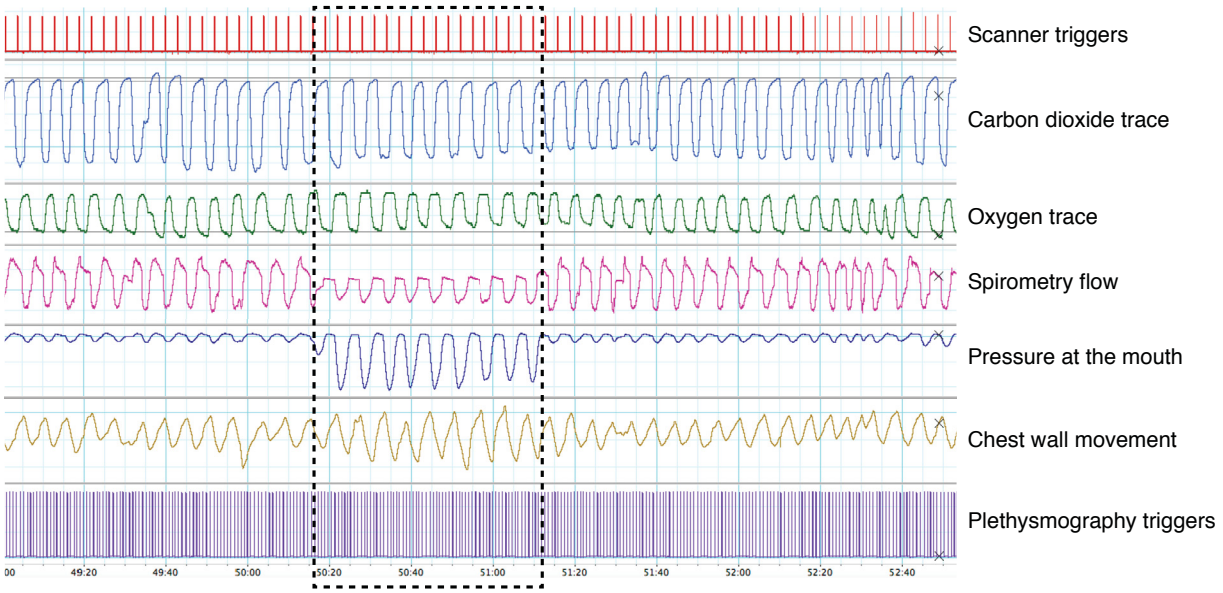


Supplementary Figure 2. Schematic illustration of experimental session and aversive conditioning paradigm. Prior to the application of each inspiratory load, the fixation cross on the screen changed to one of three shapes, a triangle, a square and a star to signal the imminent application of a stimulus (mild inspiratory load, strong inspiratory load) for eight seconds (anticipation period). The shape remained on the screen during the application of the stimulus (stimulus period) for 30-60seconds and changed back to the fixation cross when the stimulus ceased. The shapes were counterbalanced across participants. Each inspiratory load was followed by an unloaded period of between 30-60 seconds that was indicated by a third visual cue. The use of relatively long breathlessness stimuli was chosen to maximize the emotional responses associated with anticipation of breathlessness. Participants rated their respiratory intensity and unpleasantness after each stimulus. Visual stimuli were generated and presented in white on a black background using the Cogent toolbox (www.vislab.ucl.ac.uk/Cogent/) for MatLab (MathWorks Inc., Natick, MA, USA).

In an externally cued delay conditioning paradigm (Supplementary **Fig. 2**), participants learned associations between three visual cues (conditioned stimuli, (CS), either a white square, star or triangle shape presented on a black background) and resistive inspiratory loading that was intermittently applied to induce three different respiratory sensations (unconditioned stimuli, (US) - either breathlessness (strong inspiratory load, approximately - 12cmH_2O), a mild inspiratory load (approximately -3cmH_2O) or no inspiratory load). The pairing between the visual cue (CS) and respiratory load (US) was maintained constant for each participant during all 3 experimental sessions but was counterbalanced between participants. Four repeats of each of the mild and strong load, and eight repeats of the unloaded condition were performed. Immediately after each inspiratory load, participants rated the intensity and unpleasantness of their breathing on a horizontal visual analogue scale (VAS) with the anchors 'no breathlessness' on the left and 'severe breathlessness' on the right for intensity ratings, and 'not unpleasant' on the right and 'extremely unpleasant' on the left for unpleasantness ratings. There was no correlation between the pressure generated and the subjective ratings reported. The inspiratory loads did not extend to intolerable loads. Bond-Lader mood values of tension-relaxation, sedation-alertness, and discontentment-contentment (21) were obtained immediately following the breathlessness protocol using visual analogue scales (VAS) displayed on the screen and a button box. At the end of the experiment, participants were debriefed using the multidimensional dyspnea profile (MDP) questionnaire (22).

