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ANTENOR LUIZ LIMA RODRIGUES

AVALIAÇÃO, REABILITAÇÃO E SOBREVIDA NA DOENÇA PULMONAR OBSTRUTIVA CRÔNICA

Londrina
2019

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Tese apresentada ao Programa de Pós-Graduação em Ciências da Reabilitação (Programa Associado entre Universidade Estadual de Londrina [UEL] e Universidade Pitágoras-Unopar), como requisito parcial para a obtenção do título de Doutor em Ciências da Reabilitação

Orientador: Prof. Dr. Fabio de Oliveira Pitta

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Tese apresentada à UEL para sessão de defesa de Doutorado em Ciências da Reabilitação, área de concentração em Ciências da Saúde, como requisito parcial para a obtenção do título de Doutor pela Banca Examinadora composta pelos professores:

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Londrina, 10 de outubro de 2019.

Dedico este trabalho à minha família.

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“Senhor, dai pão a quem tem fome

E fome de justiça a quem tem pão...

Amém”

Padre Zezinho.

Rodrigues, Antenor Luiz Lima. **Avaliação, reabilitação e sobrevida na doença pulmonar obstrutiva crônica.** 2019. 250 f. Tese de doutorado (Programa de Pós-Graduação em Ciências da reabilitação – Programa Associado entre UEL e Pitágoras-UNOPAR) – Universidade Estadual de Londrina, Londrina, 2019.

RESUMO

Introdução: A presente tese de doutorado tem o objetivo de contribuir cientificamente para: o aprimoramento de estratégias para a suspeição/diagnóstico da fraqueza dos músculos inspiratórios; elucidar o padrão de ativação e oxigenação dos músculos respiratórios, assim como do gasto energético e oferta de oxigênio sistêmica de pacientes com DPOC quando submetidos a situações de sobrecarga; avaliar se a sobreposição da DPOC e da asma (i.e., ACO: *asma-COPD overlap)* modifica os efeitos de um programa de treinamento físico em termos de qualidade de vida, estado funcional, sintomas de ansiedade e depressão, impacto da dispneia durante atividades de vida diária e capacidade de exercício quando comparados a sujeitos com DPOC sem a sobreposição de asma; e identificar estatisticamente grupos de pacientes com DPOC que apresentem maior associação com a incidência de óbito em dois anos. **Métodos:** Quatro estudos foram desenvolvidos e são apresentados nesta tese. No primeiro estudo o impacto do uso de diferentes equações de predição para força muscular inspiratória na suspeição/diagnóstico de fraqueza muscular inspiratória foi investigado. No segundo estudo, durantes dois tipos de sobrecarga, a ativação e a oxigenação dos músculos respiratórios e o gasto energético e oferta sistêmica de oxigênio de sujeitos com DPOC foram investigadas. No terceiro estudo os efeitos de um programa de treinamento físico de alta intensidade (>60% da capacidade máxima de exercício) sobre a qualidade de vida, estado funcional, sintomas de ansiedade e depressão, impacto da dispneia durante atividades de vida diária e capacidade de exercício foram comparados em sujeitos apresentando somente DPOC e pacientes apresentando ACO. No quarto estudo, fatores que previamente demonstraram ser associados com mortalidade em pacientes com DPOC foram utilizados para agrupar pacientes com DPOC por meio da análise de *k-means* e a associação entre ser alocado nos diferentes grupos e o óbito em dois anos foi analisada. **Resultados:** O primeiro estudo mostrou que o uso de diferentes equações de referência disponíveis na literatura impacta de maneira importante na suspeição/diagnóstico de fraqueza muscular inspiratória. O segundo estudo mostrou que o diafragma e os músculos inspiratórios "não-diafragmáticos" são recrutados quando a demanda ventilatória é aumentada; no entanto, os outros músculos inspiratórios parecem ser utilizados como uma reserva quando o diafragma tem sua capacidade de gerar força limitada. O terceiro estudo mostrou que os efeitos de um programa de treinamento físico de alta intensidade são similares em pacientes com DPOC e naqueles com ACO. Finalmente, o quarto estudo mostrou que a análise de *K-means* foi capaz de identificar um grupo de indivíduos com DPOC com maior chance de óbito. **Conclusão:** Os artigos científicos apresentados nesta tese mostram que: 1) a suspeição/diagnóstico de fraqueza muscular inspiratória pode variar dependo da equação de referência utilizada; 2) apesar de tanto o diafragma quanto os músculos inspiratórios "não-diafragmáticos" serem recrutados quando a demanda ventilatória é aumentada, os outros músculos parecem ser utilizados como uma reserva

para auxiliar quando o diafragma tem sua capacidade de gerar força limitada; 3) O treinamento físico de alta intensidade promove benefícios similares sobre a qualidade de vida, estado funcional, sintomas de ansiedade e depressão, impacto da dispneia durante atividades de vida diária e capacidade de exercício em indivíduos com DPOC e com ACO; e 4) grupos de indivíduos com DPOC com maior risco de óbito podem ser identificados por meio da utilização da análise de *k-means*.

Palavras-chave: Aparelho respiratório \ Doenças. Doenças respiratórias \ Avaliação. Doenças respiratórias \ Tratamento. Doenças respiratórias \ Reabilitação.

Rodrigues, Antenor Luiz Lima. **Evaluation, rehabilitation and survival in chronic obstructive pulmonary disease.** 2019. 250 p. PhD thesis (Programa de Pós-Graduação em Ciências da Reabilitação – Programa Associado entre UEL e UNOPAR) – Universidade Estadual de Londrina, Londrina, 2019.

ABSTRACT

Introduction: This PhD thesis aims to contribute scientifically to: the improvement of strategies for the suspicion/diagnosis of inspiratory muscle weakness; to elucidate the activation and oxygenation pattern of different respiratory muscles and systemic oxygen delivery and consumption in patients with COPD when the respiratory system is submitted to overload situations; to assess whether individuals with COPD and asthma overlap achieve different responses in terms of health-related quality of life, functional status, symptoms of anxiety and depression, dyspnea in daily life and exercise capacity after an exercise training program in comparison to individuals with COPD alone (i.e., without asthma overlap); to statistically identify groups of individuals with higher association with the incidence of death in a 2-year follow-up period. **Methods**: Four studies were developed and are presented in this thesis. In the first study the impact of using different prediction equations on the suspicion/diagnosis of inspiratory muscle weakness was investigated. In the second study, during two types of overload, activation and oxygenation pattern of the respiratory muscles and systemic oxygen delivery and consumption of individuals with COPD were investigated. In the third study, responses concerning health related quality of life, functional status, symptoms of anxiety and depression, dyspnea in daily life and exercise capacity after a high-intensity (i.e., >60% of maximum exercise capacity) exercise training program were compared in subjects with COPD alone and patients with ACO. In the fourth study, factors previously known to be associated with mortality in patients with COPD were used to cluster them by k-means analysis and the association between death in two years and being allocated in the different groups was analyzed. **Results:** The first study showed that the use of different reference equations available in the literature has an important impact on the suspicion/diagnosis of inspiratory muscle weakness. The second study showed that the diaphragm and “non-diaphragmatic” inspiratory muscles are recruited when ventilatory demand is increased; however, other inspiratory muscles appear to behave as a reserve when the diaphragm ability to generate force is limited. The third study showed that the effects of a high-intensity exercise training program on health-related quality of life, functional status, symptoms of anxiety and depression, dyspnea in daily life and exercise capacity are similar in patients with COPD in comparison to those with ACO. Finally, the fourth study showed that the K-means analysis was able to identify a group of individuals with COPD with higher probability of death. **Conclusion:** The scientific papers presented herein show that: 1) the suspicion/diagnosis of inspiratory muscle weakness may vary depending on the reference equation used; 2) although both diaphragm and other “non-diaphragmatic” inspiratory muscles are recruited when ventilatory demand is increased, non-diaphragmatic inspiratory muscles appear to be used as a reserve when diaphragm ability to generate force is impaired; 3) high-intensity exercise training promotes similar benefits on health related quality of life, functional status, symptoms of anxiety

and depression, dyspnea in daily life and exercise capacity in individuals with COPD and ACO; and 4).groups of individuals with COPD at higher risk of death can be identified by using k-means analysis.

Key words: Respiratory organs \ Diseases. Respiratory diseases - \ Evaluation. Respiratory diseases - \ Treatment. Respiratory diseases - \ Rehabilitation.

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LISTA DE ABREVIATURAS

1RM	teste de uma repetição máxima
6MWT	teste de caminhada de seis minutos
ACO	*asma-COPD overlap* (sobreposição de asma e DPOC)
ATS	*American Thoracic Society*
AUC	*area under the curve* (área sob a curva)
a-vO2	diferença anterio-venosa de oxigênio
BDR	*bronchodilator response* (resposta ao uso de brondilatador)
Bf	*breathing frequency* (frequencia respiratória)
BFI	*blood flow índex* (índice de fluxo sanguíneo)
BMI	*body mass índex* (índice de massa corpórea)
BODE index	índice BODE
CaO2	conteúdo arterial de oxigênio
CO	*cardiac output* (débito cardíaco)
COPD	*chronic obstructive pulmonary disease*
DBP	*diastolic blood pressure* (pressão arterial diastólica)
DLCO	difusão de monóxido de carbono
DPOC	doença pulmonar obstrutiva crônica
EELV	*end-expiratory lung volume* (volume expiratório final)
EILV	*end-inspiratory lung volume* (volume inspiratório final)
ERS	*European Respiratory Society*
ET	*exercise training* (treinamento físico)
expPga	*expiratory gastric pressure* (pressão gástrica durante a expiração)
FEV_1	*forced expiratory volume in the first second* (volume expiratório final no primeiro segundo)
FEV_1/FVC	ratio *between forced expiratory volume in the first second and forced vital volume* (relação entre o volume expiratório forçado no primeiro segundo e a capacidade vital forçada)
FFMI	*fat-free mass índex* (índice de massa magra)
FRC	*functional residual capacity* (capacidade residual funcional)
FVC	*forced vital capacity* (capacidade vital forçada)

GINA	*Global Initiative for Asthma Management and Prevention*
GOLD	*Global Initiative for Chronic Obstructive Pulmonary Disease*
HADS	*Hospital Anxiety and Depression Scale*
Hb	hemoglobina
HR	*heart rate* (frequência cardíaca)
IC	*inspiratory capacity* (capacidade inspiratória)
IQR	*interquartile range* (intervalo interquartílico 25 – 75%)
Kg	quilograma
LCADL	*London Chest Activity of Daily Living scale*
LLN	*lower limit of normal* (limite nferior de normalidade)
MAP	*mean arterial pressure* (pressão arterial média)
MCID	*minimal clinical important difference* (mínima diferença clinicamente importante)
MEP	*maximal expiratory pressure* (pressão expiratória máxima)
MIP	*maximal inspiratory pressure* (pressão inspiratória máxima)
mMRC	*modified Medical Research Council scale*
MVV	*maximal voluntary ventilation* (ventilação voluntária máxima)
NA	*non-avaiable* (não disponível)
Pdi	*transdiaphragmatic pressure* (pressão transdiafragmática)
Pes	*esophagic pressure* (pressão esofágica)
Pga	*gastric pressure* (pressão gástrica)
PR	*pulmonary rehabilitation* (reabilitação pulmonar)
PTP	*pressure-time product* (produto pressão-tempo)
ROC	*receiver operating characteristics*
RSD	*residual standard deviation* (erro padrão da equação)
RV	*residual volume* (volume residual)
RV/TLC	*ratio between residual volume and total lung capacity* (relação entre volume residual e capacidade pulmonar total)
SBP	*sistolic blood pressure* (pressão arterial sistólica)
SD	*standard deviation* (desvio padrão)
SEE	*standard error of the estimate* (erro padrão da medida)
SGRQ	*St. George's Respiratory Questionnaire*

SpO2	saturação periférica de oxigênio
SV	sistolic volume (volume sistólico)
SVC	*systemic vascular conductance* (condutancia vascular sistêmica)
TLC	*total lung capacity* (capacidade pulmonar total)
UEL	Universidade Estadual de Londrina
UNOPAR	Universidade do Norte do Paraná
VA	volume alveolar
VC	*vital capacity* (capacidade vital)
VCO2	volume expirado dióxido de carbono
VE	volume minuto
VO2	consumo de oxigênio
WOB	*work of breathing* (trabalho respiratório)
Wpeak	*peak workload* (carga máxima de trabalho)
7th IC	*seventh intercostal* (sétimo intercostal)
ABD	*abdominal muscles* (músculos abdominais)
SCA	*scalene muscle* (músculo escaleno)

SUMÁRIO

INTRODUÇÃO

INTRODUÇÃO GERAL

O nome doença pulmonar obstrutiva crônica, comumente referida pela sigla DPOC, tem sua primeira descrição datada do ano de 1679, “pulmões volumosos”(1). A primeira descrição de casos é datada de 1769(1) e as primeiras ilustrações de “pulmões enfisematosos” nos anos 1789 e 1959(1). A nomenclatura utilizada para sua descrição inicial (i.e., enfisema), apesar de antiga, persiste até hoje como uma das alterações “mais clássicas” causada por essa doença(1, 2). As alterações brônquicas (i.e, bronquite) tem sua origem rastreadas no ano de 1814(1). A combinação das duas alterações em um mesmo pulmão datam inicialmente de 1821(1). A definição da nomenclatura “doença pulmonar obstrutiva crônica” foi mundialmente uniformizada no ano 2001, após a *Global Initiative for Chronic Obstructive Disease* - uma iniciativa da Organização Mundial da Saúde (OMS) - conhecida com GOLD, ser publicada pela primeira vez(3). A iniciativa GOLD englobou no termo DPOC o enfisema e a bronquite crônica, alterações que eram anteriormente consideradas doenças distintas, além de lançar a classificação da gravidade da doença baseada no VEF_1 (volume expiratório forçado no primeiro segundo) em porcentagem do predito(3). Desde então, enfisema e bronquite crônica são considerados fenótipos de uma mesma doença e podem apresentar-se simultaneamente ou independentemente em um mesmo indivíduo(2). A definição corrente da DPOC diz que ela é uma doença prevenível e tratável, caracterizada por sintomas respiratórios e obstrução persistente devido a anormalidades das vias aéreas ou dos alvéolos, geralmente, causada por exposição significante a partículas ou gases nocivos.(2).

O diagnóstico da DPOC é baseado em informações clínicas e na prova de função pulmonar, principalmente pela relação VEF_1/CVF (volume expiratório forçado no primeiro segundo [VEF_1] / capacidade vital forçada [CVF]) após o uso de broncodilatador(2). Caso a relação VEF_1 / CVF esteja abaixo de 0.70 e o indivíduo receba o diagnóstico de DPOC, a classificação da gravidade da doença será então baseada no valor de VEF_1 em porcentagem do predito (Figura 1), a saber: sujeitos com valores de VEF_1 maiores ou iguais a 80% do predito são classificados como GOLD I, valores entre 50 – 80% do predito GOLD II, valores entre 30 – 50% do predito GOLD III e valores abaixo de 30% do predito GOLD IV(2). Essa classificação tem papel importante tanto no tratamento quanto no prognóstico de indivíduos com DPOC(2). Outra classificação baseada nos sintomas e no número e gravidade de exacerbações é também proposta(2). Nesse caso, os pacientes são classificados como GOLD A, B, C ou D (Figura 1), a saber: sujeitos com DPOC classificados como GOLD A apresentam quadro clínico de menos sintomas e nenhuma ou uma exacerbação que necessite de internação hospitalar, enquanto sujeitos GOLD D apresentam maiores sintomas e mais e/ou pior gravidade de exacerbação (Figura 1)(2). O papel dessa classificação em relação ao prognóstico e tratamento da DPOC é atualmente investigado(2).

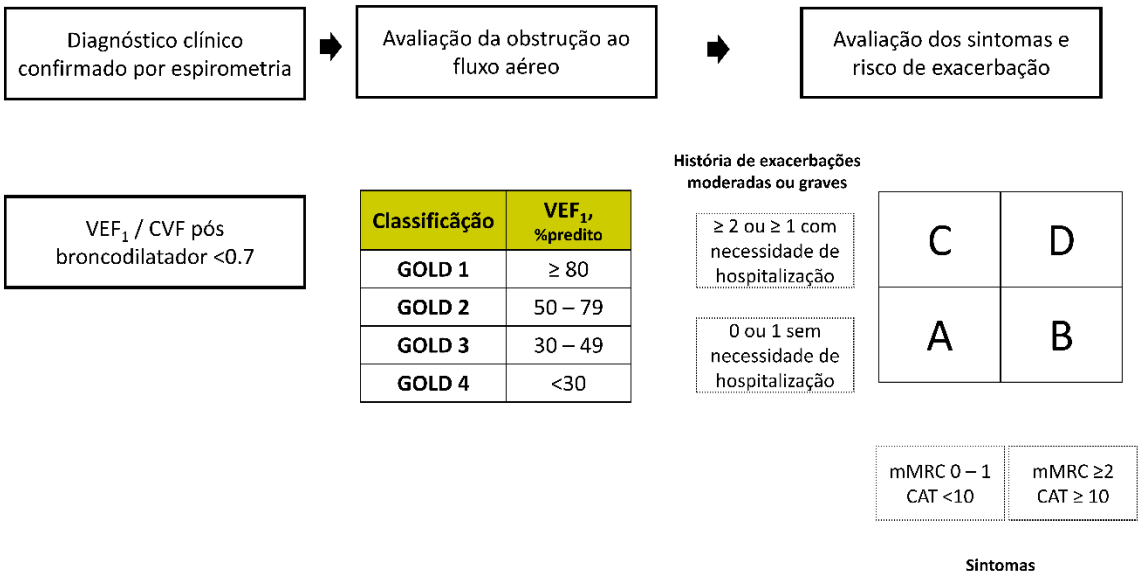

Figura 1. Classificação da Global Initiative for Chronic Obstructive Pulmonary Disease (GOLD).
Fonte: modificado da GOLD(2).

Apesar de ter o diagnóstico principalmente baseado na prova de função pulmonar e a classificação da gravidade da doença no VEF_1, ou em uma combinação de sintomas e número/gravidade de exarcebações(2), diversas alterações extrapulmonares (e.g., perda de peso não intencional, desequilíbrio nutricional, a disfunção muscular) e comorbidades (e.g., doença cardiovascular, síndrome metabólica, osteoporose, ansiedade e depressão) são associadas à DPOC(2, 4). A disfunção muscular periférica, i.e., sarcopenia, a diminuição da capacidade de gerar tensão (i.e., força) e a redução da capacidade oxidativa das fibras restantes têm causa multifatorial (e.g., inflamação sistêmica, estresse oxidativo, inatividade física, alimentação inadequada e hipóxia, entre outras) e pode contribuir para a menor capacidade de exercício e o pior estado funcional de pacientes com DPOC(4). Já a disfunção dos músculos respiratórios é associada à sensação e percepção de dispneia e, assim como a disfunção dos

músculos periféricos, tem causa multifatorial (e.g., inflamação sistêmica, estresse oxidativo)(5, 6). Os músculos respiratórios, entretanto, apresentam adaptações também em consequência do maior trabalho respiratório devido a alterações patofisiológicas que acometem o sistema respiratório de sujeitos com DPOC(5-7). Deve ser notado, porém, que a disfunção muscular pode ser ao menos parcialmente revertida(4-7). Portanto, é um foco importante de intervenções para promover a melhora da capacidade de exercício e estado geral de sujeitos com DPOC (e.g., treinamento físico, treinamento muscular inspiratório e suporte nutricional)(4-7). Com relação às comorbidades comumente diagnosticadas em pacientes com DPOC, é notório que a própria DPOC aumenta o risco de desenvolvimento de outras doenças, como por exemplo o câncer de pulmão(2). Se essa associação é (ou não) consequência de fatores de risco comuns a ambas as doenças (e.g., hábito tabágico) ainda não é totalmente sabido(2). As comorbidades podem estar presentes em pacientes com DPOC independentemente da gravidade da obstrução ao fluxo aéreo, e irão influenciar as taxas de hospitalização e de mortalidade destes sujeitos(2). Atualmente, a sobreposição da DPOC e de outras doenças bem definidas tem sido descrita como fenótipos específicos, como por exemplo a insuficiência cardíaca(8, 9) e a asma(10, 11), e.g., DPOC + insuficiência cardíaca: COPD-HF e DPOC + asma: ACO. Critérios específicos para o diagnóstico dos fenótipos criados pela sobreposição de diferentes doenças e os impactos da associação entre a DPOC e uma ou mais comorbidades no quadro clínico, na evolução do quadro clínico ao longo do tempo e em tratamentos comumente utilizados em pacientes com DPOC ainda necessitam ser claramente descritos(8, 9, 11). Porém, atualmente existem características e critérios que podem identificar indivíduos que apresentem alta probabilidade de serem classificados como pertencentes a esses grupos fenotípicos

específicos (e.g., COPD-HF ou ACO)(9, 11, 12). Quanto a possíveis impactos da associação da DPOC com uma ou mais doenças no quadro clínico dos sujeitos afetados, é possível hipotetizar que a somatória de doenças/comorbidades possa maximizar alterações que são notadas em sujeitos que não possuem comorbidades associadas. No caso de pacientes com DPOC e asma (i.e., ACO), por exemplo, é possível hipotetizar que ocorra um maior trabalho respiratório para um mesmo nível de ventilação. Essa hipótese se baseia em uma possível maior resistência das vias aéreas em sujeitos com DPOC e asma (ACO), devido à broncoconstrição decorrente da hipersensibilidade brônquica característica de sujeitos com diagnóstico de asma(11). Em conjunto com uma maior inflamação sistêmica, alterações mais acentuadas da morfologia, estrutura e função dos músculos respiratórios e locomotores também poderiam estar presentes(5, 6). Em conjunto, essas alterações poderiam, por exemplo, diminuir a tolerância ao exercício físico devido à fadiga precoce e maior percepção de dispneia e de fadiga muscular(13). Seguindo essa linha de pensamento, uma maior percepção da dispneia associada à maior intolerância ao exercício físico poderia, por exemplo, impedir que um sujeito com DPOC e asma (ACO) atingisse intensidades e durações adequadas de treinamento físico para se beneficiar deste, que é parte fundamental do tratamento de pacientes com DPOC e responsável por promover benefícios que para certos desfechos podem ser considerados superiores ao tratamento medicamentoso. Ademais, sabe-se que a presença dessas alterações sistêmicas e comorbidades contribui de maneira importante para a morbidade e o prognóstico de pacientes com DPOC(9, 12), sendo que atualmente a DPOC é a terceira maior causa de mortalidade no mundo.

Ao longo do presente documento, questões específicas sobre a disfunção dos músculos respiratórios, os efeitos do treinamento físico na sobreposição da DPOC com asma e os fatores associados à mortalidade de indivíduos com DPOC serão discutidas. Estudos resultantes dessas questões (Quadro 1) serão apresentados em formato de capítulos 1 a 4. No entanto, inicia-se abaixo com um sumário, contextualização e especificidades sobre os temas e estudos abordados em cada capítulo. Detalhes sobre características específicas de cada estudo (e.g., objetivos) estão descritos no quadro 1.

SUSPEIÇÃO/DIAGNÓSTICO DE FRAQUEZA MUSCULAR INSPIRATÓRIA

A medida da pressão inspiratória máxima (MIP) é normalmente realizada por meio do exame de manuvacuometria, um exame que mede a menor pressão estática gerada pelo sistema respiratório e reflete a maior força gerada pela contração dos músculos inspiratórios(7, 14). Durante a realização do exame o sujeito sendo testado é orientado a realizar uma inspiração forçada máxima (i.e., manobra de Muller), usualmente a partir do volume residual, contra uma via aérea artificial (e.g., bucal) ocluída(7, 14). Portanto, o exame é definido como esforço-dependente(7, 14). A avaliação da MIP por meio da manuvacuometria pode ser utilizada como um instrumento de triagem para a identificação de sujeitos com fraqueza dos músculos inspiratórios(7, 14, 15). Porém, os resultados do exame de manuvacuometria não devem ser interpretados sem a consideração de informações adicionais (e.g., história clínica e sintomas)(7, 14, 15). Para tanto, valores de MIP menores que -80 cmH_2O foram propostos como um limiar para exclusão de fraqueza muscular inspiratória clinicamente importante(7, 14). No entanto,

esse valor absoluto específico de -80 cmH_2O não leva em consideração fatores como idade e sexo, que são associados aos valores absolutos de MIP(7, 14, 16), i.e., é sabido que a MIP se reduz com o avançar da idade e é maior no sexo masculino que no feminino(7, 14, 16). Deste modo, a utilização de um valor absoluto pode sub- ou supradiagnosticar a fraqueza dos músculos inspiratórios(7, 14). Valores de referência de MIP consideram, normalmente, fatores como os supracitados (i.e., idade e sexo), e podem ser utilizados como alternativa aos valores absolutos para o diagnóstico/suspeição de fraqueza muscular inspiratória(7, 14). Neste caso, o uso do limite inferior de normalidade, uma abordagem estatística para a identificação de sujeitos (ou valores) anormais dentro de uma dada população, pode ser uma alternativa interessante(17). Porém, mesmo o uso do limite inferior de normalidade possui pontos que deve receber atenção(17): valores de normalidade devem ser baseados, ao menos, em características antropométricas da população sendo estudada; uma amostra representativa de toda a população deve ser incluída, o que implica na inclusão de amplas faixas de idade e proporção equitativa entre os sexos, por exemplo, para que os valores sejam estimativas reais de uma dada população. Ademais, valores preditos normalmente são desenvolvidos em diversos países e regiões, o que implica na existência de diferentes valores preditivos ao redor do mundo(15, 18, 19). Sendo assim, ainda que um indivíduo seja classificado baseado nas características da população a que pertence, a variabilidade de valores que podem ser considerados “anormais” dentre diferentes populações pode diminuir a capacidade de comparação de estudos desenvolvidos em diferentes regiões/populações(15, 18, 19). Por isso, saber qual é a influência do uso de diferentes equações de referência para o diagnóstico de uma determinada condição, nesse caso da fraqueza muscular inspiratória, pode guiar a escolha

de valores de referência que, apesar de baseados em populações com características similares às do sujeito sendo avaliado, forneçam valores comparáveis a outras equações disponíveis ao redor do mundo(20). Outra potencial utilização dos diversos valores de referência disponíveis é a combinação destes para produzir uma estimativa indireta de um (possível) valor de normalidade que, baseado em fatores que são associados com a força muscular inspiratória (i.e., idade e sexo) combine caraterísticas de estudos desenvolvidos em diferentes regiões do mundo (i.e., "um valor de referência" baseado na população mundial). No primeiro estudo desta tese(15), foi investigada a variabilidade gerada pelo uso de diferentes valores de referências na identificação de sujeitos com alta probabilidade de possuir fraqueza muscular inspiratória e são discutidos valores para diferentes faixas de idade e para cada sexo, baseados nos valores de referência mais citados na literatura, que podem ser utilizados para a identificação de sujeitos com alta probabilidade de fraqueza muscular inspiratória.