Conscious association between CS and US was confirmed in writing and 4 participants who did not form such associations were excluded from the study. Following the breathlessness protocol, an anaesthetist performed a medical assessment; this included a 20-minute test infusion of remifentanil to ensure tolerance and safety. Participants were allowed at least 5 minutes to get accustomed to the breathing system before the experiment commenced. Throughout the experiment, the partial pressure of expired carbon dioxide ($P_{ET}CO_2$) was maintained constant (isocapnia). This was achieved by initially increasing $P_{ET}CO_2$ by +0.3kPa from baseline by adding CO_2 to the inspired air, and then manually adjusting inspired CO_2 as necessary (23, 24). $P_{ET}CO_2$ was maintained constant for each participant, and we did not attempt to maintain equivalent $P_{ET}CO_2$

between subjects. The partial pressure of expired oxygen ($P_{ET}O_2$) was kept constant at 20kPa in a similar manner.



Supplementary Figure 3. Example trace of the physiological recordings collected throughout the experiment with healthy volunteers. Black dashed box represents an example application of an inspiratory loading stimulus, designed to induce the perception of experimental breathlessness. Scanner triggers: Output from the MR scanner to indicate the beginning of each brain volume measurement. Carbon dioxide trace: Recorded at the mouth, and using small manual additions of 5% Carbon dioxide (in air) mixture this was maintained at +0.3 kPa above baseline. Oxygen trace: Recorded at the mouth, and using small manual additions of a 15% Oxygen (balance nitrogen) mixture this was maintained at approximately 20 kPa. Both end-tidal carbon dioxide and oxygen were manually adjusted on a breath-by-breath basis to minimize fluctuations in these values. Spirometry flow: Ventilatory flow as measured by a spirometer, demonstrating a reduction in flow during the stimulus. Pressure at the mouth: Pressure trace measured at the mouth, demonstrating a marked increase in inspiratory pressure during the application of the stimulus. Chest wall movement: Measured via a respiratory bellows belt around the chest, demonstrating a small increase in tidal volume during the stimulus. Plethysmography triggers: Cardiac cycle peaks measured via pulse oximetry, demonstrating no observable difference in heart rate during the application of the stimulus.

Physiological recordings. Arterial oxygen saturation and heart rate were monitored continuously, and non-invasive blood pressure was recorded between each scan (In Vivo Research, Orlando, FL, USA). $P_{ET}CO_2$ and $P_{ET}O_2$ were determined using rapidly responding gas analysers (ADinstruments ML206) and continuously displayed and recorded with a data acquisition device (PowerLab 8, ADInstruments Ltd, Oxford, United Kingdom) connected to a laptop computer using dedicated software (Chart 5, ADInstruments Ltd, Oxford, United Kingdom)). Inspiratory gas flow was measured with a

pneumotachograph (ADinstruments Spirometer FE141) and a standard flow head. Mouth pressure was measured using a Validyne pressure transducer (Validyne Engineering, 8626 Wilbur Ave Northridge, CA 91324). An example trace of physiological recordings collected throughout an experiment is presented in Supplementary **Fig. 3**.

FMRI sessions. Two FMRI sessions were performed, one on each of the two days following the initial conditioning session, and consisted of a remifentanil and saline placebo session (counterbalanced). During acquisition of the FMRI scans, participants underwent the breathlessness protocol as described above (identical to the initial session). Additional structural scans, field maps and measures of cerebral blood flow were obtained (described in detail below).