DISFUNÇÃO MUSCULAR INSPIRATÓRIA EM PACIENTES COM DPOC.

A gênese do conhecimento da disfunção muscular respiratória em pacientes com DPOC data dos anos 1970(21). É originária de estudos que mostraram uma redução na MIP de indivíduos com DPOC em comparação àqueles sem doença pulmonar aparente(21). De modo controverso, porém, quando as medidas de força muscular inspiratória foram realizadas em volumes pulmonares semelhantes, pacientes com DPOC possuíam maior MIP do que os indivíduos sem doença pulmonar(22). Com a evolução do conhecimento sobre as alterações presentes nos

músculos inspiratórios de pacientes com DPOC, acredita-se atualmente que esse paradoxo seja uma consequência decorrente da hiperinsuflação pulmonar, que encurta as fibras musculares e aumenta a curvatura do raio do diafragma, alterando a relação comprimento-tensão das fibras musculares diafragmáticas(5, 6, 22, 23). Ademais, outras alterações do sistema respiratório causadas pela DPOC, como a maior resistência das vias aéreas e a obstrução ao fluxo aéreo, aumentam o trabalho respiratório e, consequentemente, sobrecarregam os músculos respiratórios de indivíduos com DPOC mesmo em situações de repouso(5, 6, 23). Portanto, apesar da baixa demanda metabólica dos músculos respiratórios em situações fisiológicas normais, em pacientes com DPOC essa demanda se torna progressivamente maior(5, 6). Não obstante, alterações sistêmicas associadas à DPOC somam-se a esses fatores e contribuem para a disfunção dos músculos respiratórios, como o desequilíbrio entre a oferta e a demanda de oxigênio, inflamação sistêmica, aumento do estresse oxidativo, alterações hormonais, acidose, inatividade e descondicionamento físico, desnutrição, exacerbações, envelhecimento e medicações(2, 4-6, 24). Extraordinariamente, no entanto, apesar destes fatores negativos, o produto das alterações presentes nos músculos respiratórios de pacientes com DPOC parece ser menos negativa que o provável(5, 6, 23). Indiscutivelmente, tanto o diafragma quanto outros músculos inspiratórios apresentam alterações com repercussões negativas(5-7, 23). No entanto, o fato de o diafragma de pacientes com DPOC ter a capacidade de desenvolver mais força do que o diafragma de sujeitos sem doença pulmonar quando um nível similar de hiperinsuflação é imposto para ambos permite a interpretação de que, de algum modo, as alterações presentes nos músculos respiratórios de pacientes com DPOC possam ser “não totalmente prejudiciais”(5-7, 23).

Os músculos inspiratórios, assim como os músculos “periféricos” são músculos estriados esqueléticos e, portanto, possuem capacidade de se adaptar aos estímulos aos quais são submetidos(5-7, 25). Em outras palavras, podem apresentar alterações fenotípicas em decorrência dos estímulos específicos recebidos(5-7, 25). Portanto, o maior trabalho respiratório realizado diariamente como consequência das alterações causadas pela DPOC simula, em alguma magnitude, os efeitos causados quando músculos estriados são submetidos a algum tipo de treinamento (i.e., treino de endurance)(5, 6) – princípios de especificidade e sobrecarga(26). Corroborando esse raciocínio, os músculos respiratórios de pacientes com DPOC apresentam aumento da capacidade oxidativa mitocondrial(27-29), aumento do número de capilares por fibra muscular(30), redução do comprimento de sarcômeros e maior proporção de fibras musculares tipo I em comparação a sujeitos sem doença pulmonar aparente(30, 31). Esse fato é respaldado por estudos que mostram que essas alterações são mediadas pela ocorrência de lesão muscular transitória seguida por processo muscular de reparo e remodelamento(32, 33). A presença simultânea de sinais de miopatia, entretanto, parece estar associada à menor capacidade de geração de força muscular das fibras musculares diafragmáticas de maneira isolada(34). Em conjunto, apesar de reduzir a capacidade de produção de tensão (i.e., força) por fibra muscular, essas alterações parecem também gerar efeitos positivos como a menor susceptibilidade do diafragma para o aparecimento de fadiga após a realização de exercício físico em pacientes com DPOC em comparação a sujeitos sem doença pulmonar aparente(35, 36). Porém, por tratarem-se de músculos esqueléticos, assim como os músculos periféricos, a disfunção dos músculos respiratórios pode também ser ao menos parcialmente revertida(5, 6, 25, 26).

Mesmo que um aumento da força muscular respiratória possa ser notado após a realização de treinamento físico convencional (i.e., ≈6 cmH_2O)(37, 38), aumentos maiores são gerados após treinamento específico dos músculos respiratórios (i.e., ≈16 cmH_2O)(37, 38). Porém, é importante notar que sujeitos com DPOC submetidos a treinamento muscular inspiratório "*sham*" também apresentam algum aumento da força muscular inspiratória (i.e., ≈4 cmH_2O)(37, 38). O aumento da força muscular inspiratória após treinamento físico convencional é possivelmente decorrência do aumento do trabalho respiratório gerado pela hiperpneia que acontece durante a realização de exercício físico(5, 6), ou seja, a hiperpneia durante o exercício é uma estratégia de manutenção do equilíbrio acidobásico, i.e., $V_E = 863 \times VCO_2 / PaCO_2[1-V_D / V_t]$(39). O aumento da ventilação minuto (V_E) é atingido às custas de um aumento de frequência respiratória e do volume corrente(40). O aumento da frequência respiratória causa uma consequente diminuição do tempo inspiratório (Ti), do tempo expiratório (T_E) e da relação entre o tempo inspiratório e o tempo total do ciclo respiratório (i.e., Ti/Ttot)(40). O menor tempo inspiratório vai tornar necessário o aumento da intensidade da ativação e do trabalho dos músculos respiratórios a fim de acelerar o fluxo inspiratório e atingir um maior volume corrente em um menor tempo inspiratório (maior potência)(40). Além disso, em pacientes com DPOC, a diminuição do tempo expiratório pode impedir que o sistema respiratório atinja o número de constantes de tempo necessárias para que os pulmões se esvaziem apropriadamente(41). Por conseguinte, pode acontecer o desenvolvimento da hiperinsuflação dinâmica, podendo alterar ainda mais a relação V_D/V_t e diminuir a complacência pulmonar

devido à aproximação do ponto de maior resistência da curva pressão-volume do sistema respiratório(40, 41), por conseguinte aumentando o trabalho respiratório. Já o aumento da força muscular inspiratória após treinamento muscular inspiratório específico é consequente à sobrecarga imposta por meio do aumento da resistência externa, que pode ser aplicada por diferentes dispositivos (e.g., *threshold loading*, *POWERBreath*)(42-44). Neste caso, cargas entre 30 – 50% da MIP podem ser aplicadas e os músculos respiratórios deverão gerar força suficiente para superar a resistência e gerar fluxo inspiratório por um número pré-determinado de respirações (e.g., 30 – 40 respirações). Sendo assim, enquanto durante o treinamento físico convencional os músculos respiratórios são submetidos a um estímulo de *endurance*, durante treinamento muscular inspiratório específicos eles são submetidos a um estimulo de "fortalecimento".(26) Respostas como *drive* neural respiratório e oxigenação local (i.e., específicas dos músculos respiratórios) e sistêmica desencadeadas por esses dois tipos diferentes de estímulo, entretanto, não são completamente conhecidas. Por isso, o estudo apresentado no capítulo 3 explorou e comparou a ativação e a oxigenação dos músculos respiratórios, e consumo e oferta de oxigênio a nível sistêmico durante estes dois tipos de sobrecarga dos músculos (res)inspiratórios: 1- hiperpneia, reproduzindo as demandas ventilatórias de uma sessão de treinamento físico convencional e 2- aumento da sobrecarga específica dos músculos inspiratórios, reproduzindo uma sessão de treinamento muscular específico.

SOBREPOSIÇÃO DE ASMA EM PACIENTES COM DPOC (ACO) E POSSÍVEIS EFEITOS DO TREINAMENTO FÍSICO NESSA POPULAÇÃO

A diferenciação entre asma e DPOC pode ser complexa em adultos(11, 45). Essa complexidade é decorrente do fato de que obstrução não reversível ao fluxo aéreo (i.e., após o uso de broncodilatador) pode estar presente em muitos adultos com história de asma(45, 46). Porém, em sujeitos com mais de 40 anos de idade o diagnóstico de DPOC se torna mais comum, e a diferenciação de indivíduos com diagnóstico de asma com obstrução persistente ao fluxo aéreo daqueles com DPOC se torna problemática, principalmente quando em associação à história tabágica(45, 46). Além disso, sujeitos que possuem qualquer uma destas duas doenças apresentam características que podem estar presentes em ambas (e.g., dispneia)(2, 11, 45, 46). Dados mostram que a prevalência do diagnóstico da sobreposição entre DPOC e asma pode variar de 15 – 55%(11, 46). Acredita-se que, ao menos em parte, a grande variabilidade na prevalência do diagnóstico da combinação dessas duas doenças é consequente à falta de consenso sobre sua definição e diagnóstico(11, 46). Apesar dessa falta de consenso, a sobreposição destas duas doenças parece ter um impacto negativo no prognóstico e no quadro clínico dos sujeitos que as apresentam, i.e., maior prevalência de exacerbações, pior qualidade de vida, declínio acelerado da função pulmonar, maior mortalidade e maior consumo de recursos financeiros relacionados ao cuidado com a saúde em comparação a sujeitos apresentando apenas uma das duas doenças(11, 46). Em uma tentativa de padronização da nomenclatura da sobreposição da DPOC e asma, o termo ACO (*asthma-COPD overlap*) foi sugerido pela última *Global Initiative for Asthma* (GINA)(11) e por um grupo específico de especialistas(45). Enquanto pela GINA foram propostos critérios que podem ser utilizados para

identificar sujeitos com maior probabilidade de apresentar DPOC, asma ou ACO(11), (Quadro 2) o consenso de especialistas propôs critérios que podem ser utilizados para o diagnóstico da ACO (Quadro 3)(45). Os fatores variam como a idade de início dos sinais/sintomas, exame de função pulmonar, história pregressa e história familiar, exacerbações, marcadores inflamatórios e utilização de exame de imagem pulmonar. Esses fatores podem ser considerados para que se faça uma estimativa da probabilidade um dado sujeito apresentar asma, DPOC ou ACO. Apesar das diferentes recomendações, os critérios utilizados por elas são, em sua grande maioria, semelhantes. Recomendações para o tratamento farmacológico de sujeitos apresentando ACO são também feitos considerando, principalmente, a “combinação” de algoritmos para o tratamento da DPOC e da asma em separado(11). Não existem dados prévios, porém, que descrevam de forma aprofundada os efeitos de intervenções como o treinamento físico nessa população específica.

277 **Quadro 1.** Características usuais de asma, DPOC e ACO e critérios que, quando presentes, aumentam a probabilidade em favor de

278 asma ou DPOC

				Critérios de maior probabilidade	
Característica	**Asma**	**DPOC**	**ACO**	**Asma**	**DPOC**
Idade de início	Usualmente tem início na infância, porém pode ter início em qualquer idade	Usualmente >40 anos	Usualmente idade ≥40 anos, porém pode ter tido sintomas na infância ou início da vida adulta	Início após 20 anos de idade	Início após 40 anos de idade
Sintomas respiratórios	Podem variar ao longo do dia, normalmente limitando atividade física. Frequentemente desencadeados por exercício, emoções (e.g., risos), poluição ou exposição a alérgenos	Sintomas contínuos, particularmente durante exercício, com dias "melhores" e dias "piores"	Persistentes podendo apresentar picos	Variação de sintomas ao longo de minutos, horas ou dias Pior durante a noite e início da manhã Desencadeados por exercício, emoções (e.g., risos), exposição a poeira ou alérgenos	Persistentes apesar do tratamento Dias melhores e dias piores porém por todos os dias, além de dispneia ao esforço Tosse crônica e produtiva precede o início da dispneia, independentemente dos fatores

					desencadeadores
Função pulmonar	Obstrução ao fluxo aéreo variável atual ou anterior	VEF1 pode melhorar após tratamento, porém FEV1 / CVF <0.7 persiste	Obstrução ao fluxo aéreo não reversível, porém frequentemente com história atual ou pregressa de variabilidade	Obstrução ao fluxo aéreo variável	Obstrução persistente ao fluxo aéreo
Função pulmonar entre sintomas	Pode ser normal entre a aparição de sintomas	Obstrução persistente ao fluxo aéreo	Obstrução persistente ao fluxo aéreo	Função pulmonar normal entre os aparecimento de sintomas	Função pulmonar anormal entre o aparecimento de sintomas
História passada ou familiar	Muitos pacientes possuem alergias e história pregressa de asma na infância ou na família	Exposição a partículas e gases nocivos	História de diagnóstico prévio de asma, alergias e histórico familiar de asma, e/ou histórico de exposição a partículas e/ou gases nocivos	Diagnostico prévio de asma História familiar de asma e outras alergias	Diagnóstico prévio de DPOC, bronquite crônica ou enfisema pulmonar Importante exposição tabágica ou biomass
Comportamento ao longo do tempo	Frequentemente melhora espontaneamente ou com tratamento, porém pode resultar	Geralmente com progressão lenta ao longo dos anos apesar	Sintomas são parcialmente reduzidos com o tratamento. Progressão é usual e apresenta	Não há piora de sintomas ao longo do tempo. Sintomas variam entre estações ou de ano em ano Podem apresentar melhora	Sintomas apresentam piora ao longo do tempo (anos)

	em obstrução persistente ao fluxo aéreo	do tratamento	alta necessidade de tratamento	espontânea ou resposta imediata pós uso de broncodilatador ao longo de semanas	Broncdilatadores de ação rápida possuem benefícios parciais
Raio-x	Usualmente normal	Hiperinsuflação grave e outras características de DPOC	Semelhante à DPOC	Normal	Hiperinsuflação grave
Exacerbações	Podem ocorrer, porém o risco é diminuído com tratamento	Exacerbações podem ser reduzidas através de tratamento. Comorbidades, quando presentes, contribuem para deterioração do quadro	Exacerbações podem ser mais comuns que em DPOC, porém reduzem com o tratamento. Comorbidades podem contribuir para a deterioração	Quadros em cinza descrevem características que, quando presentes, podem ser utilizadas para identificar pacientes com quadro típico de asma e DPOC. Para um dado paciente, deve-se contar o número de pontos em cada coluna. Se três ou mais pontos estão presentes para asma ou DPOC, o paciente apresenta probabilidade de ter a doença em que os pontos foram confirmados. Se o número de pontos é similar em ambas as colunas, o diagnóstico de ACO deve ser considerado.	
Inflamação das vias aéreas	Eosinófilos e neutrófilos	Neutrófilos +/- eosinófilos, linfócitos nas vias aéreas e podem apresentar inflamação sistêmica	Eosinófilos e/ou neutrófilos na secreção		

Fonte: Adaptado de Global Initiative for Asma.(11)

279 **Quadro 2.** Critérios diagnósticos para ACO

Critérios maiores	**Critérios menores**
Obstrução persistente ao fluxo aéreo (i.e., VEF1 / CVF < 0.7 ou <LIN em sujeitos com idade maior ou igual a 40 anos.	História documentada de atopia ou renite alérgica
História tabágica de ao menos 10 anos ou exposição a poluição equivalente	Responsividade do FEV1 ≥200 ml e 12% dos valores pré-broncodilatador em duas ou mais visitas
História de asma com idade menor que 40 anos ou responsividade do VEF1 maior que 400 ml após uso de broncodilatador	Contagem eosinofílica ≥300 cel.ul

280 **Fonte:** adaptado de Sin et al.(45). É recomendada a presença de três critérios maiores e ao menos um critério menos para o
281 diagnóstico de ACO. FEV1: volume expiratório forçado no primeiro segundo; CVF: capacidade vital forçada; LIN: limite inferior da
282 normalidade.

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Apesar da existência de publicações anteriores que indicavam os resultados controversos sobre os benefícios do treinamento físico em pacientes com DPOC(47), os efeitos benéficos da realização de treinamento físico nesses indivíduos são notórios desde a publicação de um emblemático estudo realizado por Casaburi *et al*. no ano de 1991(48). De maneira hábil e inteligente, Casaburi *et al*. randomizaram 19 sujeitos com DPOC para realizarem treinamento físico de moderada (i.e., 50% VO_2max) ou alta (i.e., ≥80%VO_2max) intensidade por um período de 8 semanas(48). Durante essas oito semanas, cada paciente realizava ≈45 minutos de exercício em bicicleta duas vezes por semana(48). Os resultados mostraram que após as oito semanas de exercício físico, sujeitos com DPOC que haviam realizado treinamento físico de alta intensidade apresentavam menor acúmulo de lactato, maior redução da demanda e da ineficiência ventilatória, da demanda cardíaca e do consumo de oxigênio durante a realização de atividade com carga igual em comparação aos sujeitos que realizaram exercício físico em moderada intensidade(48). Efeitos fisiológicos que proporcionaram um aumento de *endurance* da magnitude de 73% no grupo que realizou o treinamento físico em alta intensidade - um aumento de 9% foi notado no grupo que treinou em moderada intensidade(48). Esses efeitos foram creditados a uma melhora da função muscular, já que não houve mudanças em índices de função pulmonar (e.g., VEF_1) e a redução da ventilação foi associada a reduções no acúmulo de lactato(48). Os resultados desse estudo foram, ao menos em parte, responsáveis por promover uma das maiores e mais cruciais mudanças no tratamento de pacientes com doença pulmonar crônica(4). Desde então uma multitude de evidências tem ratificado os efeitos positivos do treinamento físico de alta intensidade na melhora da capacidade de exercício e na qualidade de vida de sujeitos com DPOC(49). Diferentes modalidades de treinamento físico

foram investigadas (e.g., treinamento em ambiente aquático(50), treinamento físico intervalado(51), caminhada nórdica(52)) e mostraram ser capazes de promover benefícios ao menos semelhantes ao treinamento físico convencional (i.e., exercício em bicicleta e/ou esteira)(4, 50-52). Atualmente, as recomendações para a prescrição de exercício físico para sujeitos com doença respiratória crônica são as de realização de exercício físico de alta intensidade (i.e., ≥60%max), de duas a três vezes semanais, com duração de ao menos 30 minutos ao longo de ao menos oito semanas(4). Ademais, é recomendada a realização de exercícios de *endurance* e de fortalecimento muscular em associação, devido ao fato de que benefícios específicos são obtidos consequentemente à realização de cada um deles (i.e., especificidade do treinamento físico)(4).

O acúmulo de evidências científicas indica que o nível de incerteza sobre os benefícios adquiridos por pacientes com DPOC na melhora da qualidade de vida e capacidade de exercício é atualmente considerado nulo, mesmo pelo grupo Cochrane(49). Porém os efeitos do treinamento físico em desfechos como sobrevida ainda são controversos(53-57). Ademais, é sabido que fenótipos específicos podem responder de maneiras diferentes(58), e.g., indivíduos com DPOC que apresentam depleção de massa magra corpórea parecem apresentar benefícios reduzidos na ausência de suplementação nutricional simultânea à realização de treinamento físico(58), permitindo assim que hipóteses relacionadas aos benefícios adquiridos após a realização de treinamento físico em pacientes com DPOC que apresentem comorbidades ou fenótipos específicos sejam concebíveis. Além disso, estudos já mostraram que diferentes fenótipos de DPOC podem apresentar diferentes respostas fisiológicas durante a realização de exercício físico de maneira aguda(59), corroborando a concepção de tais hipóteses. Por isso, o

estudo apresentado no capítulo quatro testou a hipótese que um protocolo de treinamento físico convencional é similarmente efetivo em sujeitos com DPOC e sujeitos com ACO (i.e., sobreposição de asma e DPOC).

MORTALIDADE EM PACIENTES COM DPOC E SEUS PREDITORES

A DPOC é uma doença com alta prevalência e, em direção oposta às outras causas mais comuns de óbito, tem incidência de mortalidade crescente(60). Acredita-se que óbitos causados pela DPOC são provavelmente subestimados, devido a dificuldades de atribuição de uma única causa de óbito, alto índice de subdiagnóstico e comorbidades sendo comumente consideradas como a causa do óbito(2, 61, 62). Apesar disso, em 1990 a DPOC foi a sexta maior causa de morte no mundo, subindo para quarta colocação no ano de 2000. Em 2012 mais de três milhões de óbitos foram creditados à DPOC (i.e., 6% de todas as mortes no mundo)(63). Não obstante, apesar de projeções estimarem a DPOC como a terceira maior causa de óbitos no ano de 2030, a terceira colocação foi atingida com mais de uma década de antecedência, em 2018(63). Portanto, a identificação de sujeitos com DPOC que compõem grupos com maior risco de mortalidade é considerada importante(2, 61-63).

Neste contexto, sabe-se que diferentes desfechos possuem capacidade de identificar sujeitos com DPOC com risco aumentado de óbito. É notório, por exemplo, que exacerbações da DPOC com necessidade de hospitalização aumentam o risco de óbito(64, 65). A prevalência de óbito pós-internação por exacerbação da DPOC pode ultrapassar 50% em 5 anos, sob influência de

maior idade, baixo IMC, comorbidades, severidade da doença, e necessidade de uso de oxigênio no momento da alta hospitalar(65-67). Adicionalmente, fatores como VEF_1, capacidade de exercício (e.g., distância percorrida no teste da caminhada de seis minutos [TC6min] e consumo máximo de oxigênio), perda de peso não-intencional, redução da tensão arterial de oxigênio, estado de saúde geral e nível de atividade física na vida diária também possuem associação com o risco de óbito em sujeitos com DPOC(2, 61, 68, 69). A combinação destas medidas para identificar pacientes com risco de morte aumentado (i.e., índices multidimensionais), porém, mostrou ser uma estratégia melhor para predizer o risco de óbito aumentado(70, 71). O índice BODE(70) é o mais comumente utilizado e inclui a composição corporal (i.e., IMC), a obstrução ao fluxo aéreo (i.e., VEF_1), a percepção de dispneia (i.e., escala modificada do *Medical Research Council*) e a capacidade de exercício (i.e., TC6min). Este índice possui capacidade preditiva maior que o uso de apenas um dos componentes que o compõe(70). Portanto, a interação de diferentes fatores preditores de mortalidade em um dado indivíduo parece ter maior capacidade de identificar o risco de óbito em comparação ao uso de um único fator isoladamente. Consequentemente, métodos capazes de verificar a associação de diferentes fatores em um dado sujeito podem ser hipotetizados como estratégias com alto potencial para identificação de maior probabilidade de óbito em sujeitos com DPOC. A análise de *K-means* é um algoritmo de aprendizado de maquinas para identificação de subgrupos dentro de uma população a partir da interação de diferentes valores, i.e., identificação de grupos de sujeitos com DPOC com base em diversas variáveis que demostraram ser associadas com a incidência de mortalidade nessa população. Por isso, o estudo contido no capítulo cinco da presente tese(68) visou identificar grupos de

371 sujeitos com DPOC a partir de diferentes variáveis que previamente demostraram ser
372 associadas com a incidência de óbito; e verificar se os grupos identificados apresentam, de fato,
373 associação com a incidência de óbito em dois anos em sujeitos com DPOC.

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1. MAXIMAL INSPIRATORY PRESSURE: DOES THE CHOICE OF REFERENCE VALUES ACTUALLY MATTER?

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"This paper is dedicated to the memory of Robert Hyatt (1925-2016)"

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393

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Abbreviations List

ATS: American Thoracic Society

AUC: area under the curve

BMI: body mass index

DLCO: lung diffusing capacity for carbon monoxide

ERS: European Respiratory Society

FEV_1: forced expiratory volume in the first second

FRC: functional residual capacity

FVC: forced vital capacity

IC: inspiratory capacity

LLN: lower limit of normal

MIP: maximum inspiratory pressure

NA: not available

MRC: Medical Research Council

ROC: receiver operating characteristics

RSD: residual standard deviation

RV: residual volume

SEE: standard error of the estimate

TLC: total lung capacity

VA: alveolar volume

VC: vital capacity

1.1 ABSTRACT

BACKGROUND: Single-point measurements of maximal inspiratory pressure (MIP) are frequently used to suggest muscle weakness in clinical practice. Although there is a large variability in "mean" predicted MIP depending on the chosen reference values, it remains unclear whether those discrepancies actually impact on the prevalence of weakness, i.e., MIP below the lower limit of normal.

METHODS: 1729 subjects (50.1% males, aged 20 to 94) who underwent MIP measurements in a clinical laboratory comprised the study group. MIP was predicted according to the most cited regression equations as of August 2015. Pre-test probability of weakness was defined by a cluster of clinical and physiological variables.

RESULTS: Prevalence of weakness ranged from 33.4 % (Enright et al.) to 66.9 % (Neder et al.). Black and Hyatt, Bruschi et al. and Neder et al. (set 2 equations) agreed well in indicating weakness (kappa (95% CI) ranging from 0.81 (0.79-0.83) to 0.83 (0.81-0.85); $p<0.01$) There was a closer agreement between higher pre-test probability of weakness and low MIP according to set 2 compared to Wilson et al., Enright et al. and Harik-Khan et al. (set 1 equations). Thus, a significant fraction of subjects with abnormal MIP according to set 1 but preserved MIP according to set 2 had higher pre-test probability of weakness ($p<0.05$).

CONCLUSION: The choice of MIP reference values strongly impacts on the prevalence of weakness. Some specific equations relate better to clinical and physiological indicators of

449 weakness suggesting that they might be particularly useful to screen subjects for advanced
450 respiratory neuromuscular assessment.