Drug infusion. An intravenous infusion of either remifentanil or saline placebo was delivered in a counterbalanced, randomised and double-blind fashion. The remifentanil dose was modelled on a concentration of 0.7 ng/ml in the brain, achieved using a target-controlled infusion (TCI) pump (Graseby 3500 TCI incorporating Diprisor, SIMS Graseby Ltd, Watford, UK). The TCI pump was pre-programmed with the three-compartment pharmacokinetic model of remifentanil (25, 26). This model controls the infusion rate of remifentanil to achieve and maintain the desired effect site concentration based on the participant's gender, age, weight and height. The total duration of the infusion was 45 minutes, which allowed for a 10-minute ramp-up period to reach the desired effect site concentration. All participants fasted for 6 hours before each visit and were monitored for an hour after termination of the infusion. Both study participants and study experimenters were blinded to the order of drug administration, with only the administering anaesthetist and the MRI scanner operator being aware of drug condition.

We used remifentanil as a model opioid because its pharmacokinetics and pharmacodynamics are ideal for a mechanistic volunteer study such as this. It is a synthetic μ-opioid agonist with a rapid onset of action, a context sensitive half-life of 3-4 min, and an elimination half-life of approximately 8-10 minutes. This means that the drug

has extremely rapid onset and offset, and when combined with a target-controlled infusion, drug levels can be easily manipulated in a short time frame. Due to its rapid onset and offset, remifentanil needs to be administered intravenously, and when this infusion is combined with a pharmacokinetic model of its action, it is possible to manipulate plasma and effect site (brain) concentrations in a predictable and consistent manner. Using a target-controlled infusion allows a constant drug effect to be maintained throughout the experiment.

In terms of volunteer safety, if adverse effects develop, the infusion can be terminated with the knowledge that the drug will wear off within minutes. We chose to deliver an effect site concentration of 0.7ng/ml based upon our extensive clinical and experimental experience with this drug (27-34). Although direct comparison of equivalence with other opioids (e.g. oral morphine) is difficult, we chose a dose that is at the lower end of efficacy for the treatment of acute pain, but which has previously been shown to have effects on respiration (29) and pain suppression (27). We would estimate that the effect site concentration of 0.7ng/ml would represent the analgesia offered by 4-7 mg oral (or 2.0-3.5 mg intravenous) morphine used to treat low-moderate pain. Remifentanil is ultra-short acting, and this means that when given as a bolus the effects would vary within the scanning session, making it difficult to dissociate primary drug effect from secondary effects such as raised $P_{ET}CO_2$. For this reason, comparison with studies employing bolus doses of remifentanil (35) is difficult.

MRI data acquisition. MRI data were acquired on a 3 Tesla Siemens Trio scanner using a 32-channel head coil. A whole-brain gradient echo, echo-planar-imaging (EPI) sequence (TR = 3000 ms, TE = 30 ms, field of view: 192x192 mm, voxel size 3x3x3 mm, 45 slices, 380 volumes) was used for functional scans. Fieldmaps were obtained using a symmetric-asymmetric spin-echo sequence before the baseline functional scans (30 ms echo time, 0.5494 ms dwell time, field of view and matrix identical to EPI) and were used to correct for magnetic field inhomogeneities. A T1-weighted structural (MPRAGE

sequence, repetition time (TR) = 1720 ms, echo time (TE) = 4.68 ms, flip angle 8°, voxel size 1x1x1 mm) image was acquired for functional image registration.

FMRI image pre-processing. FMRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) using a whole-brain approach. Pre-processing of the data was performed with MCFLIRT motion correction (36), non-brain removal using BET (37), spatial smoothing using a full-width-half-maximum Gaussian kernel of 5 mm, high pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma = 75.0 s) with 150 seconds cut-off. Registration to high resolution structural was carried out using boundary-based-registration (BBR; FSL software library), registration from high resolution structural to standard space was then further refined using nonlinear registration (FNIRT; FSL software library).