451 Word count= 250

1.2 INTRODUCTION

Inspiratory muscle weakness is a potential cause of chronic breathlessness and exercise intolerance which is frequently underappreciated in practice.[1] Volitional measurements of inspiratory muscle strength (e.g. static maximal inspiratory pressure (MIP))[2] are widely used to identify subjects who might benefit from more detailed physiological assessment.[1-4] Despite the lack of consensus regarding the definition of weakness, most experts agree that a longitudinal decrease in MIP in a symptomatic patient suggests clinically-significant weakness.[3] In practice, however, previous MIP values are rarely available and weakness is frequently suggested from a discrete point in-time measurement. Thus, the choice of an adequate source of reference values for MIP becomes particularly relevant to lessen over- or under-diagnosis of weakness.[4] There are, however, a multitude of published reference values for MIP.[5,6,7,8,9,10,11,12,13] Of note, comparative analysis among those prediction equations indicates large discrepancies among the range of normal MIP values by age and sex thereby suggesting increased risk of misdiagnosis.[4,14,15,16] Despite this state of affairs, it remains unclear whether the stated differences of "mean" predicted MIP actually impact on the diagnosis of weakness (i.e., MIP below the lower limit of normal (LLN)[4,17] in clinical practice. For instance, potential differences of "mean" predicted MIP may not hold practical relevance if the recorded value is consistently above the LLN regardless the chosen reference values. Moreover, it should be acknowledged that MIP is not routinely assessed unless there is some clinical suspicion of neuromuscular weakness, e.g. unexplained low vital capacity (VC), disproportionate dyspnea, suspected diaphragmatic paralysis, known neurological disease.[1-4] In other words, the pre-test likelihood of abnormality in subjects undergoing MIP measurements is usually greater than that observed

in those performing screening tests, such as simple spirometry. Thus, it is conceivable that occasional statistical differences in predicted MIP[4,14,15,16] may not substantially impact clinical decision making regarding the (provisional)[4] diagnosis of inspiratory muscle weakness.

We therefore reviewed a database of more than 1700 consecutive MIP measurements performed over 23 years in an outpatient-based pulmonary function testing laboratory. We firstly determined the level of agreement among the most frequently used MIP prediction equations to suggest weakness. We then contrasted the suggestion of weakness according to each of these equations with a cluster of clinical (chronic dyspnea, previous diagnosis of neuromuscular disease) and physiological (restrictive ventilatory defect, reduced slow vital capacity, significant decrement in forced vital capacity from seated to supine position) measurements suggestive of "higher" pre-test likelihood of weakness. We reasoned that these analyses would provide a clearer picture of the clinical impact of the reported MIP prediction variability[4-16] in subjects assessed in a clinical laboratory.

1.3 METHODS

Study Population

All consecutive subjects aged 20 or older who were referred for MIP measurements from May 1992 to August 2015 at the Kingston General Hospital and Hotel Dieu Hospital Pulmonary Function Test laboratory, Kingston, ON, Canada comprised the study group. In case of sequential measurements, the first assessment was recorded for analysis. Pertinent clinical data (main diagnosis hypothesis, reason for testing and Medical Research Council dyspnea score)[18] was obtained from the test requisition and additional physiological information (vital

capacity, static lung volumes and variations on forced VC from seated to supine position) was recorded. Body mass index (BMI) classified the subjects as underweight (< 18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obese (30 kg/m^2).[19] Patients were unnamed and identified by unique identification numbers. The study protocol (#6018942) was approved the Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board (FWA #00004184; IRB #00001173).

Spirometry and body plethysmography

Spirometry, body plethysmography and lung diffusion capacity for carbon monoxide (DLCO) measurements were performed using automated testing equipment (V6200 Autobox; SensorMedics; Yorba Linda, California). A restrictive ventilatory defect was established when total lung capacity (TLC) < predicted LLN[20] with or without VC <predicted LLN[21].

MIP measurements

MIP (cmH_2O) was measured according to the American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines.[3] Briefly, after familiarization, subjects breathing through a flanged mouthpiece were requested to forcefully inspire from the maximal expiratory level against an occluded valve (Vmax Series 330; SensorMedics). The apparatus has a small leak (approximately 2-mm internal diameter and 20–30 mm in length) to prevent glottic closure. Recorded MIP values were the maximum pressure sustained for 1 second as displayed in a computer monitor. The maximum value of three maneuvers that vary by less than 20% was recorded.[3]

MIP data analysis

A literature search was performed in the Web of Science in order to select the six most cited source of reference values for MIP prediction in adults as of August 2015. The following prediction equations were selected: Black and Hyatt[5] (1248 citations), Wilson et al.[6] (334), Bruschi et al.[7] (102), Enright et al.[8], Harik-Khan et al.[9] and Neder et al.[10] (e-Table 1). The LLN for each regression equation (one-tailed) was established as mean − 1.645 × standard error of the estimate (SEE) or residual standard deviation (RSD) of the regression line (e-Table 2).[7-10] For the equations where neither SEE or RSE were available, the LLN was based on age- and sex-specific cut-offs as proposed by the respective authors.[5,6] "Higher" pre-test probability of weakness was established in subjects presenting with : suspected neuromuscular and/or diaphragmatic disease as the main diagnostic hypothesis,[3] MRC dyspnea ≥2,[18] VC and/or TLC < LLN[3,22] and a significant reduction in forced VC from seated to supine position (> 20%).[23]

527 **e-Table 1.** Key features of the most-cited references values for MIP prediction in adults

Authors	Year	Subjects (M/F)	Age range (yrs)	Mouthpiece	Recorded pressure	Time	Recorded value / number of trials
Black and Hyatt et al.[5]	1969	60/60	20 - 86	Tube	Peak	At least 1s	Highest of at least 2
Wilson et al.[6]	1984	48/87	18 - 70	Flanged	Peak	At least 1.5s	2 equal of at least 3
Bruschi et al[7]	1992	242/339	18 - 70	Tube	Peak	At least 1s	Highest of at least 5
Enright et al.[8]	1994	1.269/1.602	65 - 85	Tube	Peak	2s	2 highest (within 10%) of 5
Harik-Khan et al.[9]	1998	139/128	20 - 90	Tube	Peak	2s	2 highest (within 10%) of 5
Neder et al.[10]	1999	50/50	20 - 80	Flanged	Peak	At least 1s	Highest (within 10% of 3 trials) of 3 - 5

528 **e-Table 2.** Regression equations for MIP prediction proposed by the most cited source of reference values for adult.

Equation		Gender (M=1/F=0)	Age (years)	Height (cm)	Weight (kg)	BSA	Constant	R^2	LLN
Black and Hyatt et al.[5]	Male	-	-0.55	-	-	-	143	-	NA
	Female	-	-0.51	-	-	-	104	-	NA
Wilson et al.[6]	Male	-	-1.03	-	-	-	142	0.21	NA
	Female	-	-	0.71	-	-	-43	0.05	NA
Bruschi et al[7]	Male and female	-0.26	-0.004	-	-	0.47	4.02	0.27	0.33
Enright et al.[8]	Male	-	-1.27	-	0.131	-	153	0.1	25.4
	Female	-	-0.805	-	0.133	-	96	0.08	21.5
Harik-Khan et al.[9]	Male	-	-1.028	-	0.343	-	126	0.42	22.4
	Female	-	-0.649	-0.743	0.861	-	171	0.31	18.5
Neder et al.[10]	Male	-	-0.8	-	0.48	-	119	0.47	16.7
	Female	-	-0.49	-	-	-	110	0.46	9.1

529 *lnMIP. NA= not available

530

531

532

Statistical Analysis

Continuous data are expressed as mean ± SD and categorical variables as number of subjects (n) and proportions (%). Non-paired t-test, chi-square test and univariate linear regression where used when appropriated. The Cohen's kappa (k) coefficient[18] assessed between-equations agreement in determining the presence of weakness and the agreement between weakness according to each equation vs. "higher" pre-test probability of weakness. Agreement was classified as follows: "none", k= 0; "mild", k=0-0.20; "fair", k= 0.21-0.40; "moderate", k= 0.41-0.60; "substantial", k= "0.61-0.80", and "high", k= 0.81-1.[24] Receiver operating characteristics (ROC) curve analysis was used to determine the optimal absolute (cmH_2O) and relative (% predicted according to each equation) MIP value associated with "higher" pre-test probability of weakness according to age and gender.

1.4 RESULTS

The studied sample presented with a balanced sex distribution with a wide age range (20 to 94 yrs). Most subjects were overweight or mildly-to-moderately obese. Almost half of them were deemed to present with "higher" pre-test probability of weakness (Table 1).[3,18,21,22] These subjects presented with significant lower MIP compared to their counterparts (52 ± 22 cmH_2O vs. 68 ± 34 cmH_2O, respectively; $p<0.01$). As expected, MIP decreased as a function of age being generally higher in males than females (e-Figure 1). In fact, ROC curve analyses revealed that absolute MIP values associated with higher pre-test probability of weakness decreased with aging in both sexes (Table 2). Wilson et al.[6], Enright et al.[8] and Harik-Khan et al.[9] (set 1 equations) predicted significantly lower MIP values, i.e., recorded values represented a

554 higher % of predicted compared to Black and Hyatt [5], Bruschi et al.[7] and Neder et al.[10] (set 2
555 equations) (Table 1). Thus, while the prevalence of weakness ranged from ≈35% to 45%
556 according to set 1, it was typically greater than 60% based on set 2 (Figure 1A). Additional
557 analysis performed in subjects presenting with airflow obstruction provided similar results as
558 the prevalence of weakness ranged from 32.3% to 44.7% according to set 1 and 56.2% to 68.1%
559 based on set 2 in this specific subpopulation. These results were consistent with an age-based
560 analysis where a given MIP was more frequently considered abnormally reduced according to
561 set 2 compared to set 1. Set 2 consistently agreed (>90% of subjects on each age group) to
562 suggest the presence of weakness when MIP was ≤ 60 cmH_2O (20-40 yrs), ≤ 50 cmH_2O (40-60
563 yrs) and ≤ 40 cmH_2O (60 yrs or older) for men (e-Table 3). The corresponding values for females
564 were generally lower in each age group (e-Table 4). The frequency of "higher" pre-test
565 probability of weakness was greater in these subjects compared with their counterparts with
566 higher MIP (values ranging from 52.3% to 61.4% versus 18.3% to 31.0% for males and females,
567 respectively; $p<0.05$). In line with the findings of higher predicted MIP according to set 2
568 equations, optimal % MIP thresholds to suggest the presence of weakness were lower
569 according to set 2 compared to set 1 equations across age groups for both sexes (Table 3).

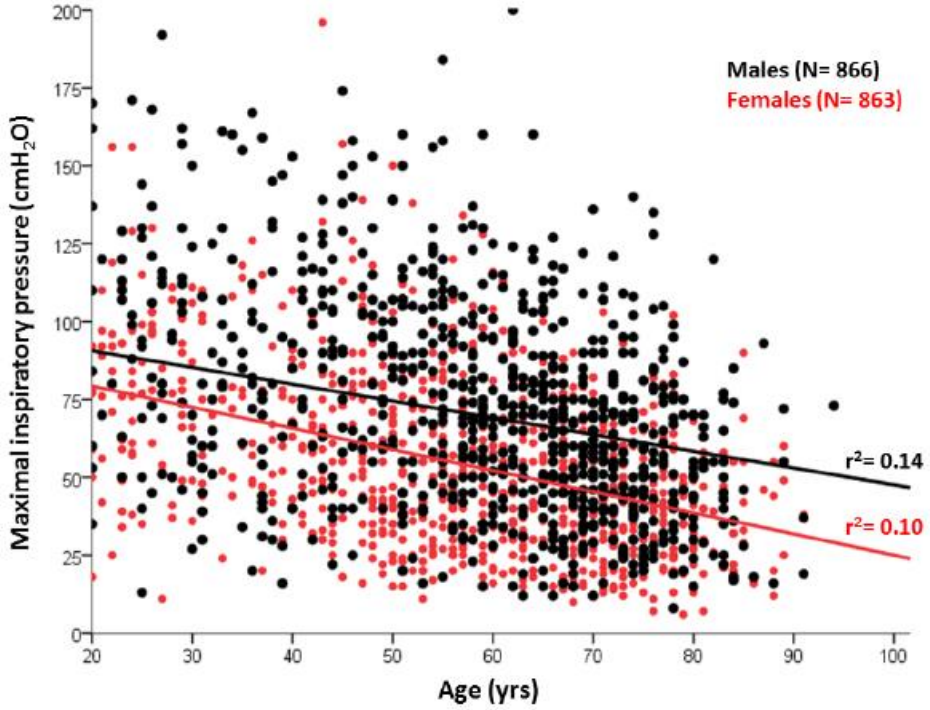
Table 1. Subjects characteristics

Variables	**Values (N= 1729)**
Anthropometrics and Demographics	
Gender, Males/Females (%)	866/863 (50.1/49.9)
Age, yrs	56.7 ± 17.7
BMI, kg/m2	28.1 ± 7.1

Underweight, n(%)	90 (5.2)
Normal weight, n(%)	501 (29)
Overweight, n(%)	552 (31.9)
Obese, n(%)	586 (33.9)
Spirometry	
FVC, L (%pred)	2.74 ± 1.16 (74.5 ± 23.4)
VC, L (%pred)	2.95 ± 1.16 (79.9 ± 22.5)
FEV_1, L (%pred)	2.02 ± 1.01 (74.7 ± 27.9)
FEV_1/FVC, %	72.4 ± 15.1
Obstructive pattern, n (%)	404 (23.3)
Lung Volumes	
TLC, L (%pred)	5.23 ± 1.57 (96.7 ± 23.7)
RV, L (%pred)	2.28 ± 1.21 (117.4 ± 55.9)
FRC, L (%pred)	3.07 ± 1.2 (105.7 ± 37.5)
Restrictive pattern, n (%)	590 (34.0)
Mixed obstructive and restrictive pattern, n (%)	145 (8.4)
Lung Diffusion Capacity	
D_LCO, ml/min/mmHg (%pred)	18.7 ± 7.2 (79.9 ± 25.4)
VA, L (%pred)	4.2 ± 1.3 (77.0 ± 17.8)
D_LCO/VA, ml/min/mmHg/L (%pred)	4.29 ± 1.22 (107.9 ± 27.9)
Maximum Inspiratory Pressure	
"Higher" pre-test probability of weakness, n (%)	815 (47.1)
Measured, cmH_2O	62 ± 32
%pred B*lack and Hyatt et al.*[5]	65.4 ± 31.8

%pre *Wilson et al.*[6]	81.7 ± 39.1
%pred *Bruschi et al*[7]	64.8 ± 30.1
%pred *Enright et al.*[8]	73.5 ± 32.7
%pred *Harik-Khan et al.*[9]	71.4 ± 31.7
%pred *Neder et al.*[10]	63.4 ± 29.4

*Values are mean ± standard deviation. BMI: body mass index; FVC: forced vital capacity; FEV_1: fored expiratory volume in the first second; VC: vital capacity; IC: inspiratory capacity; FRC: functional residual capacity; RV: residual volume; TLC: total lung capacity; VA: alveolar volume; DL_{CO}: transfer factor for carbon monoxide.



e-Figure 1. Maximal inspiratory pressure as a function of age in males and females aged 20 to 94 years.

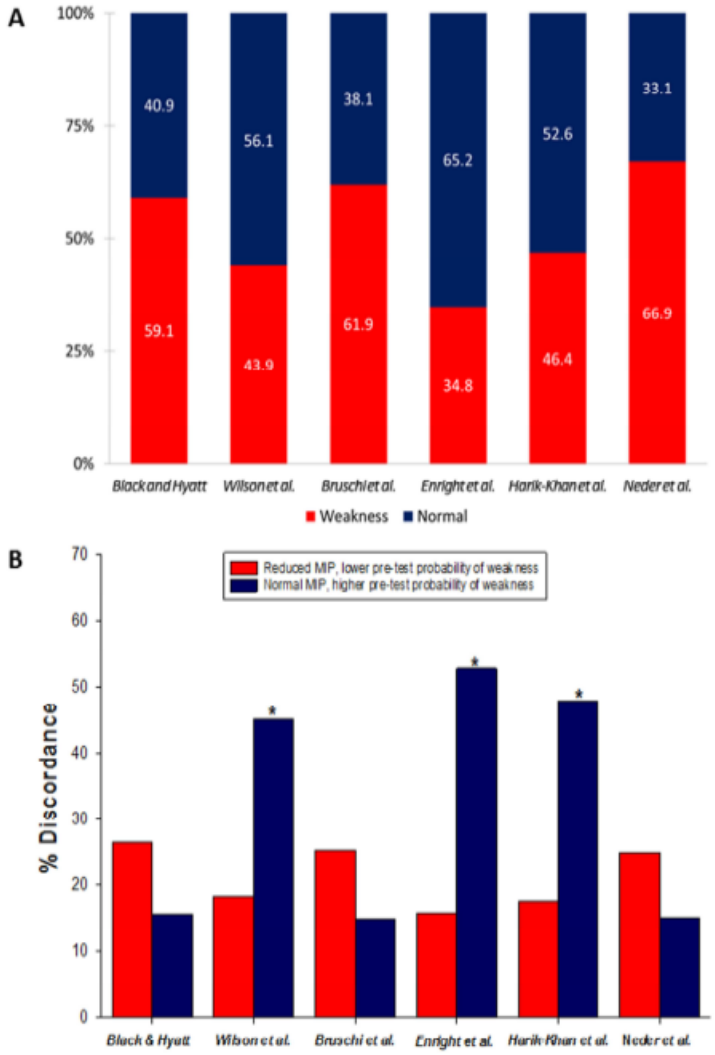
577 **Table 2.** Absolute MIP values (cmH_2O) associated with "higher" likelihood of inspiratory
578 muscle weakness by sex and age. The cut-offs were established by ROC curve analysis.

	Men*	**Women†**
< 40 yrs	63	58
40 – 60 yrs	55	50
6 – 80 yrs	47	43
>80 yrs	42	38

579 *N= 64 (<40 yrs), 302 (40 – 60 yrs), 365 (61 – 80 yrs). † N= 140 (<40 yrs), 293 (40 – 60 yrs), 387 961 – 80
580 yrs) and 43(>80 yrs).

581

582



583

584 Figure 1. Panel A shows the prevalence. Panel A shows the prevalence of weakness
585 according the most cited reference values for maximal inspiratory pressure (MIP) prediction in
586 adults. Panel B depicts within-equation discordance between presence or not of weakness and
587 its pre-test probability based on a cluster of clinical and physiological variables.

588

589 **e-Table 3.** Percent of males in whom a given maximal inspiratory pressure (MIP) was
590 considered as indicative of weakness (<LLN) by each prediction equation across age groups. The

591 lines indicate thresholds values where et 1 equations (Black and Hyatt,[5] Brusch et al.[7] and
592 Neder et al.[9] uniformly agreed that at least 90% of subjects had weakness.

Age/MIP	*Black and Hyatt et al.*[5]	*Wilson et al.*[6]	*Bruschi et al*[7]	*Enright et al.*[8]	*Harik-Khan et al.*[9]	*Neder et al.*[10]
<40 yrs (n=164)						
20 cmH_2O	100	100	100	100	100	100
30 cmH_2O	100	100	100	100	100	100
40 cmH_2O	100	98.7	100	100	97.4	100
50 cmH_2O	100	79.7	99	97	86.2	100
60 cmH_2O	92.5	52.8	90.4	83	66.9	100
70 cmH_2O	52.8	50.2	46.6	55.4	60.3	100
80 cmH_2O	52.1	22.3	36.7	49.8	56.4	70.5
90 cmH_2O	22.3	0.7	21.6	43.6	54.1	43.3
100 cmH_2O	0	0	7.9	24.9	52.1	26.9
40–60 yrs (n=302)						
20 cmH_2O	99.8	99.8	998	99.6	98.8	98.8
30 cmH_2O	99.8	99.8	99.8	99.6	98.8	99.8
40 cmH_2O	99.8	99.6	99.8	85.9	84.3	99.8
50 cmH_2O	98.8	71.1	95.9	59.5	71.3	99.8
60 cmH_2O	47	26.6	65.2	43	60.7	99.3
70 cmH_2O	47	0.7	42.5	24.1	53.9	54.6
80 cmH_2O	22.1	0	27.1	8.9	50	33.8
90 cmH_2O	0	0	12.5	1.8	35.5	16.1
100 cmH_2O	0	0	3.9	0.4	12.1	4.6
61–80 yrs (n=365)						
20 cmH_2O	100	100	100	94.7	90.6	100
30 cmH_2O	100	100	100	70.5	78.3	100

40 cmH_2O	100	99.7	99.5	43	65.3	100
50 cmH_2O	60.3	37	83.3	20.5	58	99.3
60 cmH_2O	51.4	0.1	54.6	6.1	54.2	58.7
70 cmH_2O	49.9	0.1	54.6	6.1	54.2	58.7
80 cmH_2O	0.1	0	12.7	0.1	17.4	9
90 cmH_2O	0	0	4.5	0.1	0.8	3.3
100 cmH_2O	0	0	4.5	0.1	0.3	1.2
>80 yrs (n=35)						
20 cmH_2O	100	100	100	61.5	73.4	100
30 cmH_2O	100	100	100	24.8	61.5	100
40 cmH_2O	100	76.1	96.3	1.8	54.1	100
50 cmH_2O	49.5	18.3	68.8	0	49.5	80.7
60 cmH_2O	49.5	0	45	0	40.4	15.5
70 cmH_2O	0	0	11.9	0	2.8	1.8
80 cmH_2O	0	0	1.8	0	0	0
90 cmH_2O	0	0	0	0	0	0
100 cmH_2O	0	0	0	0	0	0

593

e-Table 4. Percent of females in whom a given maximal inspiratory pressure (MIP) was considered as indicative of weakness (<LLN) by each prediction equation across age groups. The lines indicate thresholds values where et 1 equations (Black and Hyatt,[5] Brusch et al.[7] and Neder et al.[9] uniformly agreed that at least 90% of subjects had weakness.

Age/MIP	*Black and Hyatt et al.*[5]	*Wilson et al.*[6]	*Bruschi et al*[7]	*Enright et al.*[8]	*Harik-Khan et al.*[9]	*Neder et al.*[10]
<40 yrs (n=140)						
20 cmH_2O	100	100	100	100	100	100
30 cmH_2O	100	100	100	100	100	100
40 cmH_2O	100	96.1	100	100	94.9	100
50 cmH_2O	93.8	57.4	91.8	84.3	62.8	100
60 cmH_2O	85.7	51.6	79.2	62.7	57.9	85.1
70 cmH_2O	54.9	40.9	51.2	58.1	50.3	80.3
80 cmH_2O	47.3	24.9	34.0	48.0	45.3	57.2
90 cmH_2O	18.1	1.7	19.2	40.7	38.1	32.3
100 cmH_2O	0	0	6.4	21.3	23.1	20.1
40–60 yrs (n=293)						
20 cmH_2O	100	100	100	100	100	100
30 cmH_2O	100	100	100	100	100	100
40 cmH_2O	95.5	90.3	97.8	81.2	85.3	99.8
50 cmH_2O	88.9	78.5	88.7	50.4	75.7	92.8
60 cmH_2O	45.5	22.0	60.2	40.0	58.7	60.0
70 cmH_2O	40.3	5.5	39.5	27.3	50.0	44.6
80 cmH_2O	20.1	2.1	24.1	6.9	35.0	25.5
90 cmH_2O	0	0	1.2	0	15.5	1.5
100 cmH_2O	0	0	0	0	8.5	0

61 – 80 yrs (n= 387)						
20 cmH_2O	100	100	100	100	85.6	100
30 cmH_2O	100	100	100	79.5	74.8	100
40 cmH_2O	100	98.5	100	48.5	62.8	100
50 cmH_2O70.3	70.3	35.5	80.0	25.5	55.1	80.2
60 cmH_2O	52.8	4.0	55.7	7.5	47.5	65.5
70 cmH_2O	455	2.1	35.5	1.8	32.8	30.3
80 cmH_2O	10.5	0	15.1	1.0	10.4	12.0
90 cmH_2O	0	0	2.5	0.1	0.4	1.2
100 cmH_2O	0	0	2.5	0.1	0.1	0
>80 yrs (n= 43)						
20 cmH_2O	100	100	100	74.8	80.5	100
30 cmH_2O	100	100	100	56.4	73.5	100
40 cmH_2O	88.8	84.7	78.3	30.7	50.3	91.0
50 cmH_2O	75.5	34.3	65.8	10.1	40.7	72.5
60 cmH_2O	45.5	22.1	35.5	2.3	25.6	48.5
70 cmH_2O	28.2	0	15.9	0	2.0	15.9
80 cmH_2O	0	0	0	0	0	0
90 cmH_2O	0	0	0	0	0	0
100 cmH_2O	0	0	0	0	0	0

598 **Table 3.** Optimal MIP % predicted cut-off associated with increased likelihood of respiratory muscle weakness according to
599 each prediction equation across age groups applicable for both males and females. Th best cut-offs were established by ROC curve
600 analysis.

	<40 yrs (N= 304)	40 - 60 yrs (N= 595)	61 - 80 yrs (N= 752)	>80 yrs (N= 78)
***Black and Hyatt et al.*[5]**	62	56	52	50
***Wilson et al.*[6]**	77	72	68	60
***Bruschi et al*[7]**	65	58	55	52
***Enright et al.*[8]**	75	70	68	65
***Harik-Khan et al.*[9]**	72	67	65	62
***Neder et al.*[10]**	65	58	55	50

Inter-equation agreement indicating weakness is shown in Table 4. Thus, while "high"[24] agreement was consistently reached within set 2, there was poorer agreement within set 1. Of note, a significant fraction of subjects with preserved MIP according to set 1 had "higher" pre-test probability of weakness (Figure 1B; $p<0.05$). Moreover, most subjects with reduced MIP according to set 2 but preserved MIP according to set 1 had "higher" pre-test probability of weakness (221/306 (72.2%)). Thus, we found superior agreement between pre-test probability of weakness and MIP below the LLN as defined by set 2 ((k (95% CI): Black and Hyatt[5] (0.68 (0.65-0.71), Bruschi et al.[7] (0.74 (0.70-0.78), and Neder et al.[10] (0.75 (0.72-0.78)) compared to set 1 (Wilson et al.[6], (0.39 (0.35-0.43), Enright et al.[8] (0.44 (0.41-0.45) and Harik-Khan et al.[9] (0.53 (0.49-0.57)). In fact, set 2 equations typically presented with higher areas under the ROC curves to suggest the presence of weakness compared to set 1 equations (area (standard error)= 0.524 (0.154) to 0.648 (0.131) versus 0.416 (0.124) to 0.559 (0.181), respectively).