The FMRI scans were corrected for motion, scanner and cerebrospinal fluid artefacts using independent component analysis (ICA) denoising (38). BOLD images were corrected for physiological noise with physiological noise modelling integrated in FEAT (RETROICOR) (39, 40). To ensure noise was not reintroduced through the combination of ICA denoising and RETROICOR, the following stepwise process was followed:

1. ICA was used to decompose FMRI data into different spatial and temporal components using FSL's MELODIC (Multivariate Exploratory Linear Optimised Decomposition into Independent Components) using automatic dimensionality estimation. This is a data-driven approach to ICA dimensionality estimation, which allows MELODIC to choose the number of components into which the FMRI data is decomposed. This means that different FMRI scans may be decomposed into a different number of components. The noise components (classified as movement, cerebral spinal fluid (CSF) and scanner artefact) were then identified based on spatial location of signal and signal frequency (41, 42), and manually removed from the 4D FMRI data, this is referred to as the "ICA denoised data".

2. The pulse oximetry and respiratory flow measurements allowed determination of cardiac and respiratory phase with each acquired slice of each FMRI image volume. This assigned phase is then entered into a low-order Fourier expansion (40, 43) to derive time course regressors (2 cardiac, 2 respiratory and 1 interaction regressor). These regressors explain potential signal changes associated with cardiac and respiratory (or interacting) processes. These regressors were regressed against the ICA denoised data using FEAT's Physiological Noise Modelling (PNM) tool (40, 43). The residuals from this regression were subtracted from the ICA denoised data. This is referred to as the "PNM signal".

3. To combine the ICA denoising with PNM it is necessary to remove any overlap between the two. Therefore, the PNM signal was also run through ICA denoising (41, 42) and the resulting denoised PNM signal was removed from the ICA denoised data to give the final denoised data used in the analysis.

First level FMRI analysis. Linear models were used to describe the data. First level analysis used a general linear modelling (GLM) approach with multiple explanatory variables (EVs). Individual subject contrasts were generated for mild and strong anticipation periods from the beginning of the symbol presentation until the onset of the inspiratory resistive loading. 'On' loading periods for each of the mild and strong stimuli were then constructed from the onset of the resistance stimulus until the end of the loading period. Unloaded periods were modelled for the duration of the unloaded stimulus. Periods when participants rated their preceding sensations and the 5-second periods after each respiratory manipulation (fixation cross; presented to give participants a chance to recover before giving their subjective ratings) were modelled as regressors of no interest. $P_{ET}CO_2$ was entered as a separate EV in order to account for residual fluctuations in CO_2 that could affect BOLD signal (24).

To account for possible changes in the haemodynamic response function (HRF), including slice-timing delays, external timing files were convolved with an optimal basis set of three waveforms (FLOBS: FMRIB's Linear Optimal Basis Sets, default FLOBS

supplied in FSL (44)), instead of the standard gamma waveform. The second and third FLOBS waveforms, which model the temporal and dispersion derivatives, were orthogonalised to the first waveform, of which the parameter estimate was then passed up to the higher level to be used in group analysis.

Higher level FMRI analysis. Using this brain imaging data, we aimed to determine how the extent of an individual's response to remifentanil related to their brain activity at baseline (i.e. during infusion of 0.9% saline, the placebo condition). This analysis was designed to explore where the brain responses to breathlessness and anticipation during the saline condition were indicative of the subsequent magnitude of remifentanil-induced relief of breathlessness. A higher-level mixed effects analysis was conducted for the saline condition only. A principal component analysis (PCA; MATLAB 2013a) was performed on the opioid 'response' cluster from the hierarchical cluster analysis. The resulting individual scores were included within a group fMRI analysis in the saline condition only, using a general linear model ($Z > 2.3$, whole brain corrected $p < 0.05$). FMRIB's Local Analysis of Mixed Effects (FLAME 1+2 (44)) was used with automatic outlier de-weighting. Analysis was corrected for multiple comparisons across the whole brain, a cluster threshold of >2.3 and a corrected cluster significance threshold of $p = 0.05$ were used.