613 **Table 4.** Between-equations agreement in indicating inspiratory muscle weakness*

	Black and Hyatt et al.[5]	*Wilson et al.*[6]	*Bruschi et al*[7]	*Enright et al.*[8]	*Harik-Khan et al.*[9]	*Neder et al.*[10]
Black and Hyatt et al.[5]	-	0.70 (0.68–0.72)	0.81 (0.78–0.82)	0.53 (0.51–0.55)	0.60 (0.58–0.61)	0.82 (0.80-0.84)
Wilson et al.[6]	0.70 (0.68–0.71)	-	0.62 (0.60–0.64)	0.60 (0.58–0.62)	0.39 (0.37–0.41)	0.55 (0.53–0.57)
Bruschi et al[7]	0.81 (0.79–0.83)	0.62 (0.60–0.64)	-	0.45 (0.43–0.47)	0.60 (0.58–0.59)	0.83 (0.81–0.85)
Enright et al.[8]	0.53 (0.51–0.55)	0.60 (0.58–0.62)	0.45 (0.43–0.47)	-	0.57 (0.55–0.59)	0.40 (0.38-0.42)
Harik-Khan et al.[9]	0.60 (0.58–0.61)	0.39 (0.37–0.41)	0.60 (0.58–0.61)	0.57 (0.55–0.59)	-	0.50 (0.48–0.52)
Neder et al.[10]	0.82 (0.80–0.84)	0.55 (0.53–0.57)	0.83 (0.81–0.85)	0.40 (0.38–0.42)	0.50–0.52)	-

614 *Values are Kappa (k) statistics (CI 95%).

1.5 DISCUSSION

This study investigated whether potential differences in predicted MIP according to the most frequently cited reference values[5-10] would impact on the prevalence of weakness (MIP below the LLN) in adults referred to a pulmonary function testing laboratory. Our main findings were: a) the prevalence of weakness varied widely depending on the chosen set of reference values; b) superior agreement to suggest weakness (k > 0.8, i.e., "high" agreement)[24] was reached among Black and Hyatt[5], Bruschi et al.[7] and Neder et al.[10] (set 2 equations) and c) while "substantial" agreement (kappa 0.6-0.8)[24] was uniformly found between these equations and "higher" pre-test probability of weakness, Wilson et al.[6], Enright et al.[8] and Harik-Khan et al.[9] (set 1 equations) agreed only "fairly" to "moderately" (k less <0.6)[24] with the probability of weakness . Thus, our data indicate that the variability in predicted MIP does carry significant implications for clinical decision making vis-à-vis a (provisional) diagnosis of weakness. Some reference values (Black and Hyatt[5], Bruschi et al.[7] and Neder et al.[10]) are more closely associated with other markers of weakness suggesting that they might prove particularly useful to screen subjects for more advanced neuromuscular assessment.

The ample heterogeneity of normal predicted values for MIP has long been recognized.[1-4,25] This might stem from several sources including inter- and intra-subject variability of a highly effort-dependent test,[15] differences in methods of measurement and reporting (Table e-1), and biological variation in variables which are notoriously difficult to control for, such as athleticism, muscularity and central versus peripheral fat deposition.[2,26,27,28,29] This issue has been addressed from theoretical and population based perspectives[4,14,15,16] which uniformly showed substantial heterogeneity across age ranges in both sexes. The present study sought to shed

637 new light on the topic by assessing whether the previously reported between-equation
638 differences in predicted values[4,14,15,16] were large enough to impact on the prevalence of
639 weakness, i.e., MIP below the LLN. Thus, we used a Bayesian approach in which MIP for each
640 subject was compared not only with a sound statistical benchmark[17] but also with the individual
641 pre-test probability of weakness.[3,18,21,22]

642 In this context, a key result with clinical implication relates to the superior performance
643 (i.e., high inter-equation agreement and concordance with pre-test probability of weakness) of
644 a set of reference values which predicted higher MIP values (set 2)[5,7,10.] These equations,
645 therefore, increased the prevalence of weakness compared to set 1.[6,8,9] Of note, a sizeable
646 fraction of subjects with preserved MIP according to set 1 had "higher" pre-test likelihood of
647 weakness[3,18,21,22] i.e., while these subjects were considered "non-weak" by set 1, their MIP
648 values were already below the LLN according to set 2. It should be acknowledged, however,
649 that this might have been influenced by a large fraction of subjects presenting with increased
650 pre-test probability of weakness (Table 1). Although this would favors equations estimating
651 higher MIP (set 2), our sample reflected the usual population referred for those measurements
652 in practice, i.e. subjects in whom clinical and/or imaging assessment previously raised the
653 hypothesis of inspiratory weakness. Considering the close agreement among set 2 equations
654 (Table 4), the choice of an "ideal" prediction equation would likely demand a comparative
655 analysis between those specific reference values against MIP measured in a local sample of
656 males and females presenting with a wide age range.

657 The present results should be interpreted under the assumption that volitional tests of
658 inspiratory muscle strength (such as MIP) are primarily for screening purposes rather than for

659 definitive diagnosis.[30] Thus, a low MIP needs to be confirmed by more sophisticated and time-
660 consuming methods.[3,31] Using reference values which tend to over-rate MIP as "normal" (set 1)
661 may substantially increase the prevalence of false negatives for weakness thereby negating
662 more accurate measurements for subjects with increased risk of true weakness. Conversely, it
663 could be argued that equations predicting lower MIP would be clinically more useful since
664 submaximal effort is commonly seen during MIP maneuvers.[28] Moreover, equations predicting
665 higher MIP (set 2)[5,7,10] may label a greater fraction of subjects as "abnormal" even if they have
666 low pre-test probability of weakness. In the present study, however, a better overall
667 compromise was reached by set 2 since reduced MIP according to these equations but lower
668 probability of weakness was significantly less frequent than normal MIP by set 1 but higher
669 probability of weakness (Figure 1B).

670 We also determined specific MIP cut-offs in which set 2 uniformly agreed that almost all
671 subjects in each age category had weakness (e-Table 3). It is noteworthy that most of these
672 subjects had a high pre-test likelihood of weakness. Thus, we propose these thresholds as
673 general "rules of thumb" to suggest weakness in clinical practice. The fact that those MIP values
674 were still considered in the normal range by set 1[16,8,9] emphasize their potential to under-
675 estimate the presence of weakness. The Enright et al.[8] study, in particular, is the largest source
676 of reference values for MIP published to date (e-Table 1). These authors, however, restricted
677 their analysis to persons aged 65 years or older. Thus, extrapolating the reference values to
678 younger subjects may have contributed to MIP underestimation. Owing to the large confidence
679 intervals, values as low as 20-30 cmH_2O were still considered as "normal" according to this
680 specific equation. Similar results were obtained by using Harik-Khan et al.[9] and, to a lesser

extent, Wilson et al.[6] equations (e-Table 3). Considering that many patients with MIP within the expected range according to set 1 had "higher" probability of weakness, these data corroborate the assertion that these equations tend to under-estimate the presence of weakness.

Our study should be appreciated in the light of its inherent strengths and limitations. From a positive perspective, this is one of the largest series published to date of MIP measurements consecutively performed in a "real life" setting according to the ATS/ERS guidelines.[3] As mentioned, results were interpreted taking into consideration robust clinical and physiological predictors of weakness.[3,18,21,22] However, we acknowledged that the lack of criterion tests of non-volitional strength[30,32] precluded any inference regarding to diagnostic accuracy of individual reference values. It should also be recognized that MIP tends to over-diagnose weakness and combination of tests increase diagnostic precision. For instance, an influential study found that these tests reduced the diagnosis of specific diaphragm weakness by 19%.[30] However, those advanced tests are beyond the capability of most non-specialized pulmonary function testing laboratories. Thus, despite its inherent limitations, MIP measurements are unlikely to be superseded in the foreseeable future as screening tests for weakness. Finally, we were unable to assess variations in MIP over time in a sizeable number of patients in whom required clinical information was available to judge the level of intra-equation concordance in the longitudinal diagnosis of weakness.

1.6 CONCLUSION

In conclusion, the ample variability of predicted MIP according to the most frequently cited reference values significantly impacted on the prevalence of inspiratory muscle weakness

in a large sample of males and females aged 20 to 94. Some reference values (Black and Hyatt[5], Bruschi et al.[7] and Neder et al.[10]) better agreed to suggest weakness and they were more closely associated with a cluster of clinical and physiological predictors of weakness than others (Wilson et al.[6], Enright et al.[8] and Harik-Khan et al.[9]). The former set of regression equations, therefore, might prove particularly useful in identifying individuals who require more advanced clinical neuromuscular assessment.

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1.7 REFERENCES

1. Moxham J. Respiratory muscle fatigue: mechanisms, evaluation and therapy. Br J Anaesth 1990;65(1):43–53.

2. Polkey MI, Green M, Moxham J. Measurement of respiratory muscle strength. Thorax 1995;50(11):1131–1135.

3. American Thoracic Society/European Respiratory Society. ATS/ERS Statement on respiratory muscle testing. Am J Respir Crit Care Med 2002;166(4):518–624.

722 4. Evans JA, Whitelaw WA. The assessment of maximal respiratory mouth pressures in
723 adults. Respir Care 2009;54(10):1348–1359.

724 5. Black LF, Hyatt RE. Maximal respiratory pressures: normal values and relationship to
725 age and sex. Am Rev Respir Dis 1969;99(5):696–702.

726 6. Wilson SH, Cooke NT, Edwards RH, Spiro SG. Predicted normal values for maximal
727 respiratory pressures in caucasian adults and children. Thorax 1984;39(7):535–538.

728 7. Bruschi C, Cerveri I, Zoia MC, et al. Reference values of maximal respiratory mouth
729 pressures: a population-based study. Am Rev Respir Dis 1992;146(3):790–793.

730 8. Enright PL, Kronmal RA, Manolio TA, Schenker MB, Hyatt RE. Respiratory muscle
731 strength in the elderly. Correlates and reference values. Cardiovascular Health Study Research
732 Group. Am J Respir Crit Care Med 1994;149(2 Pt 1):430–438.

733 9. Harik-Khan RI, Wise RA, Fozard JL. Determinants of maximal inspiratory pressure. The
734 Baltimore Longitudinal Study of Aging. Am J Respir Crit Care Med 1998;158(5 Pt 1):1459–1464.

735 10. Neder JA, Andreoni S, Lerario MC, Nery LE. Reference values for lung function tests.
736 II. Maximal respiratory pressures and voluntary ventilation. Braz J Med Biol Res
737 1999;32(6):719–727.

738 11. Hautmann H, Hefele S, Schotten K, Huber RM. Maximal inspiratory mouth pressures
739 (PIMAX) in healthy subjects--what is the lower limit of normal? Respir Med 2000;94(7):689–
740 693.

741 12. Wohlgemuth M, Kooi EL van der, Hendriks JC, Padberg GW, Folgering HT. Face mask
742 spirometry and respiratory pressures in normal subjects. Eur Respir J 2003;22(6):1001–1006.

743 13. Windisch W, Hennings E, Sorichter S, Hamm H, Criée CP. Peak or plateau maximal
744 inspiratory mouth pressure: which is best? Eur Respir J 2004;23(5):708–713.

745 14. Sachs MC, Enright PL, Hinckley Stukovsky KD, Jiang R, Barr RG, Multi-Ethnic Study of
746 Atherosclerosis Lung Study. Performance of maximum inspiratory pressure tests and maximum
747 inspiratory pressure reference equations for 4 race/ethnic groups. Respir Care
748 2009;54(10):1321–1328.

749 15. Petrini MF, Haynes D. In search of maximum inspiratory and expiratory pressure
750 reference equations. Respir Care 2009;54(10):1304–1305.

751 16. Sclauser Pessoa IMB, Franco Parreira V, Fregonezi GAF, Sheel AW, Chung F, Reid WD.
752 Reference values for maximal inspiratory pressure: a systematic review. Can Respir J J Can
753 Thorac Soc 2014;21(1):43–50.

754 17. Culver BH. How should the lower limit of the normal range be defined? Respir Care
755 2012;57(1):136-145-145.

756 18. Task group on surveillance for respiratory hazards in the occupational setting,
757 Brooks SM (Chairman). Surveillance for respiratory hazards. News 1982;8:12–16.

758 19. World Health Organization (WHO). Technical report series 894: Obesity: Preventing
759 and managing the global epidemic. [Internet]. Available from: 2000

760 20. Crapo RO, Morris AH, Clayton PD, Nixon CR. Lung volumes in healthy nonsmoking
761 adults. Bull Eur Physiopathol Respir 1982;18(3):419–425.

762 21. Knudson RJ, Lebowitz MD, Holberg CJ, Burrows B. Changes in the normal maximal
763 expiratory flow-volume curve with growth and aging. Am Rev Respir Dis 1983;127(6):725–734.

764 22. De Troyer A, Borenstein S, Cordier R. Analysis of lung volume restriction in patients
765 with respiratory muscle weakness. Thorax 1980;35(8):603–610.

766 23. Allen SM, Hunt B, Green M. Fall in vital capacity with posture. Br J Dis Chest
767 1985;79(3):267–271.

768 24. Landis JR, Koch GG. The measurement of observer agreement for categorical data.
769 Biometrics 1977;33(1):159–174.

770 25. Karvonen J, Saarelainen S, Nieminen MM. Measurement of respiratory muscle forces
771 based on maximal inspiratory and expiratory pressures. Respir Int Rev Thorac Dis
772 1994;61(1):28–31.

773 26. Moxham J. Respiratory muscle testing. Monaldi Arch Chest Dis 1996;51(6):483–488.
774 27. Wen AS, Woo MS, Keens TG. How many maneuvers are required to measure maximal
775 inspiratory pressure accurately. Chest 1997;111(3):802–807.

776 28. Aldrich TK, Spiro P. Maximal inspiratory pressure: does reproducibility indicate full
777 effort? Thorax 1995;50(1):40–43.

778 29. Arena R, Cahalin LP. Evaluation of cardiorespiratory fitness and respiratory muscle
779 function in the obese population. Prog Cardiovasc Dis 2014;56(4):457–464.

780 30. Steier J, Kaul S, Seymour J, et al. The value of multiple tests of respiratory muscle
781 strength. Thorax 2007;62(11):975–980.

782 31. Maillard JO, Burdet L, Melle G van, Fitting JW. Reproducibility of twitch mouth
783 pressure, sniff nasal inspiratory pressure, and maximal inspiratory pressure. Eur Respir J
784 1998;11(4):901–905.

785 32. Luo YM, Hart N, Mustfa N, et al. Reproducibility of twitch and sniff
786 transdiaphragmatic pressures. Respir Physiol Neurobiol 2002;132(3):301–306.

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ARTIGO EM SEGUNDO *ROUND* DE REVISÃO NA REVISTA MEDICINE AND SCIENCE IN SPORT AND EXERCISE- FATOR DE IMPACTO 4.478

2. DIFFERENCES IN RESPIRATORY MUSCLE RESPONSES TO HYPERPNEA OR LOADED BREATHING IN COPD

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2.1 ABSTRACT

Introduction: To compare diaphragm and non-diaphragmatic inspiratory muscle acute mechanical and metabolic responses during two different types of respiratory loading in patients with COPD.

Methods:In 16 patients with COPD (age 65±13, 56% male, FEV1 60±6%pred, Pimax 82±5%pred) diaphragm and non-diaphragmatic muscles (Scalene[SCA], Sternocleidomastoid[SCM], Parasternal[ICM] and 7th Intercostal[7thIC]) electromyogram (EMG) and respiratory pressures and its derivatives (transdiaphragmatic[Pdi] and esophageal[Pes] pressures, work of breathing[WOB] and pressure-time product[PTP]) were measured in a random order in two different conditions: 1) Hyperpnea, reproducing ventilatory responses during a whole-body high-intensity endurance exercise bout; 2) Loaded breathing reproducing high-intensity inspiratory muscle training. Breathing pattern and systemic (VO2, cardiac output and oxygen delivery and extraction) and local hemodynamic and oxygenation responses (SCA, 7thIC and abdominal muscle blood flow index, tissue oxygen saturation (StiO2), deoxyhemoglobin concentration ([HHb]), oxygen delivery) were assessed.

Results: Diaphragm activation (EMG%max) was similar between hyperpnea and loaded breathing (P=0.33). During hyperpnea, there were moderate-intensity load (Pes), increased breathing frequency, higher PTP/min and higher ventilation (P<0.05 for all). VO2 and non-diaphragmatic inspiratory muscle oxygen requirement ([HHb]) and delivery were increased (P<0.05 for all). Muscular oxygen delivery/utilization ratio (StiO2) was preserved. During loaded

834 breathing, there were higher loads and increased non-diaphragmatic inspiratory muscle
835 activation (EMG%max) ($P<0.05$). Breathing frequency, ventilation, Ti/Ttot and PTP/min were
836 lower ($P<0.05$ for all). There were intermittent increases in non-diaphragmatic inspiratory
837 muscle oxygen requirements ([HHb]), lower VO2 and systemic and local oxygen delivery ($P<0.05$
838 for all). Muscular oxygen delivery/utilization ratio was impaired (i.e., lower SCA and IC StiO2;
839 $P<0.05$ for all).

840 **Conclusion:** Loaded breathing evoked greater non-diaphragmatic muscle activation and
841 reduction in tissue oxygen saturation. Hyperpnea increased systemic responses and
842 metabolism without restricting non-diaphragmatic muscle oxygen saturation.

843

844 Key Words: RESPIRATORY MUSCLE ACTIVATION, RESPIRATORY MUSCLE LOADING,
845 RESPIRATORY MUSCLE METABOLISM, RESPIRATORY MUSCLE TRAINING.

2.2 INTRODUCTION

Improvements in both respiratory muscle endurance and strength can be observed in patients with COPD after whole-body endurance exercise training.(1-3) However, larger improvements in inspiratory muscle strength (i.e., pressure generating capacity) might be reported after specific inspiratory muscle training (IMT) by the addition of "high external resistance"(i.e. increases of 16 vs 6 cmH2O in MIP, respectively).(4, 5) Improvements after endurance training are probably due to the increased ventilatory demands during hyperpnea, thereby providing a high flow / low resistance stimulus to the respiratory muscles with a high number of repetitions (e.g. 30 breaths per minute for 15-30 minutes).(2, 3) During some types of IMT such as loaded breathing by tapered flow resistive loading, however, inspiratory muscle loading is a consequence of the addition of a "high external resistance" (e.g., loads equaling about 30-50% of maximal inspiratory pressure – MIP) that needs to be overcome by the respiratory muscles for a limited number of breathing cycles per session (e.g. 30-40 full vital capacity breaths).(4) Therefore, as much as limb muscles respond distinctively to endurance and strengthening stimuli,(6, 7) it can also be expected that different responses are induced when the respiratory muscles are experiencing "endurance" (i.e., exercise hyperpnea) or "strengthening" (i.e., loaded breathing) stimuli. Differences in specific loads imposed on the different components of the respiratory pump, muscle recruitment and activation patterns as well as local and systemic oxygenation responses evoked by increasing either ventilatory demand by exercise training (i.e., hyperpnea) or loaded breathing have however, to the best of our knowledge, never been comprehensively characterized. Therefore, we aimed to explore and compare the responses of a number of physiological variables during these two different

868 types of inspiratory muscle loading in patients with COPD namely 1) normocapnic hyperpnea
869 reproducing the breathing pattern during exercise training and 2) loaded breathing reproducing
870 the stimulus provided during an specific IMT session.

871 ## 2.3 METHODS

872 Subjects. Sixteen symptomatic patients (Baseline Dyspnea Index 6 ± 1)(8) with a clinical
873 diagnosis of COPD according to the Global Initiative for Chronic Obstructive Pulmonary Disease
874 (GOLD),(9) aged between 55 and 74 years (see subjects in the online supplement for more
875 details) were included in the study. The study was approved by the University Hospital Ethics
876 Committee (S58513). Before participation in the study, all patients were informed of any risks
877 and discomforts associated with the experiments and provided written, informed consent.

878 Study design. Experiments were performed in two visits. During the first visit (i.e., initial
879 testing) patients performed comprehensive pulmonary function testing.(10, 11) Maximal
880 inspiratory muscle strength was measured by maximum inspiratory mouth pressures (MIP).(12,
881 13) An incremental cardiopulmonary exercise test (CPET)(14) and a constant work rate cycle
882 endurance test (CWRT)(14) were also performed during this visit (please, see initial testing at
883 the supplemental material for more details). During the second visit, patients performed in a
884 random order a Normocapnic Hyperpnea trial (hyperpnea)(13, 15) reproducing the ventilatory
885 responses (i.e., mean tidal volume, breathing frequency and minute ventilation)(15) recorded
886 for each patient during the CWRT and a Tapered Flow Resistive Loading task (loaded breathing)
887 reproducing a high-intensity IMT session.(13, 16) Both tasks were performed for five minutes.
888 Breathlessness was measured by the modified Borg scale(17) at rest and at the end of each

889 task. Additionally, during the final 60 seconds of the hyperpnea and loaded breathing tasks,
890 respiratory muscle perfusion,(18) and oxygen delivery,(19) respiratory muscle activation (root
891 mean square EMG%max) and respiratory effort were assessed.(13, 20-23) Metabolic and
892 ventilatory variables were also assessed breath by breath during both tasks by a metabolic cart
893 (Vmax 229; Sensor Medics, Anaheim, CA, USA) (see below for detailed description of additional
894 assessments).

895 Normocapnic Hyperpnea trial (Hyperpnea). Patients were requested to maintain tidal
896 volume, breathing frequency and minute ventilation reproducing their own breathing
897 responses recorded during the CWRT for five minutes.(15) Thus, during the test investigators
898 provided continuous verbal guidance aiming to maintain a maximum variation in minute
899 ventilation of 5% throughout the test.(15) Visual feedback on breathing parameters was also
900 provided on a screen displayed in front of the patient so as to adjust his/her breathing
901 frequency and tidal volume to the level required by the investigator. Normocapnia was
902 maintained by having subjects inspire from a Douglas bag containing 5% CO2, 21% O2 and 74%
903 N2 for balance, connected to a two-way non-rebreathing valve (model 2700, Hans Rudolph) by
904 a piece of tubing.(15)

905 Tapered Flow Resistive Loading trial (Loaded breathing). The loaded breathing training
906 session was performed in accordance with previously published protocols of IMT using the
907 electronic POWERbreathe KH2 device(16) Subjects were requested to breathe out completely
908 (i.e., until residual volume) through a loaded breathing device (POWERbreathe KH2) for 30
909 breaths or for a minimum of five minutes.(16) The external resistance was set at ~50% of
910 patients MIP, reflecting the highest tolerable resistance that still allowed subjects to perform

full vital capacity inspirations. Thereby loading the inspiratory muscles throughout their full range of motion in accordance with a previously published method.(16)

Respiratory muscles pressures, work of breathing and activation during hyperpnea and loaded breathing. Respiratory muscle pressures and diaphragm activation (EMGdi) were measured by a combined multipair esophageal electrode catheter with esophageal- and gastric-balloons (Yinghui Medical Equipment Technology Co. Ltd., Guangzhou, China) nasally inserted after topical anesthesia. Procedures for optimal positioning of the catheter and signal processing have already been published(20) and are described in detail in Respiratory muscle recruitment, respiratory effort and diaphragm activation in the supplemental material. EMGdi was converted into root mean square (RMS), normalized by its maximum activation during inspiratory capacity maneuvers (ICs) and reported as percentage of maximum activation (EMGdi, %max) (detailed information is provided in Respiratory muscle recruitment, respiratory effort and diaphragm activation in the supplemental material. Continuous measurements of esophageal (Pes), gastric (Pga) and transdiaphragmatic (Pdi, i.e., Pga - Pes) pressures and its derivatives were performed (for more details see Respiratory muscle recruitment, respiratory effort and diaphragm activation in the supplemental material). Inspiratory Pes, Pga and Pdi max were obtained during inspiratory sniff maneuvers.(20) Expiratory Pga max, however, was obtained during forced expiratory capacity maneuvers. Non-diaphragmatic respiratory muscle activation was measured by surface electromyography (sEMG) (Desktop Direct Transmission (DTS), NORAXON, Scottsdale, USA).(21) Electrodes were placed (1) on the posterior left triangle of neck at the level of cricoid process for scalene muscle measurements (EMGsca), (2) at the midpoint along the long axis of the right sternocleidomastoid muscle between the mastoid

process and the medial clavicle for sternocleidomastoid muscle measurement (EMGscm), (3) at the right parasternal space of the 2nd and 3rd rib 3 cm lateral to the sternum for parasternal intercostal muscle measurements (EMGpicm), (4) at the line between 7th and 8th intercostal space at mid axillary line for 7th Intercostals measurements (EMG 7th icm). Total Inspiratory Neural Drive (IND) was estimated as the sum of the maximum activation of each respiratory muscle (i.e., IND(RMS)= EMGdi(RMS) + EMGsca(RMS) + EMGscm(RMS) + EMGicm(RMS) + EMG 7th ICM (RMS)), and the partial contribution of each respiratory muscle to the respiratory pump (i.e., %IND) was measured as its respective activation divided by the IND (e.g., EMGdi, %IND= EMGdi(RMS)/IND). More detailed methods about sEMG acquisition, processing and normalization are reported in Respiratory muscle recruitment, respiratory effort and diaphragm activation in the supplemental material.

Central hemodynamic responses during loaded breathing and hyperpnea. Cardiac output, heart rate and stroke volume were continuously measured by impedance cardiography device (PhysioFlowPF50; Manatec Biomedical, Macheren, France) previously validated for COPD patients(24) and are described in details in Central hemodynamic responses during hyperpnea and loaded breathing in the supplemental material. Estimated systemic oxygen delivery was calculated by the product of cardiac output and arterial oxygen content; the latter was calculated as the product of 1.39 x hemoglobin concentration [Hb] and %SpO2,(25) whilst arterio-venous oxygen content (a-vO2) difference was calculated by dividing oxygen uptake by cardiac output. The systemic oxygen extraction ratio was calculated as the ratio of the arterio-venous oxygen content (a-vO2) difference to arterial oxygen content. In addition, systemic

954 vascular conductance was calculated by dividing cardiac output with mean arterial blood
955 pressure

956 Respiratory muscles perfusion and oxygenation responses. Non-diaphragmatic muscles
957 (i.e., 7th Intercostal, Scalene and Abdominal) blood flow index (BFI)(18) was calculated by using
958 two commercial Near-Infrared Spectroscopy (NIRS; NIRO-200 and a NIRO-200NX;
959 HAMAMATSU Photonics KK) and the light-absorbing indocyanine green dye (ICG) that was
960 injected through a peripheral venous catheter in line as previous validated for patients with
961 COPD(18) (see Respiratory muscles perfusion and oxygenation responses in the supplemental
962 material for further information).(24) NIRS optodes were placed at the left 7th intercostal
963 space, the right posterior triangle of the neck and abdomen to respectively measure Intercostal,
964 Scalene and Rectus Abdominis muscles perfusion. ICG injections for calculating BFI were
965 performed during the last 5 breaths during loaded breathing and during the last 30 seconds of
966 exercise during hyperpnea trial.