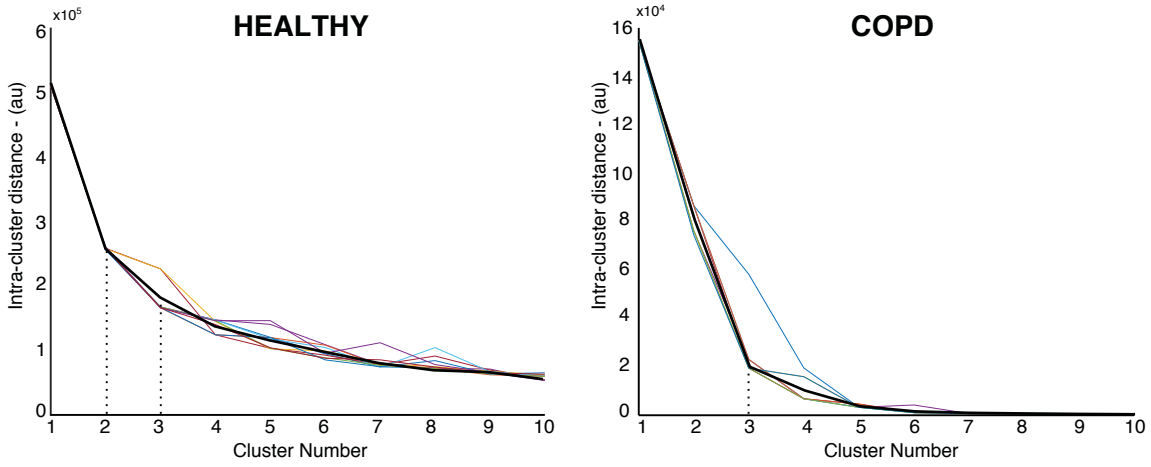
This analysis differs from that presented in Hayen et al. (2), which examined group mean differences rather than exploring potential mechanisms underlying inter-individual variability in response. The brain imaging data collected during remifentanil administration was not analysed and is not presented in this manuscript.

Hierarchical cluster threshold techniques

For both hierarchical cluster analyses performed in this manuscript, the elbow method and visual inspection of dendrogram structure were utilized for cluster thresholding and separation. The elbow method is a validated clustering technique in which the percentage

of explained variance is described as a function of the number of clusters. Considering the variable set as initially one large cluster, the algorithm then divides the variables into increasing numbers of clusters. With each additional cluster, the percentage of explained variance is expected to increase. While initially this increase is sharp, after a certain number of clusters the gain will become marginal. When this relationship is plotted, as the sum of intra-cluster distance against cluster number, the point at which additional clusters add only marginally to the explained variance can be seen as a sharp bend or elbow in the graph. The number of clusters corresponding to this elbow point is thus the number of most statistically distinct clusters in the dendrogram.

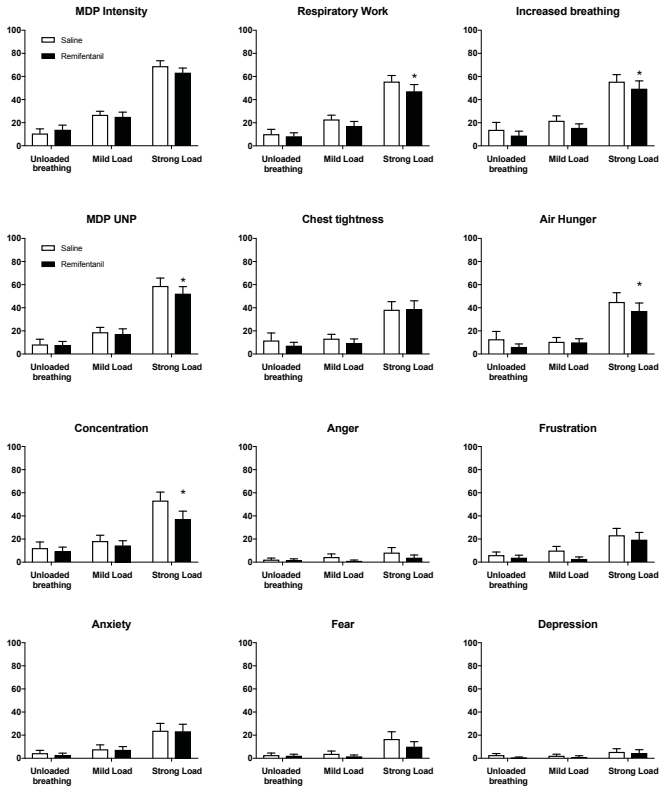
The elbow method is, however, fundamentally limited by the stability of the variables within a cluster. When situations arise where one or more variable sits at the border of two clusters the method can give unreliable results. In the current investigation, the elbow method was trialed and assessed for test-retest reliability (a marker of cluster stability), across 10 trials. Although there was clear variance across the trials in both the healthy and COPD datasets, the overall trend indicated 2 or 3 major clusters within the healthy population, and 3 major clusters within the COPD population (Supplementary **Fig. 4**). Whilst additional modeling techniques such as confirmatory factor analysis (CFA) could then be employed to validate clusters and sub-clusters, there are insufficient sample sizes in these datasets for a valid CFA to be conducted. Therefore, in this work, the elbow method was applied and followed by visual inspection of the dendrogram structure.