967 NIRS-derived changes in local respiratory muscle deoxyhemoglobin concentration
968 ([HHb]) was used as an index of respiratory muscle oxygen extraction.(26) NIRS-derived tissue
969 oxygen saturation index (i.e., StiO2) was measured as a measure of the dynamic balance
970 between local tissue oxygen delivery and utilization(27) and therefore local muscle capacity to
971 match oxygen supply relative to its metabolic demand (see Respiratory muscles perfusion and
972 oxygenation responses in the supplemental material for further details).

973 Statistical analysis. For sample size considerations, please see Data analysis in the
974 supplemental material. Data are expressed as mean ± SE or mean difference (95% confidence

975 interval). Mean respiratory muscle activation, respiratory pressures and its derivatives,
976 breathing pattern variables and central hemodynamic and metabolic variables during the last
977 60 seconds of rest, hyperpnea and loaded breathing were compared by one-way repeated
978 measures ANOVA when normal distribution was not violated. Otherwise, the Friedman test was
979 used. When statistical significance was met ($P < 0.05$) pairwise comparisons with Holm
980 correction were performed as post-hoc analysis. Changes in respiratory muscles perfusion and
981 oxygenation responses from rest to hyperpnea versus rest to loaded breathing were compared
982 by paired T test when normally distributed or by Mann-Whitney test if normal distribution
983 criteria was not met (see Data analysis in the supplemental material for more details).

984 ## 2.4 RESULTS

985 *Subjects characteristics.* Subjects' characteristics are described in detail in table 1. The
986 sample was well balanced regarding sex and composed by patients classified as having mild to
987 very severe COPD presenting resting lung hyperinflation (i.e., increased RV/TLC) (see Subjects
988 characteristics in the supplemental material for more details). Six out of the sixteen included
989 subjects did not have diaphragm EMG and respiratory pressures data, due to difficulties during
990 the insertion of the esophageal catheter (n= 1) or did not agree (n= 5) to swallow the
991 esophageal catheter. Three patients did not have respiratory muscle perfusion measured for
992 technical reasons (n=1) or because of contraindications regarding ICG injections (n=2). Hence,
993 nine out of the sixteen patients had concurrent measurements of diaphragm EMG, respiratory
994 pressures and respiratory muscle perfusion. There were no differences regarding pulmonary
995 function, peak exercise and inspiratory muscle capacity between subjects with EMGdi and

996 respiratory pressures measurements versus those subjects not able or not willing to undergo
997 for this specific experimental procedure.

998

999 **Table 1.** Subjects' characteristics, pulmonary function and peak exercise and inspiratory
1000 muscle capacity data

	n: 16
Demographics / Anthropometrics	
Sex, male/female	9 / 7
Age, yrs	65 ± 13
BMI, kg/m^2	27 ± 1.6
Pulmonary function	
FEV$_1$, L	1.44 ± 0.15
FEV$_1$, %pred	60 ± 6
FVC, L	3.23 ± 0.22
FVC, %pred	99 ± 8
FEV$_1$/FVC, %	44 ± 3
MVV, L/min	52 ± 5
MVV, %pred	65 ± 8
TLC, L	6.4 ± 0.46
TLC, %pred	118 ± 5
RV, L	3.45 ± 0.33
RV, %pred	155 ± 12

RV/TLC, %	54 ± 2
VC, L	2.9 ± 0.2
TLCO, mmol/min/kpa	4.3 ± 0.4
TLCO, %pred	56 ± 4
Peak exercise data and inspiratory muscle capacity	
Wpeak, W	81 ± 7
Wpeak, %max	71 ± 5
VO_2, peak, L/min	1.371 ± 0.116
VO_2, peak, %max	87 ± 8
COpeak, L/min	12.0 ± 0.5
MIP, cmH_2O	74 ± 4
MIP, %pred	82 ± 5
MIP <LLN, n(%)	9 (56)
Hb, g/dl	14.5 ± 0.3

Data are mean ± SE or n (%). FEV_1: forced expiratory volume in the first second; FVC: forced - vital capacity; MVV: maximum voluntary ventilation; TLC: total lung capacity; RV: residual volume; TLCO: transfer factor for carbon monoxide; MIP: maximal inspiratory pressure; Insp. mm. weakness: maximum inspiratory pressure bellow the lower limit of normality; Wpeak; peak exercise capacity; VO2peak: peak oxygen consumption; COpeak; peak cardiac output; LLN: lower limit of normality.

Respiratory symptoms during hyperpnea and loaded breathing tasks. Neither breathlessness nor respiratory effort' sensation were statistically different between hyperpnea and loaded breathing (5 ± 1 vs. 4 ± 1, P 0.15 and 5 ± 1 vs. 5 ± 1, P 0.93, respectively).

Respiratory muscle activation. Inspiratory neural drive was significantly increased during loaded breathing in comparison to hyperpnea (figure 1a). We found similar diaphragm activation (EMG%max) (figure 1b), between hyperpnea and loaded breathing (P= 0.35), however, activation of non-diaphragmatic inspiratory muscles was significantly higher during loaded breathing as compared to hyperpnea (figures 1b – 1f). Nevertheless, when considering the contribution of each respiratory muscle activation to the total inspiratory neural drive the results indicate that diaphragm contribution was again similar between hyperpnea and loaded breathing (P= 0.42) (supplemental material figure E1a). At the same time we found significant greater contributions to the total inspiratory neural drive of the non-diaphragmatic inspiratory muscles during loaded breathing in comparison to hyperpnea (supplemental material figure E1). There were no statistical significant differences between expiratory activation of the abdominal muscle between hyperpnea and loaded breathing (EMGabd, %max: 33 ± 4 vs. 30 ± 6, respectively; P=0.27).

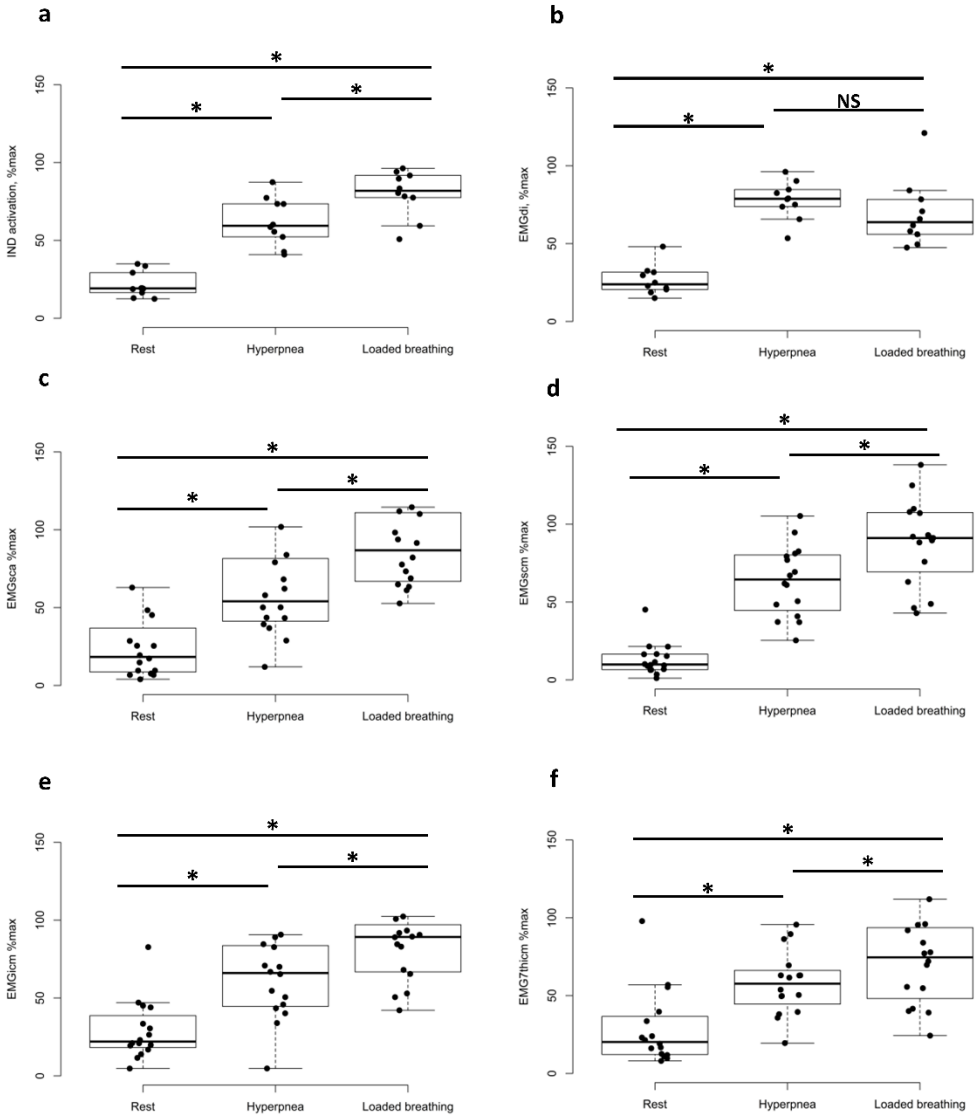


Figure 1. Comparisons between the EMG activation among the different tasks. RND, %max: relative total respiratory neural drive activation; EMGdi, %max: relative diaphragmatic activation; EMGsca, %max: relative scalenes activation; EMGscm, %max: relative sternocleidomastoid activation; EMGicm, %max: relative parasternal intercostal activation; EMG 7th icm, %max: relative 7th intercostal activation. Boxplots shows median at central line, first and third quantiles for lower and upper box's limits, respectively, and minimum and maximum values for

lower and upper limits. Dots are single patients' values. Dots outside the limits are outliers' values. *P <0.05; NS; P >0.05. EMGdi: n= 10; sEMG n= 16.

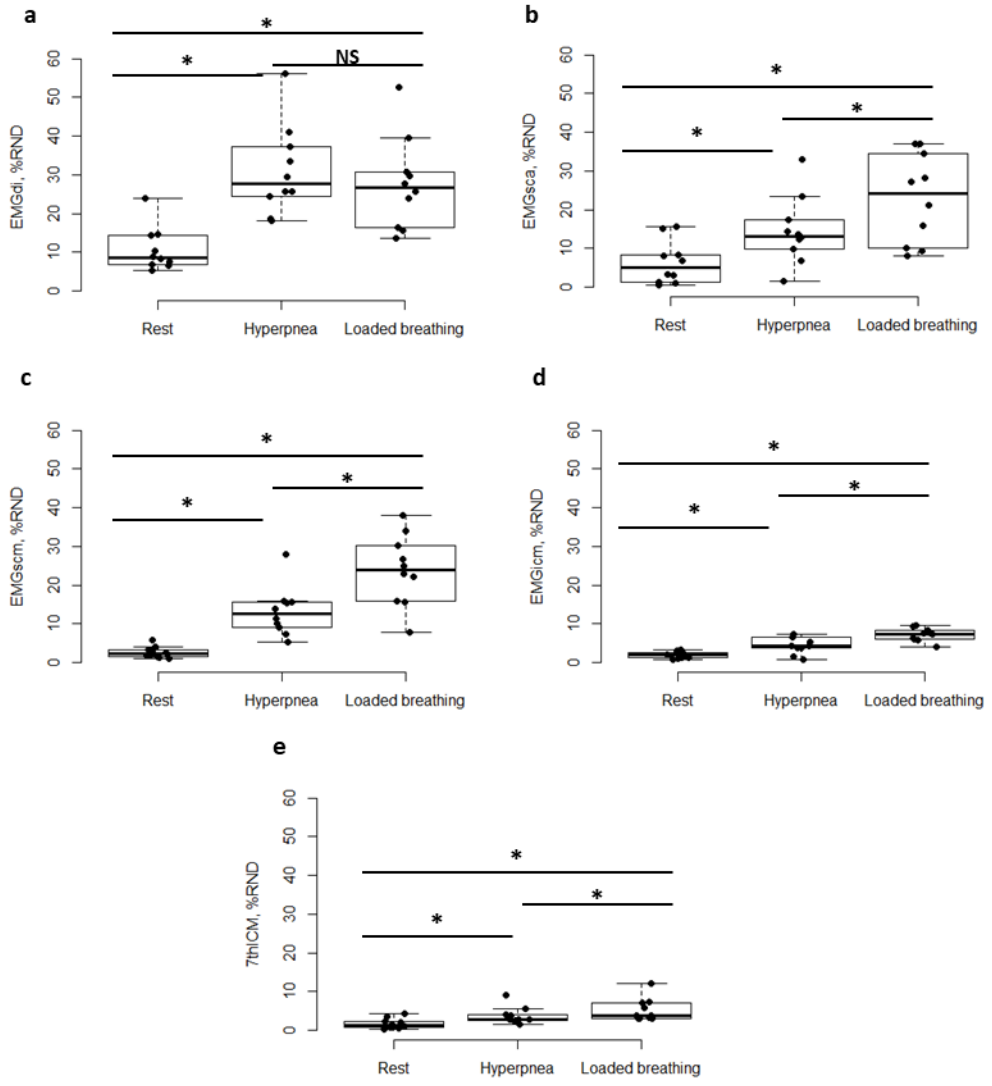


Figure E1. Boxplots of the contribution of each muscle to the respiratory neural drive. EMGdi, %RND: relative diaphragmatic contribution; EMGsca, %RND: relative scalenes contribution; EMGscm, %RND: relative sternocleidomastoid contribution; EMGicm, %RND: relative parasternal intercostal contribution; EMG 7th icm, %RND: relative 7th intercostal contribution. Boxplots shows median at central line, first and third quantiles for

lower and upper box's limits, respectively, and minimum and maximum values for lower and upper limits. Dots are single patients' values. Dots outside the limits are outliers' values. *P <0.05; NS; P >0.05. EMGdi: n= 10; sEMG n= 16.

Respiratory pressures and work of breathing. Diaphragmatic and esophageal pressures per breath were significantly higher during loaded breathing in comparison to hyperpnea, gastric pressures, however, were similar (P= 0.64) (table 2). Pes PTP and Pes WOB/b were significantly higher during loaded breathing in comparison to hyperpnea (table 2). For diaphragmatic and gastric pressures, only Pga and Pdi WOB/b were significantly greater during loaded breathing as compared to hyperpnea (table 2). Pes and Pdi WOB/min were significantly increased during hyperpnea in comparison to loaded breathing but Pga WOB/min was similar (P= 0.06). Pes, Pga and Pdi PTP/min responses during hyperpnea were significantly higher as compared to loaded breathing (table 2). There were no statistical differences between expiratory Pga between hyperpnea and loaded breathing (P= 0.83; table 2).

Table 2. Respiratory pressures and work of breathing and breathing pattern data during hyperpnea and loaded breathing

				Mean diff (95% CI)		
	Rest	Hyperpnea	Loaded breathing	Hyperpnea - Rest	Loaded breathing - Rest	Loaded breathing - Hyperpnea
Respiratory pressures and work of breathing (n= 10)						
Pes, cmH$_2$O	-9 ± 1	-15 ± 1	-35 ± 2	-6 (-11 - -2)*	-26 (-30 - -21)*	-19 (-24 - -15)*
Pes, %max	14 ± 2	23 ± 2	54 ± 5	10 (-2 – 21)*	40 (27 – 51)*	30 (18 – 41)*
Pga, cmH2O	10 ± 2	12 ± 2	15 ± 4	1 (-9 – 12)	5 (-5 – 15)	3 (-7 – 13)
expPga, cmH2O	10 ± 1	21 ± 4	21 ± 4	10 (-1 – 21)	11 (0 – 22)	1 (-10 – 12)
Pga, %max	21 ±	22 ± 4	26 ± 6	1 (-15 – 17)	5 (-11 – 21)	4 (-12 – 20)
Pdi, cmH$_2$O	19 ± 1	27 ± 2	50 ± 4	7 (17 - -2)*	30 (40 – 20)*	22 (32 – 12)*
Pdi, %max	21 ± 2	28 ± 1	53 ± 4	7 (-2 – 16)*	32 (22 – 41)*	24 (15 – 34)*
Pes WOB, L/cmH$_2$O	7 ± 1	20 ± 3	78 ± 11	13 (-11 – 37)	72 (48 – 96)*	59 (35 – 82)*
Pga WOB,, L/cmH2O	8 ± 2	17 ± 6	37 ±12	9 (-18 – 37)	29 (2 – 55)*	19 (-8 – 46)
Pdi WOB, L/cmH$_2$O	15 ± 2	37 ± 8	115 ± 20	23 (-21 – 66)*	100 (56 – 144)*	78 (34 – 122)*
PTP Pes, cmH2O/s//b	4 ± 0	6 ± 0	8 ± 1	2 (0 – 4)*	4 (-2 – 4)*	2 (0 – 4)*
PTP Pga, cmH2O/s/b	4 ± 1	4 ± 1	3 ± 1	0 (-3 – 3)	-1 (4 – 2)	-1 (-4 – 2)
PTP Pdi, cmH2O/s/b	8 ± 1	10 ± 1	11 ± 1	2 (0 – 6)	3 (0 – 6)	1 (-2 – 4)
Pes WOB, L/cmH$_2$O/min	121 ± 14	614 ± 76	431 ± 57	494 (299 – 688)*	310 (116 – 505)*	-183 (-377 – 12)*

Pga WOB, L/cmH2O/min	137 ± 22	541 ± 175	189 ± 47	403 (34 - 773)*	51 (-318 – 420)	-352 (-721 – 17)
Pdi WOB, L/cmH_2O/min	257 ± 21	1154 ± 240	620 ± 79	897 (384 – 1411)*	363 (-150 – 877)*	-534 (-1047 - -21)*
PTP Pes, cmH_2O/s/min	71 ± 12	184 ± 16	49 ± 9	112 (69 – 157)*	-21 (-66 – 22)	-135 (-179 – 91)*
PTP Pga, cmH2O/s/min	84 ± 18	142 ± 28	21 ± 7	58 (-12 – 127)*	-62 (-132 – 7)*	-120 (-190 - -51)*
PTPPdi, cmH_2O/s/min	154 ± 26	325 ± 35	68 ± 13	171 (79 – 262)*	-85(-177 – 6)*	-256 (-348 - -1654.73)*
Breathing pattern (n= 16)						
	Rest	**hyperpnea**	**loaded breathing**	**hyperpnea - Rest**	**loaded breathing - Rest**	**loaded breathing - hyperpnea**
VE, L	13 ± 1	38 ± 3	12 ± 1	25 (18 – 32)*	-1 (-8 - 5)	-26 (-33 - -19)*
Insp. vol., L	0.74 ± 0.06	1.17 ± 0.11	1.9 ± 0.21	0.43 (-0.05 – 0.91)*	1.16 (0.68 – 1.64)*	0.73 (0.25 – 1.21)*
Bf, b/min	20 ± 1	34 ± 1	7 ± 1	14 (10 – 18)*	-13 (-17 - -8)*	-27 (-31 - -22)*
Insp. peak flow, L/sec	0.91 ± 0.05	2.47 ± 0.18	2.23 ± 0.2	1.56 (1.03- 2.09)*	1.32 (0.80 - 1.85)*	-0.24 (-0.77 – 0.28)
Insp. time, s	1.27 ± 0.1	0.67 ± 0.04	2.26 ± 0.22	-0.60 (-1.09 – 0.11)*	0..99 (0.50 – 1.47)*	1.58 (1.10 – 2.07)*
Ti/Ttot, %	38 ± 1	37 ± 1	24 ± 2	-2 (-6 - 4)	-14 (-19 - -8)*	-12 (-18 - -7)*
EELV, %VC	30 ± 2	45 ± 2	0 ± 0	14 (8 - 20)*	-30 (-37 – 24)*	–45 (-51 - -38)*
EILV, %VC	54 ± 2	81 ± 2	59 ± 4	26 (16 – 38)*	5 (-6 - 16)	-22 (-33 - 11)*

Data are mean ± SE or mean difference (95% confidence interval). EELV: end inspiratory lung volume; EILV: end inspiratory lung volume; Ti/Ttot: duty cycle of respiration; Bf: breathing frequency; Pes: Esophageal pressure; Pdi: Transdiaphragmatic pressure; WOB: work of breathing; PTP: Pressure Time Product. * P <0.05.

Breathing pattern. In comparison to hyperpnea, ventilation was lower, absolute and relative inspiratory volumes were higher and breathing frequency was significantly lower during loaded breathing ($P < 0.05$). Peak inspiratory flow was similar ($P = 0.20$) and accompanied by longer inspiratory time and lower duty cycle during loaded breathing ($P < 0.05$; table 2). EELV and EILV were significantly lower during loaded breathing in comparison to hyperpnea (table 2).

Central hemodynamic and metabolic responses. Cardiac output and oxygen consumption responses were significantly greater during hyperpnea in comparison to loaded breathing. During loaded breathing arteriovenous oxygen difference was significantly lower as compared to hyperpnea (table 3). Systemic vascular conductance responses were significantly greater during hyperpnea as compared to loaded breathing (table 3).

Table 3. Central hemodynamic and metabolic responses

				Mean diff (95% CI)		
	Rest	**hyperpnea**	**loaded breathing**	**hyperpnea - Rest**	**loaded breathing - Rest**	**loaded breathing - hyperpnea**
HR, bpm	76 ± 3	90 ± 4	89 ± 4	14 (2 – 26)*	13 (1 – 25)*	-1 (-13 – 11)
SV, ml	70 ± 4	84 ± 6	73 ± 4	15 (-1 – 31)*	4 (-13 – 20)	-11 (-27 – 5)*
CO, L/min	5 .2 ± 0.3	7.5 ± 0.5	6.5 ± 0.4	2.3 (0.9 – 3.7)*	1.1 (0.2 – 2.6)*	-1.1 (-.4 – 0.3)*
CO, %max	44 ± 3	62 ± 4	54 ± 4	19 (6 – 32)	10 (-3 – 23)	-8 (-21 – 5)
VO_2, ml/min	283 ± 20	625 ± 42	443 ± 34	342 (229 – 454)*	161 (46 – 275)*	-181 (-296 - -67)*
VO_2, %max	25 ± 4	54 ± 7	39 ± 5	29 (10 – 48)*	13 (-6 – 32)*	-16 (-34 – 3)*
VCO_2, ml/min	224 ± 14	412 ± 69	409 ± 32	188 (35 – 341)*	185 (29 – 340)*	-4 (-159 – 151)
$CaO_2 ml O_2/100ml$	18.9 ± 0.5	19.2 ± 0.5	19.2 ± 0.4	0.3 (-1.2 – 1.9)	0.3 (-1.2 – 1.9)	0 (-1.5 – 1.6)
O_2 delivery, LO_2/min	0.98 ± 0.05	1.42 ± 0.1	1.23 ± 0.07	0.44 (0.17 – 0.71)*	0.25(-0.01 – 0.52)*	-0.18 (-0.45 – 0.08)*
O_2 extraction, %	29 ± 2	46 ± 4	38 ± 3	16 (6 - 26*	8 (-2 – 19)*	-8 (-18 – 3)*
a-vO_2 difference , $ml O_2/100ml$	5.6 ± 03	8.73 ± 0.75	7.3 ± 0.7	3.2 (1.1 – 5.3)*	1.7 (-0.4 – 3.9)*	-1.4 (-3.5 – 0.7)*
SVC, ml/min/mmHg	56 ± 3	74 ± 5	63 ± 4	18 (5 – 32)*	7 (-7 – 21)*	-11 (-26 – 3)*
SpO_2, %	94 ± 1	95 ± 1	94 ± 1	2 (-1 – 4)	0 (-2 – 3)	-1 (-4 – 1)
SBP, mmHg	120 ± 3	139 ± 6	133 ± 5	19 (4 – 33)*	13 (-3 – 28)*	-5.9(-22 – 10)
DBP, mmHg	80 ± 2	88 ± 2	90 ± 4	8 (-1 – 17)	10 (1 – 20)*	2 (-8 – 12)
MAP	93 ± 2	105 ± 3	104 ± 4	12 (2 – 22)*	11 (1 – 21)*	-1 (-11 – 10)

Data are mean ± SE or mean difference (95% confidence interval). HR: heart rate; SV: stroke volume; CO: cardiac output; VO_2: oxygen consumption; VCO_2: carbon dioxide production; CaO_2; arterial oxygen content; a-vO_2 difference: arterio-venous oxygen difference; SVC: systemic vascular conductance SpO_2: peripheral oxygen saturation; SBP: systolic blood pressure; DBP: diastolic blood pressure; Vasc. Cond.: systemic vascular conductance. *P <0.05.

Respiratory muscle perfusion and oxygenation responses. The increase in scalene muscle BFI from rest was significantly lower during loaded breathing as compared to hyperpnea (table 4). Scalene muscle oxygen extraction (i.e., [HHb]) responses were significantly greater and tissue oxygen saturation was significantly lower during loaded breathing in comparison to hyperpnea. BFI and oxygen delivery also increased less during loaded breathing (table 4). Additionally, despite the absence of significant differences in 7th intercostal muscle BFI and oxygen delivery between loaded breathing and hyperpnea (P= 0.27 and P=0.26, respectively), 7th Intercostal muscle oxygen extraction tended to be higher during loaded breathing (P=0.56) leading to a significantly greater decrease in Intercostal muscle tissue oxygen saturation as compared to hyperpnea (table 4). No significant changes in perfusion (P=0.09), oxygen extraction (P=0.11), oxygen delivery (P=0.10) and tissue oxygen saturation (P=0.50) responses were observed for the abdominal muscles between loaded breathing and hyperpnea.

Table 4. Respiratory muscles perfusion and oxygenation responses during hyperpna and loaded breathing

			Mean diff (95% CI)
	hyperpnea	**loaded breathing**	**loaded breathing - hyperpnea**
Respiratory muscle perfusion, n= 13			
ΔSCA BFI, nmol/L	4.67 ± 1.3	2.81 ± 1.17	-1.86 (-3.2 - -0.5)*
Δ7th IC BFI, nmol/L	0.76 ± 0.2	0.5 ± 0.2	0.27 (-0.78 - 0.2)
ΔABD BFI, nmol/L	1.2 ± 0.5	0.4 ± 0.3	-0.8 (-1.7 – 0.2)
Respiratory muscle O2delivery			

ΔSCA O2del, au	90 ± 24	54 ± 22	-36 (-11 - -62)*
Δ7th IC O2del, au	14 ± 4	10 ± 5	-5 (4 - -14)
ΔABD O2del, au	23 ± 10	8 ± 6	-14 (3 – 33)
Respiratory muscle oxygen saturation, n= 15			
ΔSCA StiO2, %	1.25 ± 0.9	-2.84 ± 1.27	-4.1 (-6 - -2.1)*
Δ7th IC StiO2, %	1.5 ± 0.71	-1.52 ± 0.86	-3 (-4.9 - -1.3)*
ΔABD StiO2, %	1.00 ± 1.00	-0.40 ± 1.52	-1.38 (-3.6 - 0.9)
Respiratory muscle oxygen extraction, n= 15			
ΔSCA [HHb], µmol/L	2.94 ± 1.33	7.68 ± 2.08	4.73 (1.88 -7.58)*
Δ7th IC [HHb], µmol/L	0.42 ± 0.61	1.9 ± 0.87	1.48 (-0.05 - 3)
ΔABD [HHb], µmol/L	-1.67 ± 0.86	0.03 ± 1.1	1.70 (-0.82 - 3.48)

Data are mean ± SE or mean difference (95% confidence interval). Δ: changes from rest; SCA: Scalenes; 7th IC: 7th Intercostal; ABD: Rectus Abdominis; [HHb]: deoxyhemoglobin concentration; $StiO_2$: Tissue oxygen saturation index; BFI: blood flow index. *P <0.05.

2.5 DISCUSSION

Main findings

Our key findings are the differences in both local (i.e., respiratory muscle) and central responses evoked by different patterns of respiratory muscle loading provided during hyperpnea reproducing the ventilatory requirement of exercise hyperpnea and loaded breathing in patients with COPD. While loaded breathing elicits greater non-diaphragmatic inspiratory muscle activation and oxygen requirement (figure 1 and table 4), during hyperpnea

local and systemic hemodynamic responses were increased in association with an increased systemic oxygen requirement, (tables 3 and 4). The increased non-diaphragmatic inspiratory muscle activation and oxygen requirements during loaded breathing (figure 1 and table 4) reveals the additional loading imposed by this loading regimen on these muscles in comparison to an endurance loading stimulus (table 2). This became evident by additional activation of rib cage (i.e., Intercostals) and neck (i.e., Scalenes and Sternocleidomastoid) inspiratory muscles. Enhanced diaphragmatic activation was of similar magnitude during hyperpnea and loaded breathing (figure 1).

Respiratory muscle activation during loaded breathing and hyperpnea

Increased recruitment of the diaphragm as well as non-diaphragmatic inspiratory muscles is an expected strategy in humans when the respiratory demands are increased.(28) Therefore, the observed increased in the total inspiratory neural drive can be explained given the increased loads imposed on the respiratory muscles during both hyperpnea and loaded breathing (figure 1 and table 2). Moreover, the contribution of non-diaphragmatic muscles to the act of breathing is amplified at higher lung volumes, and this “dependency” is further heightening with increased inspiratory flows.(29-31) Additionally, breathing at increased lung volumes and its ensuing rib cage displacement are known to impair the length-tension relationship of the diaphragm, consequently to moving its fiber length to a far position from its optimal length and by increasing its radius curvature.(32-34) Thereupon, decreasing diaphragm capacity to generate pressure.(32) - Of note, besides the resting hyperinflation (i.e., increased

RV/TLC; table 1), whilst higher EILV occurs towards the end of each inspiration during loaded breathing due to “full” vital capacity inspirations during hyperpnea higher EILV might be consequential to dynamic hyperinflation ensuing an increased end expiratory lung volume (table 2). - Notoriously, as compared to diaphragm, increased lung volumes ensue less length-tension impairment at non-diaphragmatic inspiratory muscles (i.e., 30 - 35 vs. 3 - 10%, respectively), i.e., “less” sub-optimal length at higher volumes,(35-37) thereby preserving their pressure generating capacity.(37)

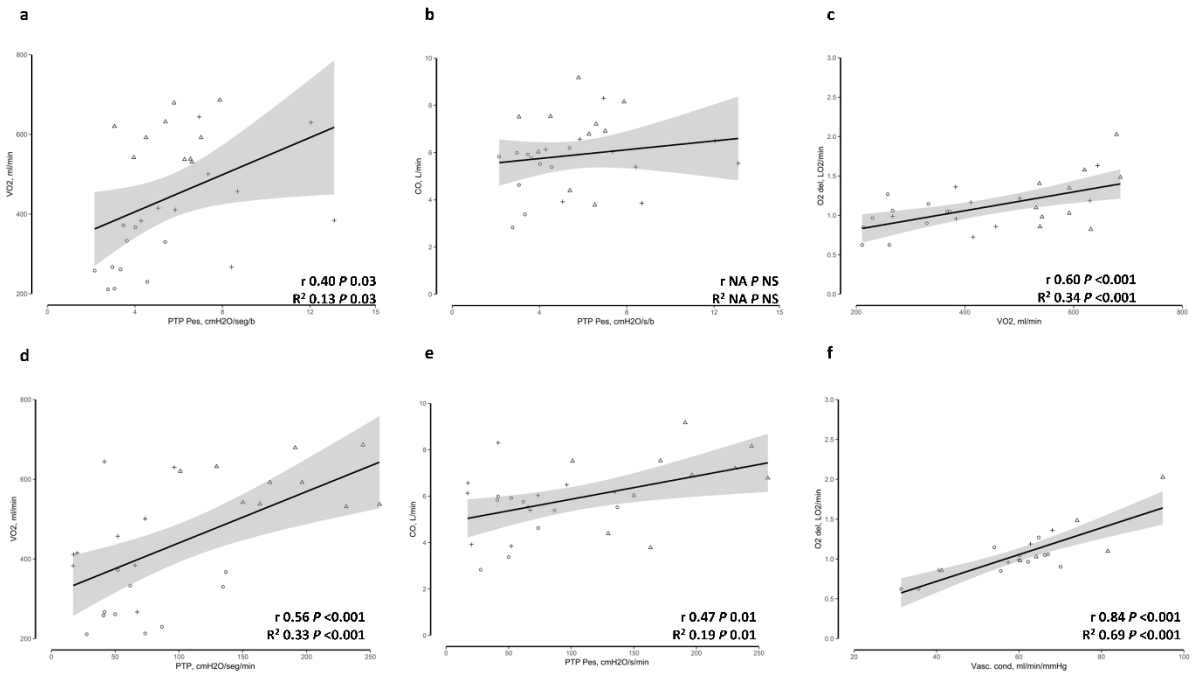
Thus, non-diaphragmatic inspiratory muscle recruitment corollary allows the respiratory system to compensate for the lost efficiency of the diaphragm. In fact, in subjects presenting impaired diaphragmatic ability to match the ventilatory demand (i.e., diaphragmatic paralysis), Intercostal and neck muscle contraction may result in slight higher changes in airway opening pressure.(38) Moreover, non-diaphragmatic inspiratory muscles aid the diaphragm by increasing lateral, dorsoventral (i.e., Intercostals) and cranial (i.e., Scalenes and Sternocleidomastoid) displacement of the rib cage, whilst the diaphragm increases the caudal and lateral displacement.(32) Thusly, while diaphragm ability to decrease pleural pressure and consequently generate flow might face a ceiling effect with increasing end inspiratory lung volumes (EILV) (i.e., “flattened” dome),(32) non-diaphragmatic inspiratory muscles recruitment cleverly behaves as a reserve to overcome increasing demands inflicting the respiratory system.(35, 36) The recruitment of non-diaphragmatic muscles as a reserve is further highlighted by its additional recruitment during loaded breathing (Figure 1 and supplemental material figure E1) when an additional external load was imposed to the respiratory system, therefore, inflicting increased respiratory demands (i.e., inspiratory pressures, WOB and PTP;

table 2). Interesting, despite the significant increase in Pdi and its derivatives (table 2), the absence of increased inspiratory Pga highlights the overload imposed during loaded breathing being de facto overcame by non-diaphragmatic muscles, i.e., changes in Pdi were due to increased Pes and not Pga (see Gastric pressure behavior during hyperpnea and loaded breathing supplemental material for more details).

Local and systemic metabolism responses during loaded breathing and hyperpnea

During "resting" hyperpnea the only muscles to develop additional work in comparison to rest are the respiratory muscles. Therefore, increases in systemic oxygen requirements (e.g., VO2) have been interpreted as an indirect measure of respiratory muscle oxygen cost of breathing (i.e., VO2resp: respiratory muscle oxygen consumption).(39) Thus, changes in systemic oxygen requirements not only during hyperpnea, but during the two tasks used herein (i.e., hyperpnea and loaded breathing) are due to additional respiratory muscle work ensuing changes in oxygen requirements of the respiratory muscles. Therefore, to increase respiratory muscle oxygen consumption, respiratory muscle perfusion may rise and/or a high level of oxygen extraction has to be present according to Fick's principle,(39) i.e., $VO_2 = Q (CaO_2 - CvO_2)$, where Q is the perfusion (i.e., blood flow) of the respiratory muscles and $(CaO_2 - CvO_2)$ is the arteriovenous oxygen content difference.(39) Moreover, it is know that the blood flow to the skeletal muscle increases proportionally to work and VO2.(40) Respiratory muscle blood flow, therefore, increases as respiratory muscle oxygen consumption and ventilation increases.(39) Of note, increases in VO2 during hyperpnea and loaded breathing appear to have

strong association with PTP expressed per minute rather than per breath (Figure 2). In addition, blood flow to the respiratory muscle has been shown to increase as a joint function of cardiac output and increased frequency and strength of contractions.(41) Likewise VO2, increases in cardiac output during “resting” respiratory muscle loading (i.e., hyperpnea and loaded breathing) were associated with PTP/min but independently of PTP/b (figure 2).



1173

1174 Figure 2. Relationship between contraction-volume and exercise-volume with oxygen consumption (VO2;
1175 a and d, respectively) and cardiac output (CO; b and e, respectively); and between systemic oxygen delivery (O2
1176 del) and oxygen consumption (VO2) and vascular conductance (Vasc. cond.; c and f, respectively). r: Pearson
1177 coefficient correlations; R2: Adjusted R squared (univariate linear regression); NA: not applicable; NS: P >0.05 (non-
1178 significant). Lines are the best-fitting line and shadow areas are 95% confidence interval. Circles: rest; triangles:
1179 normocapnic hyperpnea; cross: tapered flow resistive loading.

1180

1181

The increased energy requirement of the respiratory muscles during hyperpnea (table 2 and 3), as per Fick's principle, would elicit an increased level of oxygen delivery (i.e., blood flow) or oxygen extraction (i.e., CaO2 – CvO2). Notable, the increase from rest in the level of systemic and local oxygen extraction (i.e., a-vO2 difference and O2 extraction and [HHb], respectively) was simultaneously accompanied by an increased level of systemic and local oxygen delivery (i.e., O2del and mO2del, respectively). Hence, elegantly preserving respiratory muscle oxygen delivery/utilization ratio (i.e., StiO2; table 4). During loaded breathing, however, despite higher pressures and PTP per breath (table 2), PTP/min was lower (table 3). Therefore, the oxygen cost of breathing (i.e., VO2) and the increase in cardiac output, that are associated with PTP/min (figure 2), were less (table 3). The lower "systemic" oxygen requirements (i.e., VO2 and a-vO2 difference) during loaded breathing were accompanied of lower increase in respiratory muscle blood flow and oxygen delivery (i.e., mO2del; table 4). Nevertheless, the latter responses during loading breathing induced an imbalance between non-diaphragmatic muscle oxygen delivery/utilization ratio as indicated by greater increase in muscle oxygen extraction (i.e., HHB) and significantly lower muscle tissue oxygen saturation compared to hyperpnea (table 4). Thus, revealing an insufficient convective O2 delivery during loaded breathing. In addition, the high intramuscular tension (i.e., PTP/b; table 2) imposed during loading breathing (table 2), maybe another potential factor limiting the increase in local (i.e., non-diaphragmatic muscle) blood flow and oxygen delivery compared to hyperpnea (table 4). Further support of the notion of the high intramuscular pressures during loading breathing compared to hyperpnea might be provided when considering that mean arterial pressure did not statistically differ between the two conditions (i.e., loaded breathing vs hyperpnea) (table 3). One could expect the lower

central hemodynamic responses during loaded breathing compared to hyperpnea to be accompanied by a lower increase in mean arterial blood pressure. However, this was not the case in our study as mean arterial blood pressure during loaded breathing increased from rest and did not significantly differ compared to hyperpnea (table 3). Indeed, this finding is important when taking into account that increases in intramuscular pressure during dynamic exercise can reflexively increase mean arterial blood pressure from rest (via the activation of a pressure-sensitive i.e., mechanoreceptor-mediated reflex within the skeletal muscle) (42, 43)

In summary, during hyperpnea the moderate intensity, rhythmic and constant patter of respiratory muscle contraction (i.e., higher breathing frequency) ensues increased PTP/min (table 2) and, consequently, oxygen delivery (i.e., cardiac output and respiratory muscle blood flow). During loaded breathing, the contractions are of much higher intensities but interspersed by longer periods of “relaxation” (i.e., expiration) of the inspiratory muscles (i.e., lower duty cycle; table 2). Therefore, despite local oxygen requirements are acutely increased during each breath of loaded breathing (table 4), the “systemic” oxygen requirements are lower (table 3). The high intensity loads imposed to the inspiratory muscles during each breath evokes acute (i.e., during contraction time) and interspersed perturbations of the muscular oxygen delivery/utilization ratio (i.e., StiO2). Thus, resulting in reduction in their tissue oxygen saturation, that can only be revealed during the real-time measurement of local muscle tissue oxygen metabolism (i.e., NIRS).

General considerations

Collectively, these results seem to support the notion that additional inspiratory pressures generated during loaded breathing are mainly a consequence of increased loading and activation of non-diaphragmatic inspiratory muscles. The behavior of the “respiratory effort-recruitment” ratio, i.e., the “matching” between respiratory muscle effort (e.g., Pes, %max) and the recruitment of different inspiratory muscles (EMG, %max), is noteworthy. While during resting breathing a higher ratio indicates a “predominantly diaphragm contribution to breathing”, with increasing load (i.e., hyperpnea and loaded breathing), the ratio becomes similar between diaphragm and non-diaphragmatic muscles, thereby indicating that non-diaphragmatic muscle contribution to breathing becomes equally important as that of the diaphragm (supplemental material figure E3).

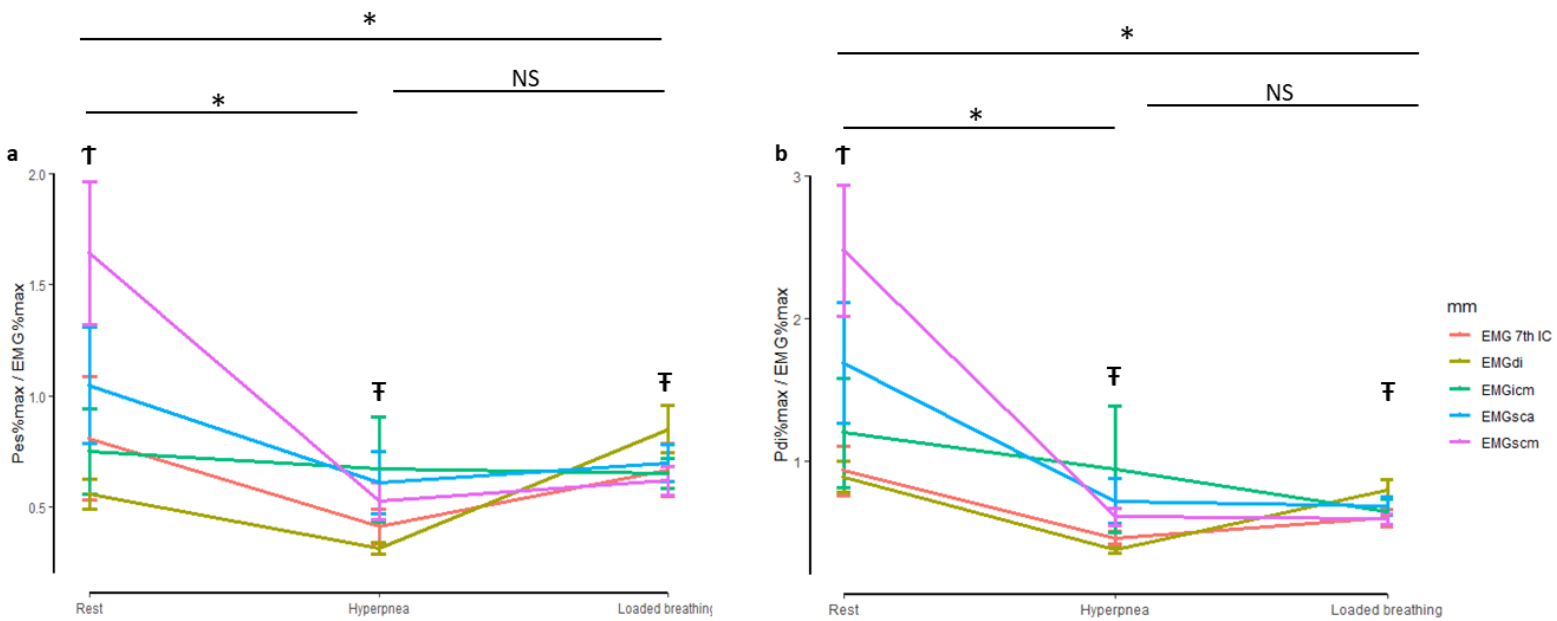


Figure E3. Ratio between relative global (a) and diaphragmatic (b) inspiratory muscle effort and inspiratory muscles recruitment during rest, hyperpna and loaded breathing. *P <0.05 between tasks (i.e., rest, hyperpna, loaded breathing). ŦP <0.05 for between tasks ratio for EMGscm (i.e., Pes, %max / EMGscm, %max; and Pdi, %max / EMGscm, %max); NS: P>0.05 (i.e., non-significant). whilst during resting breathing a higher ratio indicates a "mismatch" between non-diaphragmatic inspiratory muscles recruitment and inspiratory muscle effort during the act of breathing, with increasing load (i.e., hyperpnea and loaded breathing), the ratio becomes similar between diaphragm and non-diaphragmatic muscles, indicating that under these circumstances the "matching" between

non-diaphragmatic inspiratory muscle recruitment and the respiratory effort becomes similar to the diaphragmatic effort-recruitment ratio, i.e., their contribution to breathing becomes equally important as that of the diaphragm.

In addition to revealing the responses evoked by the different stimuli challenging the inspiratory muscles during hyperpnea and loaded breathing (figure 1 and table 2 and 3), these findings indicate that increased loading, recruitment and metabolism of the respiratory muscles likely constitute a true overload, which after a training period might mediate the ensuing improvements in inspiratory muscle strength (i.e., MIP) and endurance. Moreover, the increased load and volume of work imposed on the inspiratory muscles during both tasks (table 2) further corroborate this effect, i.e., load and work volume are known to be determinants of muscle improvements after exercise programs.(44) Furthermore, according to the specificity and overload principles of training,(44) in response to a low load (i.e., pressures), high repetition (i.e., breathing frequency) and high exercise-volume (i.e., PTP cmH2O/s/min) (table 2) stimulus as hyperpnea, an endurance benefit would be expected. While after loaded breathing, improvements in strength would be anticipated as consequence of the high load (i.e., pressures), low repetition (i.e., breathing frequency) and high contraction-volume (PTP cmH2O/s/b) stimulus imposed by this regimen (table 2). Noteworthy the additional recruitment of only non-diaphragmatic inspiratory muscles (figure 1 and supplemental material figure E1) with the increased load imposed during loaded breathing in comparison to hyperpnea (table 2) was accompanied by an increased metabolic burden placed upon non-diaphragmatic muscles (table 3). Thus, as consequence of the additional load of loaded breathing being overcome mostly by using non-diaphragmatic inspiratory muscles (figure 1, supplemental material figure E1, table 2), these muscles are more likely to benefit from this additional stimulus (i.e., increased load).(44) Despite being recently under investigation in healthy,(45) the effects of IMT on non-diaphragmatic inspiratory muscles of patients with COPD are scant. Noteworthy,

1271 when responses of non-diaphragmatic inspiratory muscles to IMT were studied, it was observed
1272 that IMT evoked increases in the proportion of type I fibers and in the size of both type I and II
1273 (intercostal) muscle fibers.(46)

1274

1275 Implications

1276 Accordingly, we demonstrated that patients with COPD recruit non-diaphragmatic
1277 inspiratory muscle to assist breathing when inflicted by increased respiratory demands as
1278 hyperpnea and loaded breathing (Figures 1, supplemental material figures E2 and E3, Tables 2
1279 and 3). When the respiratory system is overburdened their recruitment is probably the
1280 consequence of the inability to increase the contribution of a mechanically limited and less
1281 efficient diaphragm.(29, 32) Thus, under these circumstances non-diaphragmatic muscles
1282 endure an increased metabolic demand (table 3). Nevertheless, it is important to stress that the
1283 hyperpnea used herein resembles the load imposed to the respiratory system during exercise
1284 hyperpnea (i.e., 70% MVV) and not necessarily loads imposed during specific respiratory muscle
1285 endurance training (i.e., 50 - 70% MVV).(4) Besides revealing physiological responses evoked by
1286 different types (and intensities) of inspiratory muscle loading, our results pragmatically raise
1287 doubt on an “erstwhile” concept in respiratory physiology: that the absence of post-exercise
1288 diaphragmatic fatigue in patients with COPD is a consequence of diaphragm adaptations to
1289 disease’s consequences.(47, 48) Or is it a consequence of additional non-diaphragmatic
1290 inspiratory muscle recruitment “unloading” the diaphragm when the respiratory system is
1291 overburdened? In fact, increased non-diaphragmatic muscle recruitment and contribution to

the total inspiratory neural drive (i.e., contribution to tidal breathing) with increasing overload of the respiratory system has already being pointed out as a protective factor for the development of diaphragmatic fatigue in weaning-failure patients.(49) Moreover, the stimulus imposed during the loaded breathing used herein, that resemble one type of IMT session, seems to be a good complimentary training stimulus for the respiratory muscles during PR, since it provides a different additional load to the respiratory muscles in comparison to exercise hyperpnea

Strengths, limitations and technical considerations

Our analysis has to be interpreted in light of its strengths and limitations. The multitude of variables simultaneously collected is a strength of the study. It allows the concurrent investigation of the behavior of respiratory muscle activation, load and metabolism under the same stimulus. Unfortunately, however, assessments of blood flow and oxygen requirements of the diaphragm were not performed due to methodological and safety issues. A limitation of our study is the small sample size due to the complexity and the invasiveness of its methods and the fact that not all subjects were able or willing to undergo all experimental procedures. However, the sample was powered sufficiently (see Data analysis in the supplemental material for more details) to detect differences in a wide variety of physiological markers. Arterial oxygen content, arteriovenous oxygen content difference and systemic oxygen extraction were estimated using continuous SpO2 measurements at the expense of acceptable reduced accuracy in the hypoxemic patients compared with invasive arterial blood sampling. In addition,

it is known that the EMG signal from the costal diaphragm can generate noise on the activation of the 7th Intercostal we measured herein. However, the different pattern of diaphragm and 7th Intercostal activation between loaded breathing and hyperpnea suggested that this was not the case in our data. Nevertheless, there is possibility that the EMG signal measured at the 7th intercostal space could have, at least in part, contaminated from nearby activity. In our patients, the contribution of diaphragmatic blood flow to the overall NIRS signal on the 7th intercostal space are probably limited considering that adipose tissue thickness (fat + skin layer) (measurements were performed using a Harpenden skinfold caliper) indicated a mean value of 8.2 ± 3.7 mm. Therefore, the maximum penetration depth of NIRS light to the muscle tissue was reduced to approximately 12 mm. Taking into account the substantial distance between the sampling point of NIRS on the skin and the diaphragmatic appositional area compared with the shorter distance to the intercostals we believe that perfusion and oxygenation measures in our study at this site reflected mostly the external and internal intercostal muscles.

2.6 CONCLUSION

During hyperpnea, the higher breathing frequency and volume of respiratory muscle work elicited increased systemic oxygen requirements (to maintain high levels of ventilation) that were matched by an adequate increased in oxygen supply (i.e., blood flow) to the respiratory muscles, therefore preserving the ratio between muscle oxygen delivery and utilization (StiO2). During loaded breathing, despite the higher loads imposed to the respiratory muscles per breath, the interspersed breathes elicited lower respiratory muscle work per

minute that was accompanied by lower increase in systemic oxygen requirements and, therefore, delivery. During each breath, however, the respiratory muscles exhibited acute greater increases in oxygen utilization that were not matched by analogous increase in oxygen delivery causing significant reduction in muscles oxygen saturation compared to hyperpnea

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Conflict of interest

The authors have no conflict of interest to disclose. The results presented herein do not constitute endorsement by ACSM and are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

REFERENCES

1. Charususin N, Gosselink R, Decramer M, et al. Randomised controlled trial of adjunctive inspiratory muscle training for patients with COPD. Thorax. 2018;73(10):942-50. doi: 10.1136/thoraxjnl-2017-211417. PubMed PMID: 29914940.

2. Mador MJ, Deniz O, Aggarwal A, Shaffer M, Kufel TJ, Spengler CM. Effect of respiratory muscle endurance training in patients with COPD undergoing pulmonary rehabilitation. Chest. 2005;128(3):1216-24. doi: 10.1378/chest.128.3.1216. PubMed PMID: 16162709.

3. Illi SK, Held U, Frank I, Spengler CM. Effect of respiratory muscle training on exercise performance in healthy individuals: a systematic review and meta-analysis. Sports Med. 2012;42(8):707-24. Epub 2012/07/07. doi: 10.2165/11631670-000000000-00000. PubMed PMID: 22765281.

4. Gosselink R, De Vos J, van den Heuvel SP, Segers J, Decramer M, Kwakkel G. Impact of inspiratory muscle training in patients with COPD: what is the evidence? The European respiratory journal. 2011;37(2):416-25. doi: 10.1183/09031936.00031810. PubMed PMID: 21282809.

5. Beaumont M, Forget P, Couturaud F, Reychler G. Effects of inspiratory muscle training in COPD patients: A systematic review and meta-analysis. The clinical respiratory journal. 2018;12(7):2178-88. doi: 10.1111/crj.12905. PubMed PMID: 29665262.

6. Richardson RS, Sheldon J, Poole DC, Hopkins SR, Ries AL, Wagner PD. Evidence of skeletal muscle metabolic reserve during whole body exercise in patients with chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine. 1999;159(3):881-5. doi: 10.1164/ajrccm.159.3.9803049. PubMed PMID: 10051266.

7. Shoemaker JK, Hodge L, Hughson RL. Cardiorespiratory kinetics and femoral artery blood velocity during dynamic knee extension exercise. Journal of applied physiology. 1994;77(6):2625-32. doi: 10.1152/jappl.1994.77.6.2625. PubMed PMID: 7896601.