Supplementary Figure 4. Elbow plots - where variability within a cluster (y-axis, au - arbitrary units) is plotted as a function of the number of clusters (x-axis) for both the healthy (left) and COPD (right) datasets. The point at which intra-cluster distance is no longer sharply decreased by adding further clusters can be visualised as an "elbow" in the plot. Each coloured line represents one trial (10 trials total), while the thicker black line represents the average of all trials. Dotted lines highlight the "elbow joint" of the graph. The elbow plot for the healthy population contains two possible joint points, one at 2 clusters and one at 3 clusters, while the average elbow line clearly bends at 3 clusters within the COPD population dataset.

RESULTS

The inspiratory resistive loads used in the healthy control study induced a sensation of breathing intensity and unpleasantness. Compared to unloaded breathing, the mild and strong inspiratory loads significantly increased breathlessness intensity (mean VAS%; unloaded vs. mild loading: 12 vs. 32%; unloaded vs. strong loading: 12 vs. 71%) and breathlessness unpleasantness (mean VAS%; unloaded vs. mild loading: 10 vs. 25%; unloaded vs. strong loading: 10 vs. 61%)). Furthermore, the inspiratory resistive load increased the work-effort and air hunger sensations (Supplementary **Fig.** 5).



Supplementary Figure 5. Sensory and affective dimensions of dyspnea, as measured with the multidimensional dyspnea profile (MDP), during saline and remifentanil. Mean±SEM. *significant difference between saline and remifentanil condition at $p<0.05$.

References

1. Abdallah SJ, Wilkinson-Maitland C, Saad N, Li PZ, Smith BM, Bourbeau J, Jensen D. Effect of morphine on breathlessness and exercise endurance in advanced COPD: a randomised crossover trial. *The European respiratory journal* 2017; 50.
2. Hayen A, Wanigasekera V, Faull OK, Campbell SF, Garry PS, Raby SJM, Robertson J, Webster R, Wise RG, Herigstad M, Pattinson KTS. Opioid suppression of conditioned anticipatory brain responses to breathlessness. *Neuroimage* 2017; 150: 383-394.
3. Ries AL. Minimally clinically important difference for the UCSD Shortness of Breath Questionnaire, Borg Scale, and Visual Analog Scale. *Copd* 2005; 2: 105-110.

4. Johnson MJ, Bland JM, Oxberry SG, Abernethy AP, Currow DC. Clinically important differences in the intensity of chronic refractory breathlessness. *Journal of pain and symptom management* 2013; 46: 957-963.
5. Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, Celli BR, Chen R, Decramer M, Fabbri LM, Frith P, Halpin DM, Lopez Varela MV, Nishimura M, Roche N, Rodriguez-Roisin R, Sin DD, Singh D, Stockley R, Vestbo J, Wedzicha JA, Agusti A. 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: 557-582.
6. 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: 581-586.
7. 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: 751-758.
8. McGavin CR, Artvinli M, Naoe H, McHardy GJ. Dyspnoea, disability, and distance walked: comparison of estimates of exercise performance in respiratory disease. *Br Med J* 1978; 2: 241-243.
9. Zigmond AS, Snaith RP. The Hospital Anxiety and Depression Scale. *Acta psychiatrica Scandinavica* 1983; 67: 361-370.
10. Jones PW, Harding G, Berry P, Wiklund I, Chen WH, Leidy NK. Development and first validation of the COPD Assessment Test. *European Respiratory Journal* 2009; 34: 648-654.
11. Jensen D, Alsuhail A, Viola R, Dudgeon DJ, Webb KA, O'Donnell DE. Inhaled fentanyl citrate improves exercise endurance during high-intensity constant work rate cycle exercise in chronic obstructive pulmonary disease. *Journal of pain and symptom management* 2012; 43: 706-719.
12. Borg GAV. Psychophysical Bases of Perceived Exertion. *Medicine and science in sports and exercise* 1982; 14: 377-381.