8. Mahler DA, Weinberg DH, Wells CK, Feinstein AR. The measurement of dyspnea. Contents, interobserver agreement, and physiologic correlates of two new clinical indexes. Chest. 1984;85(6):751-8. PubMed PMID: 6723384.

9. Singh D, Agusti A, Anzueto A, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease: The GOLD Science Committee Report 2019. The European respiratory journal. 2019. doi: 10.1183/13993003.00164-2019. PubMed PMID: 30846476.

10. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. The European respiratory journal. 2005;26(2):319-38. doi: 10.1183/09031936.05.00034805. PubMed PMID: 16055882.

11. Wanger J, Clausen JL, Coates A, et al. Standardisation of the measurement of lung volumes. The European respiratory journal. 2005;26(3):511-22. doi: 10.1183/09031936.05.00035005. PubMed PMID: 16135736.

12. Black LF, Hyatt RE. Maximal respiratory pressures: normal values and relationship to age and sex. The American review of respiratory disease. 1969;99(5):696-702. doi: 10.1164/arrd.1969.99.5.696. PubMed PMID: 5772056.

13. Laveneziana P, Albuquerque A, Aliverti A, et al. ERS Statement on Respiratory Muscle Testing at Rest and during Exercise. The European respiratory journal. 2019. doi: 10.1183/13993003.01214-2018. PubMed PMID: 30956204.

14. Puente-Maestu L, Palange P, Casaburi R, et al. Use of exercise testing in the evaluation of interventional efficacy: an official ERS statement. The European respiratory journal. 2016;47(2):429-60. doi: 10.1183/13993003.00745-2015. PubMed PMID: 26797036.

15. Vogiatzis I, Athanasopoulos D, Habazettl H, et al. Intercostal muscle blood flow limitation during exercise in chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine. 2010;182(9):1105-13. doi: 10.1164/rccm.201002-0172OC. PubMed PMID: 20622032.

16. Langer D, Charususin N, Jacome C, et al. Efficacy of a Novel Method for Inspiratory Muscle Training in People With Chronic Obstructive Pulmonary Disease. Physical therapy. 2015;95(9):1264-73. doi: 10.2522/ptj.20140245. PubMed PMID: 25858974.

17. Borg GA. Psychophysical bases of perceived exertion. Medicine and science in sports and exercise. 1982;14(5):377-81. Epub 1982/01/01. PubMed PMID: 7154893.

18. Louvaris Z, Habazettl H, Wagner H, Zakynthinos S, Wagner P, Vogiatzis I. Near-infrared spectroscopy using indocyanine green dye for minimally invasive measurement of

1412 respiratory and leg muscle blood flow in patients with COPD. Journal of applied physiology.
1413 2018;125(3):947-59. doi: 10.1152/japplphysiol.00959.2017. PubMed PMID: 29927736.

1414 19. Piiper J, Haab P. Oxygen supply and uptake in tissue models with unequal
1415 distribution of blood flow and shunt. Respiration physiology. 1991;84(2):261-71. PubMed PMID:
1416 1876763.

1417 20. Langer D, Ciavaglia C, Faisal A, et al. Inspiratory muscle training reduces
1418 diaphragm activation and dyspnea during exercise in COPD. Journal of applied physiology.
1419 2018;125(2):381-92. doi: 10.1152/japplphysiol.01078.2017. PubMed PMID: 29543134.

1420 21. Hawkes EZ, Nowicky AV, McConnell AK. Diaphragm and intercostal surface EMG
1421 and muscle performance after acute inspiratory muscle loading. Respiratory physiology &
1422 neurobiology. 2007;155(3):213-9. doi: 10.1016/j.resp.2006.06.002. PubMed PMID: 16846758.

1423 22. American Thoracic Society/European Respiratory S. ATS/ERS Statement on
1424 respiratory muscle testing. American journal of respiratory and critical care medicine.
1425 2002;166(4):518-624. doi: 10.1164/rccm.166.4.518. PubMed PMID: 12186831.

1426 23. Faisal A, Alghamdi BJ, Ciavaglia CE, et al. Common Mechanisms of Dyspnea in
1427 Chronic Interstitial and Obstructive Lung Disorders. American journal of respiratory and critical
1428 care medicine. 2016;193(3):299-309. Epub 2015/09/26. doi: 10.1164/rccm.201504-0841OC.
1429 PubMed PMID: 26407036.

24. Louvaris Z, Spetsioti S, Andrianopoulos V, et al. Cardiac output measurement during exercise in COPD: A comparison of dye dilution and impedance cardiography. The clinical respiratory journal. 2019;13(4):222-31. doi: 10.1111/crj.13002. PubMed PMID: 30724023.

25. Borghi-Silva A, Oliveira CC, Carrascosa C, et al. Respiratory muscle unloading improves leg muscle oxygenation during exercise in patients with COPD. Thorax. 2008;63(10):910-5. doi: 10.1136/thx.2007.090167. PubMed PMID: 18492743.

26. Grassi B, Quaresima V. Near-infrared spectroscopy and skeletal muscle oxidative function in vivo in health and disease: a review from an exercise physiology perspective. Journal of biomedical optics. 2016;21(9):091313. doi: 10.1117/1.JBO.21.9.091313. PubMed PMID: 27443955.

27. Boushel R, Langberg H, Olesen J, Gonzales-Alonzo J, Bulow J, Kjaer M. Monitoring tissue oxygen availability with near infrared spectroscopy (NIRS) in health and disease. Scandinavian journal of medicine & science in sports. 2001;11(4):213-22. PubMed PMID: 11476426.

28. Aliverti A, Cala SJ, Duranti R, et al. Human respiratory muscle actions and control during exercise. Journal of applied physiology. 1997;83(4):1256-69. doi: 10.1152/jappl.1997.83.4.1256. PubMed PMID: 9338435.

29. Washino S, Kanehisa H, Yoshitake Y. Neck inspiratory muscle activation patterns during well-controlled inspiration. European journal of applied physiology. 2017;117(10):2085-97. doi: 10.1007/s00421-017-3699-5. PubMed PMID: 28823081.

30. Campbell EJ. The role of the scalene and sternomastoid muscles in breathing in normal subjects; an electromyographic study. Journal of anatomy. 1955;89(3):378-86. PubMed PMID: 13251968; PubMed Central PMCID: PMC1244766.

31. Raper AJ, Thompson WT, Jr., Shapiro W, Patterson JL, Jr. Scalene and sternomastoid muscle function. Journal of applied physiology. 1966;21(2):497-502. doi: 10.1152/jappl.1966.21.2.497. PubMed PMID: 5934453.

32. De Troyer A, Boriek AM. Mechanics of the respiratory muscles. Comprehensive Physiology. 2011;1(3):1273-300. doi: 10.1002/cphy.c100009. PubMed PMID: 23733642.

33. De Troyer A, Wilson TA. Effect of acute inflation on the mechanics of the inspiratory muscles. Journal of applied physiology. 2009;107(1):315-23. doi: 10.1152/japplphysiol.91472.2008. PubMed PMID: 19265064.

34. De Troyer A, Leduc D, Cappello M, Mine B, Gevenois PA, Wilson TA. Mechanisms of the inspiratory action of the diaphragm during isolated contraction. Journal of applied physiology. 2009;107(6):1736-42. doi: 10.1152/japplphysiol.00753.2009. PubMed PMID: 19797686.

35. Decramer M, De Troyer A. Respiratory changes in parasternal intercostal length. Journal of applied physiology: respiratory, environmental and exercise physiology. 1984;57(4):1254-60. doi: 10.1152/jappl.1984.57.4.1254. PubMed PMID: 6501034.

36. Farkas GA, Rochester DF. Contractile characteristics and operating lengths of canine neck inspiratory muscles. Journal of applied physiology. 1986;61(1):220-6. doi: 10.1152/jappl.1986.61.1.220. PubMed PMID: 3733607.

37. Farkas GA. Mechanical properties of respiratory muscles in primates. Respiration physiology. 1991;86(1):41-50. PubMed PMID: 1759052.

38. Legrand A, Schneider E, Gevenois PA, De Troyer A. Respiratory effects of the scalene and sternomastoid muscles in humans. Journal of applied physiology. 2003;94(4):1467-72. Epub 2003/03/11. doi: 10.1152/japplphysiol.00869.2002. PubMed PMID: 12626472.

39. Roussos C, Macklem PT. The respiratory muscles. The New England journal of medicine. 1982;307(13):786-97. doi: 10.1056/NEJM198209233071304. PubMed PMID: 7050712.

40. Andersen P, Saltin B. Maximal perfusion of skeletal muscle in man. The Journal of physiology. 1985;366:233-49. PubMed PMID: 4057091; PubMed Central PMCID: PMC1193029.

41. Rochester DF, Pradel-Guena M. Measurement of diaphragmatic blood flow in dogs from xenon 133 clearance. Journal of applied physiology. 1973;34(1):68-74. doi: 10.1152/jappl.1973.34.1.68. PubMed PMID: 4572509.

42. Williamson JW, Crandall CG, Potts JT, Raven PB. Blood pressure responses to dynamic exercise with lower-body positive pressure. Medicine and science in sports and exercise. 1994;26(6):701-8. PubMed PMID: 8052110.

43. Gallagher KM, Fadel PJ, Smith SA, et al. Increases in intramuscular pressure raise arterial blood pressure during dynamic exercise. Journal of applied physiology. 2001;91(5):2351-8. doi: 10.1152/jappl.2001.91.5.2351. PubMed PMID: 11641380.

44. Kraemer WJ, Ratamess NA. Fundamentals of resistance training: progression and exercise prescription. Medicine and science in sports and exercise. 2004;36(4):674-88. PubMed PMID: 15064596.

45. Ramsook AH, Molgat-Seon Y, Schaeffer MR, et al. Effects of inspiratory muscle training on respiratory muscle electromyography and dyspnea during exercise in healthy men. Journal of applied physiology. 2017;122(5):1267-75. doi: 10.1152/japplphysiol.00046.2017. PubMed PMID: 28255085; PubMed Central PMCID: PMC5451532.

46. Ramirez-Sarmiento A, Orozco-Levi M, Guell R, et al. Inspiratory muscle training in patients with chronic obstructive pulmonary disease: structural adaptation and physiologic outcomes. American journal of respiratory and critical care medicine. 2002;166(11):1491-7. doi: 10.1164/rccm.200202-075OC. PubMed PMID: 12406842.

47. Polkey MI, Ambrosino N. Inspiratory muscle training in COPD: can data finally beat emotion? Thorax. 2018;73(10):900-1. doi: 10.1136/thoraxjnl-2018-212070. PubMed PMID: 29945956.

48. Polkey MI, Moxham J, Green M. The case against inspiratory muscle training in COPD. Against. The European respiratory journal. 2011;37(2):236-7. doi: 10.1183/09031936.00095510. PubMed PMID: 21282808.

49. Tobin MJ, Laghi F, Jubran A. Ventilatory failure, ventilator support, and ventilator weaning. Comprehensive Physiology. 2012;2(4):2871-921. doi: 10.1002/cphy.c110030. PubMed PMID: 23720268.

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2.7 ONLINE SUPPLEMENT

Differences in respiratory muscle responses to hyperpnea or resistive loading in COPD

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METHODS

Subjects. Subjects analyzed in this study were patients with COPD recruited for a randomized clinical trial (RCT) investigating the effects of Inspiratory Muscle Training, by Tapered Flow Resistive Loading, on shortness of breath and postural control (Clinical Trial Identifier: NCT03240640). Consequently, the inclusion criteria for this analysis were the same of the above cited RCT: clinical diagnose of COPD according to the Global Initiative for Chronic Obstructive Pulmonary Disease (GOLD),(1) age between 40 and 90 years, breathlessness during the performance of daily living activities according to Baseline Dyspnea Index (BDI)(2) score lower than seven, peripheral muscle fatigue present after cardiopulmonary exercise testing, no participation in exercise-training programs in the previous year, no long-term oxygen use and not presenting: major cardiovascular conditions limiting exercise capacity more than pulmonary function impairments, severe orthopedic conditions with major impact on daily life activities, psychiatric or cognitive disorders and progressive neurological or neuromuscular disorders. Patients were excluded in case of development of conditions precluding the proper execution and completion of any assessment during the measurements used in the present analysis.

Initial testing. Spirometry and whole body plethysmograph (Vmax Autobox, Sensor Medics, Bilthoven, The Netherlands) were performed according to the European Respiratory Society guidelines for pulmonary function testing.(3, 4) Maximal voluntary inspiratory pressure (MIP) was assessed at residual capacity according to the technique proposed by Black and

Hyatt.(5) Reference values from Neder et al.(6) were used to define percentages of expected maximal inspiratory pressures and lower limit of normality (LLN) was estimated to classify patients presenting inspiratory muscle weakness.(7, 8)

Maximal incremental cardiopulmonary exercise testing (CPET) on an electrically braked cycle-ergometer was performed according to the American Thoracic Society(ATS)/American College of Chest Physicians (ACCP) standards(9). Continuous measurements of ventilatory and metabolic variables were performed by a metabolic cart unit (SensorMedics, Vs229d, yorba Linda, CA, USA) during the test. After a resting period a constant work rate cycle endurance test CWRT test was performed at 80% of the peak work rate obtained during the maximal test (80% Wpeak).(10) Measurements were performed during the CWRT as previously described during the CPET. Mean tidal volume (L), breathing frequency (breaths/minute) and minute ventilation (L/min) during the last 60 seconds of the CWRT were registered to be used as target during the NH trial.(11)

Respiratory muscle recruitment, respiratory effort and diaphragm activation. A combined multipair esophageal electrode catheter with esophageal- and gastric-balloons (Yinghui Medical Equipment Technology Co. Ltd., Guangzhou, China) was, after topical anesthesia, inserted nasally and swallowed by the patients. The catheter is approximately 60 centimeters long, two mm in diameter and has five diaphragm electromyography electrodes pairs (EMGdi). Procedures for optimal positioning of the catheter have already been published.(12, 13) Briefly, patients were requested to perform several slow inspiratory capacity (IC) maneuvers through the mouth. The positioning was considered optimal when the largest EMGdi signal amplitudes were at the outer electrodes pairs.(12, 14, 15) After optimal

placement, the catheter was fixed at patient's nose with tape. The EMGdi signals were sampled at 2000 HZ (Micro1401-3, Cambridge Electronic Design Limited, Cambridge, UK), amplified (Biomedical amplifier, Guangzhou, China) and recorded by data acquisition software (Spike 2, Cambridge Electronic Design Limited, Cambridge, UK). The raw EMGdi signal data was stored to be off-line processed. Raw EMGdi data treatment was done first by high pass filtered at 20 HZ and subsequently transformed into "Root Mean Squared" (RMS). Smoothing was applied to reduce signal noise. Additionally, the Least Mean Squared (LMS) Adaptive Filter was used to filter out the electrocardiographic (ECG) signal from this remaining "raw" EMGdi signal. LMS adaptive filter is the algorithm available in LabVIEW software for pattern recognition. The LMS filter was tuned to comply with the minimum error and, consequently, deliver the best result to remove the ECG "noise" from the EMGdi signal. An additional ECG channel was recorded synchronously to tune the coefficients of the Finite Impulse Response (FIR) filter continuously. Thus, removal of ECG noise from EMGdi signal was precise despite heart rhythm variations throughout time. After the filtering process the processed EMGdi RMS signal was anew imported into the acquisition software (Spike 2, Cambridge Electronic Design Limited, Cambridge, UK). Thereafter, based on flow and volume signal continuously and synchronically recorded from Vmax229d system, inspiration and expiration recordings of every each breath were automatically marked. The mean RMS signal of each inspiration throughout the NH and TFRL tasks were, therefore, automatically retrieved. EMGdi signal was normalized by presenting values relative to its maximum activation (EMGdi, %max)(13, 16) and used as measure of diaphragm activation.(12)

Maximum activation of the diaphragm has previously being reported as the maximum activation obtained during IC maneuvers performed at rest or during exercise.(13, 16-20) Despite not reported in this analysis, patients included herein we recruited for participating in a RCT (Clinical Trial Identifier: NCT03240640) in which, in addition to the data reported here, they performed a high-intensity CWR with the same equipment and data processing used for EMGdi measurement during NH and TFRL tasks. Thus, to maintain the same methodological approach widely used in the previous literature,(13, 16-20) allowing comparisons of our results with previous studies on this subject, the maximal EMGdi was considered the obtained from IC maneuvers performed during this high-intensity CWR testing.

In addition, the esophageal and gastric balloon catheters were connected to differential pressure transducers for continuous measurement of esophageal (Pes), gastric (Pga) and transdiphragmatic (Pga - Pes) pressures. Data was continuously recorded by the acquisition software (Spike 2, Cambridge Electronic Design Limited, Cambridge, UK) simultaneously with EMGdi, flow and volume data for off-line analysis. Maximal inspiratory esophageal (Pes,max), gastric (Pga,max) and transdiaphragmatic (Pdi,max) pressures were measured from FRC during sniff maneuvers. Maximal expiratorygastric pressure was measured from TLC during forced expiratory capacity maneuvers.Inspiratory Pes/Pes,max (i.e., Pes %max) and Pdi/Pdi,max (i.e., Pdi, %max) were used as indices of global inspiratory muscle effort and diaphragmatic effort, respectively.(13, 21) Inspiratory Pga/Pga,max (i.e., Pga %max) was used to verify the diaphragm contribution to changes in Pdi. Expiratory Pga/Pga,max (i.e., expPga %max) was considered the expiratory effort performed by the abdominal muscle. Esophageal, gastric and transdiaphragmatic work of breathing per breath (Pes WOB, Pga WOB and Pdi WOB,

respectively) were calculated by integrating volume and pressure generated per breath (e.g., Pes WOB= Pes x tidal volume), and were further multiplied by breathing frequency to calculate ("cumulative") work of breathing per minute (e.g., Pes WOB/min= Pes WOB x bf).(22) WOB per breath was considered as a measure of contraction "volume-load" of the inspiratory muscles, while WOB per minute as a measure of exercise "volume-load" of the inspiratory muscles.(22) The Pressure Time Product (PTP), i.e., the product of the pressure developed by all the respiratory muscles (PTP Pes) or by the diaphragm (PTP Pdi) multiplied by the time of muscle contraction (cmH2O*s), was used to estimate the "contraction-volume" per breath and per minute (i.e., PTP cmH2O/s/b, PTP cmH2O/s/min, respectively) imposed to the inspiratory muscles.(22)

Inspiratory neural drive

Inspiratory neural drive (RND) was estimated as the sum of the maximum activation of each respiratory muscle (i.e., RND(RMS)= EMGdi(RMS) + EMGsca(RMS) + EMGscm(RMS) + EMGicm(RMS) + EMG 7th ICM (RMS)), and the partial contribution of each respiratory muscle to the respiratory pump (i.e., %RND) was measured as its respective activation divided by the RND (e.g., EMGdi, %RND= EMGdi(RMS)/RND).

Central hemodynamic responses during hyperpnea and loaded breathing

Cardiac output, heart rate and stroke volume was continuously measured by impedance cardiography device (PhysioFlowPF50; Manatec Biomedical, Macheren, France) previously validated for COPD patients (ref).(23) The PhysioFlow device and its methodology have been

thoroughly described elsewhere (ref).(24) After shaving and cleaning the skin, two pairs of electrodes were firmly positioned at the left base of the neck (one vertically above the other over the carotid artery above the supraclavicular fossa) at the back over the xiphoid level for transmitting and receiving electrical currents. Two electrodes were also placed on the chest (V1/V6 position) for the ECG signal. Cardiac output values were then recorded at 1 second intervals. Data points were excluded when signal quality was less than 90% and were smoothed using a 5-point moving average (ref).(23) The cardiac output values were then time-aligned with the metabolic, ventilatory and NIRS data (see below). The cardiac output values used for comparison between NH and loaded breathing was the average of all smoothed values obtained over a 30-second period during each trial. Estimated systemic oxygen delivery was calculated by the product of cardiac output and arterial oxygen content; the latter was calculated as the product of 1.39 x hemoglobin concentration [Hb] and %SpO2 ,(25) whilst arterio-venous oxygen content (a-vO2) difference was calculated by dividing oxygen uptake by cardiac output. The systemic oxygen extraction ratio was calculated as the ratio of the arterio-venous oxygen content (a-vO2) difference to arterial oxygen content. In addition, systemic vascular conductance was calculated by dividing cardiac output with mean arterial blood pressure the latter was calculated by an automated pump sphygmomanometer. Metabolic and ventilatory variables were measured breath by breath by a metabolic cart (Vmax 229; Sensor Medics, Anaheim, CA, USA). Data were averaged every 30 seconds and time-aligned with the other physiological measures

Respiratory muscles perfusion and oxygenation responses

Non-diaphragmatic muscles (i.e.,7th Intercostal, Scalene and Abdominal) blood flow index [BFI] (26) was calculated by using two commercial Near-Infrared Spectroscopy that incorporate the Spatially Resolved Spectroscopy method (SRS) and the Modified Beer-Lambert method (MBL) in a near-infrared spectrum of 735, 810, and 850 nm (NIRS; NIRO-200 and a NIRO-200NX; HAMAMATSU Photonics KK) and the light-absorbing indocyanine green that was injected through a peripheral venous catheter according to previous validated for patients with COPD methodology (24). Three sets of NIRS optodes were placed at the left 7th intercostal space, the right posterior triangle of the neck and abdomen to respectively measure Intercostal, Scalenes and Rectus Abdominis muscles perfusion. In healthy and lean subjects, blood flow measured over the seventh intercostal space using NIRS and ICG has been considered as perfusing mostly the external and internal intercostal muscles and to a lesser extent the costal segment of the diaphragm.(21) However, in our patients, the contribution of diaphragmatic blood flow to the overall NIRS signal might not be important considering that adipose tissue thickness (fat + skin layer) (measurements were performed using a Harpenden skinfold caliper on the 7th intercostal space) indicated a mean value of 8.2 ± 3.7 mm. Therefore, the maximum penetration depth of NIRS light to muscle tissue was reduced to approximately 12 mm. Taking into account the substantial distance between the sampling point of NIRS on the skin and the diaphragmatic appositional area compared with the shorter distance to the intercostals we believe that perfusion and oxygenation measures in our study reflected mostly the external and internal intercostal muscles. Each injection was contained 5 mg of ICG dissolved in 1 ml of sterile water (5mg/mL) followed by a rapid 10-ml flush of isotonic saline. NIRS-ICG derived BFI as relative measurement of local tissues perfusion was calculated by dividing the muscle ICG

peak concentration by the rise time from 10 to 90% of peak(26) BFI was measured during the last 5 breaths during TFRL and during the last 30 seconds of exercise during NH trail. Oxygen delivery for each muscle was calculated by multiplying BFI with arterial oxygen content and expressed in arbitrary units.

NIRS-derived changes in Intercostal, Scalene and Abdominal muscle deoxyhemoglobin concentration[HHb] was used as an index of respiratory muscles oxygen extraction.(27) In addition, NIRS-derived tissue oxygen saturation index (i.e., StiO2) was measured in Intercostal, Scalene and Abdominal muscles as an absolute index of fractional tissue oxygenation. This non-invasive index is essentially the ratio of microvascular oxygenated (HbO2) to total tissue hemoglobin concentration (tHbO2) expressed as percentage [(HbO2/tHbO2) * 100] and reflects the dynamic balance between local tissue oxygen supply and utilization (Boushel, 2001) and therefore tissue capacity to match oxygen supplies relative to its metabolic demands (ref).(28) NIRS oxygenation data were averaged every 30 seconds and time-aligned with the other physiological measures.

Data analysis. The results we present herein are an exploratory analysis of an interventional study investigating the effects of IMT on symptoms, balance, diaphragmatic and non-diaphragmatic muscles recruitment and load and non-diaphragmatic muscles metabolism during exercise in patients with COPD, that is currently under investigation by our group (Clinical Trial Identifier: NCT03240640). For the afore mentioned study, a sample size calculation was done to detect a difference of one unit in dyspnea Borg score scale, assuming a

SD of 1 unit in the change between pre and post measurement to detect a power of 80% at a significance level of 5%. A sample size of 16 participants in the intervention group and 8 for the control group was revealed in this case. Despite a sample size calculation was not done for the exploratory analysis we present herein, a power calculation based on the difference between Sternocleidomastoid muscle activation between the three tasks (i.e., rest, hyperpnea and loaded breathing) revealed a power >99% (considering 16 subjects, a f value of 21.95, 1 group (within-effect design), 3 measurements, an alpha error of 5% and nonsphericity correction coefficient of 0.7). Therefore supporting the statistical strength of our results

Data handling was done on the softwares Excel and R© version 3.5.0 (The R Foundation for Statistical Computing Platform). Normality in data distribution was evaluated with the Shapiro-Wilk test and by visual inspection of Q-Q plots and histograms. Variables were expressed as Mean ± systemic error of measurement. Differences between rest, hyperpnea and loaded breathing were expressed as mean difference (95% confidence interval). Nominal and categorical variables were expressed as number of cases (relative values). Respiratory muscle activation, respiratory pressures and its derivatives, breathing pattern variables and central hemodynamic and metabolic variables Gaussian-distributed variables were compared by the one-way repeated measures ANOVA. As by convention, statistical significance level was set at 0.05. For continuous non-Gaussian-distributed variables, Friedman test was used instead. When statistical significance was met pairwise comparisons with Holm correction were performed as post-hoc analysis. Changes in respiratory muscles perfusion and oxygenation responses from rest to hyperpnea versus rest to loaded breathing were compared by paired T test when normally distributed or by Mann-Whitney test if normal distribution criteria was not met.

Correlations were investigated by Pearson Coefficient Correlations. The strength of correlations (r) were expressed only when statistical significance was met (i.e., P <0.05) and classified as week (r <0.5), moderate (r 0.5 - 0.7) or strong (r >0.7). Associations were investigated by univariate linear regression models and, whether significance was statistical (P <0.05) its strength (R2) was classified as week (R2 <0.25), moderate (R2 0.5) or substantial (R2 0.7). Estimated adjusted R2 was used to estimate the variation of the dependent variable that is explained by the independent variable.