13. Banzett RB, Moosavi SH. Measuring dyspnoea: new multidimensional instruments to match our 21st century understanding. *The European respiratory journal* 2017; 49.
14. Radloff LS. The CES-D scale: A self-report depression scale for research in the general population. *Applied psychological measurement* 1977; 1: 385-401.
15. Spielberger CD. State-Trait anxiety inventory. Wiley Online Library; 2010.
16. Reiss S, Peterson RA, Gursky DM, McNally RJ. Anxiety sensitivity, anxiety frequency and the prediction of fearfulness. *Behaviour research and therapy* 1986; 24: 1-8.
17. Watson D, Clark LA. The PANAS-X: Manual for the positive and negative affect schedule-expanded form. 1999.
18. Reynolds M, Wells A. The Thought Control Questionnaire--psychometric properties in a clinical sample, and relationships with PTSD and depression. *Psychological medicine* 1999; 29: 1089-1099.
19. Muris P, Merckelbach H. The short version of the Defense Style Questionnaire: Factor structure and psychopathological correlates. *Pers Indiv Differ* 1996; 20: 123-126.
20. Levenson H. Multidimensional locus of control in psychiatric patients. *J Consult Clin Psychol* 1973; 41: 397-404.
21. Bond A, Lader M. Use of Analog Scales in Rating Subjective Feelings. *Brit J Med Psychol* 1974; 47: 211-218.
22. Banzett RB, O'Donnell CR, Guilfoyle TE, Parshall MB, Schwartzstein RM, Meek PM, Gracely RH, Lansing RW. Multidimensional Dyspnea Profile: an instrument for clinical and laboratory research. *The European respiratory journal* 2015; 45: 1681-1691.
23. Hayen A, Herigstad M, Wiech K, Pattinson KTS. Subjective evaluation of experimental dyspnoea - Effects of isocapnia and repeated exposure. *Resp Physiol Neurobi* 2015; 208: 21-28.
24. Wise RG, Pattinson KT, Bulte DP, Chiarelli PA, Mayhew SD, Balanos GM, O'Connor DF, Pragnell TR, Robbins PA, Tracey I, Jezzard P. Dynamic forcing of end-tidal carbon dioxide and oxygen applied to functional magnetic resonance

imaging. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism* 2007; 27: 1521-1532.

25. Minto CF, Schnider TW, Egan TD, Youngs E, Lemmens HJM, Gambus PL, Billard V, Hoke JF, Moore KHP, Hermann DJ, Muir KT, Mandema JW, Shafer SL. Influence of age and gender on the pharmacokinetics and pharmacodynamics of remifentanil .1. Model development. *Anesthesiology* 1997; 86: 10-23.
26. Minto CF, Schnider TW, Shafer SL. Pharmacokinetics and pharmacodynamics of remifentanil .2. Model application. *Anesthesiology* 1997; 86: 24-33.
27. Bingel U, Wanigasekera V, Wiech K, Mhuircheartaigh RN, Lee MC, Ploner M, Tracey I. The Effect of Treatment Expectation on Drug Efficacy: Imaging the Analgesic Benefit of the Opioid Remifentanil. *Sci Transl Med* 2011; 3.
28. MacIntosh BJ, Pattinson KTS, Gallichan D, Ahmad I, Miller KL, Feinberg DA, Wise RG, Jezzard P. Measuring the effects of remifentanil on cerebral blood flow and arterial arrival time using 3D GRASE MRI with pulsed arterial spin labelling. *J Cerebr Blood F Met* 2008; 28: 1514-1522.
29. Mitsis GD, Governo RJM, Rogers R, Pattinson KTS. The effect of remifentanil on respiratory variability, evaluated with dynamic modeling. *Journal of Applied Physiology* 2009; 106: 1038-1049.
30. Pattinson KT, Rogers R, Mayhew SD, MacIntosh BJ, Lee MC, Wise RG. Remifentanil-induced cerebral blood flow effects in normal humans: dose and ApoE genotype. *Anesthesia and analgesia* 2008; 106: 347; author reply 347-348.
31. Wanigasekera V, Lee MC, Rogers R, Hu P, Tracey I. Neural correlates of an injury-free model of central sensitization induced by opioid withdrawal in humans. *J Neurosci* 2011; 31: 2835-2842.
32. Wanigasekera V, Lee MC, Rogers R, Kong Y, Leknes S, Andersson J, Tracey I. Baseline reward circuitry activity and trait reward responsiveness predict expression of opioid analgesia in healthy subjects. *Proceedings of the National Academy of Sciences of the United States of America* 2012; 109: 17705-17710.
33. Wise RG, Williams P, Tracey I. Using fMRI to quantify the time dependence of remifentanil analgesia in the human brain. *Neuropsychopharmacology : official*

publication of the American College of Neuropsychopharmacology 2004; 29: 626-635.

34. Wise RG, Rogers R, Painter D, Bantick S, Ploghaus A, Williams P, Rapeport G, Tracey I. Combining fMRI with a pharmacokinetic model to determine which brain areas activated by painful stimulation are specifically modulated by remifentanil. *Neuroimage* 2002; 16: 999-1014.
35. Leppa M, Korvenoja A, Carlson S, Timonen P, Martinkauppi S, Ahonen J, Rosenberg PH, Aronen HJ, Kalso E. Acute opioid effects on human brain as revealed by functional magnetic resonance imaging. *Neuroimage* 2006; 31: 661-669.
36. Jenkinson M, Bannister P, Brady M, Smith S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage* 2002; 17: 825-841.
37. Smith SM. Fast robust automated brain extraction. *Hum Brain Mapp* 2002; 17: 143-155.
38. Kelly RE, Alexopoulos GS, Wang ZS, Gunning FM, Murphy CF, Morimoto SS, Kanellopoulos D, Jia ZR, Lim KO, Hoptman MJ. Visual inspection of independent components: Defining a procedure for artifact removal from fMRI data. *J Neurosci Meth* 2010; 189: 233-245.
39. Brooks JCW, Beckmann CF, Miller KL, Wise RG, Porro CA, Tracey I, Jenkinson M. Physiological noise modelling for spinal functional magnetic resonance imaging studies. *Neuroimage* 2008; 39: 680-692.
40. Harvey AK, Pattinson KTS, Brooks JCW, Mayhew SD, Jenkinson M, Wise RG. Brainstem Functional Magnetic Resonance Imaging: Disentangling Signal From Physiological Noise. *J Magn Reson Imaging* 2008; 28: 1337-1344.
41. Griffanti L, Salimi-Khorshidi G, Beckmann CF, Auerbach EJ, Douaud G, Sexton CE, Zsoldos E, Ebmeier KP, Filippini N, Mackay CE, Moeller S, Xu JQ, Yacoub E, Baselli G, Ugurbil K, Miller KL, Smith SM. ICA-based artefact removal and accelerated fMRI acquisition for improved resting state network imaging. *Neuroimage* 2014; 95: 232-247.

42. Salimi-Khorshidi G, Douaud G, Beckmann CF, Glasser MF, Griffanti L, Smith SM. Automatic denoising of functional MRI data: combining independent component analysis and hierarchical fusion of classifiers. *Neuroimage* 2014; 90: 449-468.
43. Brooks JCW, Faull OK, Pattinson KTS, Jenkinson M. Physiological noise in brainstem fMRI. *Front Hum Neurosci* 2013; 7.
44. Woolrich MW, Behrens TEJ, Beckmann CF, Jenkinson M, Smith SM. Multilevel linear modelling for FMRI group analysis using Bayesian inference. *Neuroimage* 2004; 21: 1732-1747.