RESULTS

Subjects characteristics. Subjects' characteristics are described in detail in table 1. The sample was well balanced regarding sex and composed by patients classified as having mild to very severe COPD presenting resting lung hyperinflation (i.e., increased RV/TLC). Six out of the sixteen included subjects did not have diaphragm EMG and respiratory pressures data, due to difficulties during the insertion of the esophageal catheter (n= 1) or did not agree (n= 5) to swallow the esophageal catheter. Three patients did not have respiratory muscle perfusion measured for technical reasons (n=1) or because of contraindications regarding ICG injections (n=2). Hence, nine out of the sixteen patients had concurrent measurements of diaphragm EMG, respiratory pressures and respiratory muscle perfusion. There were no differences regarding pulmonary function, peak exercise and inspiratory muscle capacity between subjects with EMGdi and respiratory pressures measurements versus those subjects not able or not willing to undergo for this specific experimental procedure.

Respiratory symptoms during hyperpna and loaded breathing tasks. Breathlessness at rest was 0 ± 0 and increased to 5 ± 1 at the end of hyperpna and 4 ± 1 at the end of loaded breathing. Likewise, respiratory effort increased from 0 ± 0 at rest to 5 ± 1 during hyperpna as well as during loaded breathing. Breathlessness and respiratory effort' sensation were statistically different between hyperpna and loaded breathing compared to rest ($P < 0.05$).

Respiratory muscle activation. In comparison to rest, respiratory muscle activation significantly increased during both hyperpnea and loaded breathing (figure 1a).

Respiratory pressures and work of breathing. Diaphragmatic and non-diaphragmatic muscles pressures significantly increased during hyperpnea and loaded breathing as compared to rest (table 2). Pes PTP and Pes WOB of the non-diaphragmatic muscles were significantly increased during hyperpna and loaded breathing in comparison to rest (table 2). For diaphragmatic contractions, Pdi PTP and Pdi WOB were significantly increased during hyperpna and loaded breathing in comparison to rest. PTP/min and WOB/min of diaphragmatic and non-diaphragmatic inspiratory muscles were found significant increased during hyperpnea as compared to rest. With regard to loaded breathing, we observed only a significant increase in Pes WOB/min from rest, whilst PTP Pes/min did not significantly change from rest (table 2).

Breathing pattern. During hyperpnea, ventilation, absolute and relative inspiratory volumes, breathing frequency and peak inspiratory flow were significantly increased whilst inspiratory time was significantly shorter in comparison to rest. (table 2) Duty cycle, however, did not change between hyperpna and rest (table 2). Moreover, both end-expiratory and end-inspiratory lung volumes (EELV and EILV, respectively) were significantly higher during

hyperpnea (table 2). During loaded breathing, ventilation was similar with rest, absolute and relative inspiratory volumes were significantly higher, breathing frequency was significantly lower and inspiratory flow significantly higher. In addition, inspiratory time was significantly longer in comparison to rest. Noteworthy, duty cycle also decreased during loaded breathing. EELV was significantly reduced and EILV was similar during loaded breathing in comparison to rest.

Central hemodynamic and metabolic responses. During both hyperpnea and loaded breathing, cardiac output was significantly increased from rest secondary to a significant increase in heart rate (table 4). Similarly, oxygen consumption and carbon dioxide production were also increased during hyperpnea and loaded breathing as compared to rest. Arterial oxygen content did not change from rest ether during hyperpnea or loaded breathing. Systemic oxygen delivery, arteriovenous oxygen content difference and systemic oxygen extraction significantly increased from rest during hyperpnea (table 4). During loaded breathing arteriovenous oxygen content difference significantly increased from rest (table 4). Systemic vascular conductance increased from rest during both hyperpnea and loaded (table 4).

Associations between physiological variables. PTP/b was weakly and significantly correlated with VO2 and weakly and non-significantly correlated with cardiac output (P= 0.4); (figure 2a and 2b), while moderate and weak significant correlations between Pes PTP/min with oxygen consumption and cardiac output (Figure 2d and 2e, respectively) were observed. Pes PTP/min explains 33 and 19% of oxygen consumption and cardiac output variability, respectively. Associations between oxygen consumption and systemic vascular conductance with systemic oxygen delivery revealed 34 and 79% of systemic oxygen delivery variability being

explained by the former and the latter, respectively (figure 2c and 2f). Correlations between respiratory muscle activation and local or systemic hemodynamic and metabolic responses were weak and not statistically significant (P 0.1 – 0.8), therefore were not considered (see Data analysis in the online supplement for more details). Respiratory muscle activation presented associations with respiratory muscle effort (figure E2, online supplement), with 13 – 59% of the variability in Pes and 26 – 78% of Pdi variability being explained by inspiratory muscle recruitment.

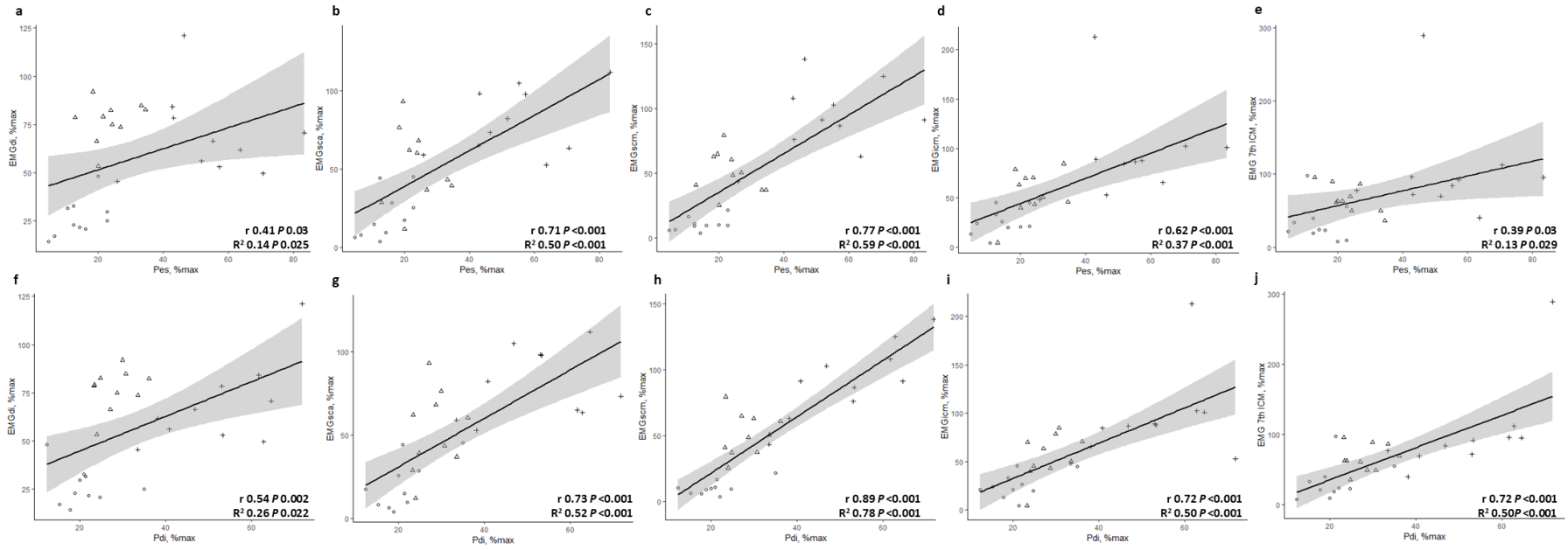

Figure E2. Relationship between diaphragm (a and f), scalenes (b and g) , sternocleidomastoide (c and h), parasternal intercostal (d and i) and 7th intercostal (e and j) muscle activation with esophageal (a – e) and transdiaphragmatic (f – j) pressures (i.e., global and diaphragmatic respiratory effort, respectively). r: Pearson coefficient correlations; R2: Adjusted univariate linear regression R squared. Lines are the best-fitting line and shadow areas are 95% confidence interval. Circles: rest; triangles: normocapnic hyperpnea; cross: tapered flow resistive loading.

DISCUSSION

Gastric pressure behavior during hyperpnea and loaded breathing. Interesting, despite the significant increase in Pdi and its derivatives (table 2), the absence of increased Pga highlights the overload imposed during loaded breathing being de facto overcame by non-diaphragmatic muscles, i.e., changes in Pdi were due to increased Pes and not Pga. Therefore, interpreting changes in Pdi without measuring the "real" contribution of the diaphragm (i.e., Pga) might mislead conclusions of diaphragm contribution to the act of breathing. This finding is in line with the higher associations between non-diaphragmatic muscle activation with Pdi (Figure E2) that in theory is assumed as an index of diaphragm contraction. In addition, whether one could argue that the increased Pga WOB/b (table 2) would be a signal that the diaphragm performed more work during the loaded breathing, it important to note that there was no changes in Pga PTP/b (table 2). Thus, the changes in Pga WOB/min are a consequence of the higher tidal volumes during loaded breathing not increased Pga, since WOB= (Pga*inspiratory volume). Therefore, in situations in which breathing volumes are not "matched" PTP would be a better index to measure the "real" work that is performed by the respiratory muscles -Accordingly, PTP is used as the marker of respiratory muscle work throughout our discussion -. Thus, the increased activation of both diaphragm and non-diaphragmatic inspiratory muscles during hyperpnea and loaded breathing simultaneously with the increased Pes and the absence of changes in Pga shows that the non-diaphragmatic muscle were the main responsible for decreasing pleural pressures and therefore, increase inspiratory volumes (table 2),(1) whilst the increased diaphragmatic activation with no "real" change in Pga shows the diaphragm

contribution may be related to stabilizing (i.e., isometric contraction) the abdominal part of the rib cage due to the increase negative pressure generated by non-diaphragmatic inspiratory muscles contractions, with no or minimal contribution to changes in pleural pressure and in generating inspiratory volume.(1) Thus, showing a possible diaphragmatic dynamic dysfunction that would be unnoted by investigation of Pdi not simultaneously with Pga.

REFERENCES

1. "Global strategy for asthma management and prevention: GINA executive summary." E.D. Bateman, S.S. Hurd, P.J. Barnes, J. Bousquet, J.M. Drazen, J.M. FitzGerald, P. Gibson, K. Ohta, P. O'Byrne, S.E. Pedersen, E. Pizzichini, S.D. Sullivan, S.E. Wenzel and H.J. Zar. Eur Respir J 2008; 31: 143-178. Eur Respir J. 2018;51(2). doi: 10.1183/13993003.51387-2007. PubMed PMID: 29386342.

2. Mahler DA, Weinberg DH, Wells CK, Feinstein AR. The measurement of dyspnea. Contents, interobserver agreement, and physiologic correlates of two new clinical indexes. Chest. 1984;85(6):751-8. PubMed PMID: 6723384.

3. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. The European respiratory journal. 2005;26(2):319-38. doi: 10.1183/09031936.05.00034805. PubMed PMID: 16055882.

4. Wanger J, Clausen JL, Coates A, et al. Standardisation of the measurement of lung volumes. The European respiratory journal. 2005;26(3):511-22. doi: 10.1183/09031936.05.00035005. PubMed PMID: 16135736.

5. Black LF, Hyatt RE. Maximal respiratory pressures: normal values and relationship to age and sex. The American review of respiratory disease. 1969;99(5):696-702. doi: 10.1164/arrd.1969.99.5.696. PubMed PMID: 5772056.

6. Neder JA, Andreoni S, Lerario MC, Nery LE. Reference values for lung function tests. II. Maximal respiratory pressures and voluntary ventilation. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas. 1999;32(6):719-27. PubMed PMID: 10412550.

7. Rodrigues A, Da Silva ML, Berton DC, et al. Maximal Inspiratory Pressure: Does the Choice of Reference Values Actually Matter? Chest. 2017;152(1):32-9. doi: 10.1016/j.chest.2016.11.045. PubMed PMID: 27940276.

8. Culver BH. How should the lower limit of the normal range be defined? Respiratory care. 2012;57(1):136-45; discussion 43-5. doi: 10.4187/respcare.01427. PubMed PMID: 22222132.

9. American Thoracic S, American College of Chest P. ATS/ACCP Statement on cardiopulmonary exercise testing. American journal of respiratory and critical care medicine. 2003;167(2):211-77. doi: 10.1164/rccm.167.2.211. PubMed PMID: 12524257.

10. Puente-Maestu L, Palange P, Casaburi R, et al. Use of exercise testing in the evaluation of interventional efficacy: an official ERS statement. The European respiratory journal. 2016;47(2):429-60. doi: 10.1183/13993003.00745-2015. PubMed PMID: 26797036.

11. Vogiatzis I, Athanasopoulos D, Habazettl H, et al. Intercostal muscle blood flow limitation during exercise in chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine. 2010;182(9):1105-13. doi: 10.1164/rccm.201002-0172OC. PubMed PMID: 20622032.

12. Luo YM, Moxham J, Polkey MI. Diaphragm electromyography using an oesophageal catheter: current concepts. Clinical science. 2008;115(8):233-44. doi: 10.1042/CS20070348. PubMed PMID: 18782085.

13. Langer D, Ciavaglia C, Faisal A, et al. Inspiratory muscle training reduces diaphragm activation and dyspnea during exercise in COPD. Journal of applied physiology. 2018;125(2):381-92. doi: 10.1152/japplphysiol.01078.2017. PubMed PMID: 29543134.

14. Luo YM, Moxham J. Measurement of neural respiratory drive in patients with COPD. Respiratory physiology & neurobiology. 2005;146(2-3):165-74. doi: 10.1016/j.resp.2004.12.014. PubMed PMID: 15766905.

15. Luo YM, Li RF, Jolley C, et al. Neural respiratory drive in patients with COPD during exercise tests. Respiration; international review of thoracic diseases. 2011;81(4):294-301. doi: 10.1159/000317136. PubMed PMID: 20588000.

16. Sinderby C, Beck J, Spahija J, Weinberg J, Grassino A. Voluntary activation of the human diaphragm in health and disease. J Appl Physiol (1985). 1998;85(6):2146-58. doi: 10.1152/jappl.1998.85.6.2146. PubMed PMID: 9843538.

17. 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. Respiratory physiology & neurobiology. 2018;247:116-25. doi: 10.1016/j.resp.2017.10.001. PubMed PMID: 28993264.

18. Elbehairy AF, Guenette JA, Faisal A, et al. Mechanisms of exertional dyspnoea in symptomatic smokers without COPD. Eur Respir J. 2016;48(3):694-705. doi: 10.1183/13993003.00077-2016. PubMed PMID: 27492828.

19. Faisal A, Alghamdi BJ, Ciavaglia CE, et al. Common Mechanisms of Dyspnea in Chronic Interstitial and Obstructive Lung Disorders. Am J Respir Crit Care Med. 2016;193(3):299-309. doi: 10.1164/rccm.201504-0841OC. PubMed PMID: 26407036.

20. Guenette JA, Chin RC, Cheng S, et al. Mechanisms of exercise intolerance in global initiative for chronic obstructive lung disease grade 1 COPD. The European respiratory journal. 2014;44(5):1177-87. doi: 10.1183/09031936.00034714. PubMed PMID: 25142487.

21. American Thoracic Society/European Respiratory S. ATS/ERS Statement on respiratory muscle testing. American journal of respiratory and critical care medicine. 2002;166(4):518-624. doi: 10.1164/rccm.166.4.518. PubMed PMID: 12186831.

22. Laveneziana P, Albuquerque A, Aliverti A, et al. ERS Statement on Respiratory Muscle Testing at Rest and during Exercise. The European respiratory journal. 2019. doi: 10.1183/13993003.01214-2018. PubMed PMID: 30956204.

23. Louvaris Z, Spetsioti S, Andrianopoulos V, et al. Cardiac output measurement during exercise in COPD: A comparison of dye dilution and impedance cardiography. The clinical respiratory journal. 2019;13(4):222-31. doi: 10.1111/crj.13002. PubMed PMID: 30724023.

24. Charloux A, Lonsdorfer-Wolf E, Richard R, et al. A new impedance cardiograph device for the non-invasive evaluation of cardiac output at rest and during exercise: comparison with the "direct" Fick method. European journal of applied physiology. 2000;82(4):313-20. doi: 10.1007/s004210000226. PubMed PMID: 10958374.

25. Borghi-Silva A, Oliveira CC, Carrascosa C, et al. Respiratory muscle unloading improves leg muscle oxygenation during exercise in patients with COPD. Thorax. 2008;63(10):910-5. doi: 10.1136/thx.2007.090167. PubMed PMID: 18492743.

26. Louvaris Z, Habazettl H, Wagner H, Zakynthinos S, Wagner P, Vogiatzis I. Near-infrared spectroscopy using indocyanine green dye for minimally invasive measurement of respiratory and leg muscle blood flow in patients with COPD. Journal of applied physiology. 2018;125(3):947-59. doi: 10.1152/japplphysiol.00959.2017. PubMed PMID: 29927736.

27. Grassi B, Quaresima V. Near-infrared spectroscopy and skeletal muscle oxidative function in vivo in health and disease: a review from an exercise physiology perspective. Journal of biomedical optics. 2016;21(9):091313. doi: 10.1117/1.JBO.21.9.091313. PubMed PMID: 27443955.

28. Louvaris Z, Habazettl H, Asimakos A, et al. Heterogeneity of blood flow and metabolism during exercise in patients with chronic obstructive pulmonary disease. Respiratory physiology & neurobiology. 2017;237:42-50. doi: 10.1016/j.resp.2016.12.013. PubMed PMID: 28057577.

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EXERCISE TRAINING IN ASTHMA-COPD OVERLAP

3. WHAT ABOUT THE EFFECTS OF HIGH-INTENSITY EXERCISE TRAINING IN PATIENTS WITH COPD AND ASTHMA OVERLAP?

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Declaration of interests

None.

3.1 ABSTRACT

Background: This study aimed to investigate whether patients with COPD presenting asthma overlap (ACO) benefit similarly in comparison to patients with only COPD after a 12-week high-intensity exercise training (ET) program.

Methods: Subjects were evaluated before and after a high-intensity ET program composed by walking and cycling plus strengthening exercises of the upper- and lower-limbs (3 days/week, 3 months, 36 sessions). Assessments included spirometry, bioelectrical impedance, six-minute walk test (6MWT), London chest activity of daily living scale (LCADL), Hospital anxiety and depression scale, modified Medical Research Council scale (mMRC), Saint-George Respiratory questionnaire (SGRQ) and respiratory and peripheral muscle strength (manovacuometry and 1-repetition maximum test [quadriceps femoris, biceps and triceps brachialis], respectively). Asthma overlap was defined according to Sin et al. (Eur Respir J, 2016)

Results: Out of 74 consecutive subjects analyzed, 12 (16%) were classified as having ACO. The sample was composed by 57% of male subjects, age 67±8 years, BMI 26 [21 -32] kg/m² and classified as having moderate to very severe COPD (FEV1 47±17 %predicted). Both groups improved pulmonary function, 6MWT, peripheral and inspiratory muscle strength, LCADL and SGRQ after ET ($P < 0.005$ for all). There was no significant interactions between the presence of Asthma overlap in patients with COPD on ET effects ($P > 0.05$ for all). Likewise, there was no difference in the proportion of patients achieving the minimum clinical important difference for 6MWT and mMRC .

Conclusion: High-intensity exercise training generates similar benefits in patients with COPD regardless whether presenting asthma overlap or not.

Key-words: Pulmonary Disease, Chronic Obstructive; Asthma; Pulmonary Rehabilitation.

Abbreviations list

1RM: one-repetition maximum test

6MWT: six-minute walk test

ACO: Asthma-chronic obstructive pulmonary disease overlap

BDR: bronchodilator response

COPD: chronic obstructive pulmonary disease

ET: exercise-training

FEV1/FVC: forced expiratory volume in the first second per forced vital capacity

FEV1: forced expiratory volume in the first second

FVC: forced vital capacity

GINA: Global Initiative for Asthma Management and Prevention

GOLD: Global Initiative for Chronic Obstructive Pulmonary Disease

HADS: Hospital Anxiety and Depression Scale

IQR: 25-75 interquartile range

Kg: kilogram

LCADL: London Chest Activity of Daily Living scale

MCID: minimal clinical important difference

MEP: maximum expiratory pressure

MIP: maximum inspiratory pressure

mMRC: modified Medical Research Council scale

PR: pulmonary rehabilitation

SD: standard deviation

SGRQ: St. George's Respiratory Questionnaire

UEL: Londrina State University

UNOPAR: University of Northern Parana

3.2 INTRODUCTION

Exercise training (ET) has long been recognized as the cornerstone of pulmonary rehabilitation (PR) programs.1 This status quo was solidly accomplished due to the multitude of research performed on this topic.2 Noteworthy, this has led the Cochrane group to the unusual decision of closing a topic of systematic review, due to the myriad of evidence highlighting its outstanding ability in improving exercise capacity and quality of life in subjects with chronic obstructive pulmonary disease (COPD).3 Nevertheless, it is not possible to assume the same for other relevant outcomes such as physical activity in daily life or prognosis. Furthermore, the impact of specific comorbidities (e.g., heart failure) or phenotypes (e.g., body composition phenotypes) may influence the effects of exercise-training programs.4 As an example, despite "classic" high-intensity ET programs being considered as a rule of thumb also for COPD subjects presenting fat-free mass depletion, improvement in fat-free mass was only possible after nutritional support in this specific phenotype.5 Among these specific phenotypes in COPD, a considerable proportion of subjects with Asthma-COPD overlap (ACO) has also been described6, with its incidence ranging from 15 to 55%.6,7

The overlap of these two distinct diseases is known to increase exacerbations rate,6,8 fasten lung function decline and increase mortality,6,9 and has been associated with worse quality of life.6,8,10 Although ET has proven to enhance exercise capacity, quality of life and symptoms burden in subjects with COPD or asthma alone,11-13 it is yet unknown whether the overlap of asthma in subjects with COPD would lessen or boost improvements achieved after an ET program. In fact, it has not been elucidated whether or not COPD subjects presenting asthma overlap benefit similarly to subjects presenting only COPD. Nevertheless, it would be

reasonable to hypothesize that these two phenotypes benefit similarly since ET is highly effective in both diseases separately. Therefore, the objective of this study was to investigate the authors' hypothesis that subjects with COPD presenting or not asthma overlap would benefit similarly after a high-intensity ET program.

3.3 MATERIAL AND METHODS

Design and Subjects

This was a multicenter study with prospective data collection and retrospective analysis. All data were obtained from the database of subjects with COPD assessed for admission in two outpatient ET programs (State University of Londrina [UEL]) and University of Northern Parana [UNOPAR], Brazil), between May 2006 and May 2018. Subjects analyzed in this study should have been included in the high intensity ET program in one of the two centers by meeting the following inclusion criteria, which were the same in the two centers: diagnosis of COPD according to the Global Initiative for Chronic Obstructive Pulmonary Disease (GOLD) criteria;7 clinical stability (i.e., absence of acute exacerbations of COPD) for at least one month prior to inclusion in the program; absence of severe comorbidities or cognitive impairments that could hinder the performance of the assessments and intervention; and no participation in any ET program in the previous year. Subjects who did not complete the ET program, those without pre-bronchodilator baseline spirometry or missing information regarding previous diagnosis of asthma were excluded. All participants signed an informed consent form and the

study was approved by the Ethics Committee of both centers (UEL n. 123/09 and UNOPAR n. 0377/10).

Group allocation: ACO or COPD

Diagnosis of asthma-COPD overlap was adapted from Sin et al.14 Thus, to be classified as having ACO, subjects should present three major criteria (i.e., 1: post-bronchodilator FEV1/FVC <0.70; 2: at least 10 pack-years of tobacco smoking or equivalent indoor or outdoor air pollution exposure; 3: documented history of asthma before 40 years of age or bronchodilator response [BDR] of 400ml or more in FEV1) and one minor criteria (i.e., FEV1 BDR >200 ml and 12% from baseline values).

Outcomes

Lung function (FEV1 and FEV1/FVC) was assessed by spirometry15 (Spirobank spirometer, version 3.6 MIR, Rome, Italy), and the reference values for the Brazilian population were used.16 Exercise capacity was measured by the six-minute walk test (6MWT) in a flat 30m corridor according to international recommendations17 and Brazilian reference values from Britto et al. were used.18 Dyspnea in daily life was measured by the modified Medical Research Council Scale (mMRC),19 functional status (i.e., the performance in activities of daily living) by the London Chest Activity of Daily Living Scale (LCADL), quality of life by the St. George's Respiratory Questionnaire (SGRQ)20 and symptoms of anxiety and depression by the Hospital

Anxiety and Depression Scale (HADS). Body composition (fat-free mass index [FFMI] and fat-mass index [FMI]) was measured by bioelectrical impedance (Biodynamics, EUA)21 and peripheral muscle strength was measured for the knee extensors and elbow flexors and extensors by the one-repetition maximum (1RM) test in a multi gym station with increasing load.22 Respiratory muscle strength was measured by manuvacuometry23 concerning maximum inspiratory (MIP) and expiratory (MEP) pressures, and Brazilian reference values were used.24

Exercise training program

The high-intensity ET protocol followed international recommendations of the American Thoracic Society/European Respiratory Society guidelines:1,4 12 weeks (3 days/week) of lower-limb cycling and walking exercises, as well as muscle strengthening for upper-limb (elbow flexors and extensors) and lower limbs (knee extensors). Walking intensity was initially set at 75% of the average speed of the initial 6MWT,25 aiming to reach 110% of the baseline 6MWT after 12 weeks of training.26 For the lower limb cycling, the training intensity was initially 60% of the maximum initial work rate and the goal was to reach 85% of the maximum load after 12 weeks of training.27 Both for the walking and the cycling exercises, target exercise time started with 10 minutes and increased up to 16 minutes until the last week of training. Once again, both for cycling and walking exercises, ET intensity increments were established a priori, so that loads were determined from tailored prescriptions, based on the initial tests by specialized physiotherapists,26,27 and adjustments were done according to subjects' perception of

dyspnea and fatigue by the modified version of the Borg scale (0 – 10), where the target was between 4 and 6.1,4 For strengthening exercises, subjects performed 3 sets of 8 - 12 repetitions with the initial load of 70% of the 1RM test for elbow flexors and extensors and knee extensors. The target was to increase the load from 3 to 6% per week in order to reach 121% of the 1RM test at the end of the program.26,27 Supplemental oxygen was offered to subjects who desaturated more than 4% or below 90% during the sessions, according to the aforementioned recommendations.1,4

Statistical Analysis

Data handling was done on the software Excel and statistical analysis was performed with the Statistical Package of Social Sciences (SPSS) 17.0 (SPSS Inc., Chicago, IL, USA). Normality in data distribution was evaluated with the Shapiro-Wilk test. Continuous Gaussian-distributed variables were expressed as mean ¬± standard deviation (SD) and (95% confidence interval). Non-Gaussian distributed continuous variables were expressed as median (25th – 75th quartiles). Nominal and categorical variables were expressed as number of cases (relative values).

Interactions between being classified as having only COPD or COPD plus asthma, time (pre and post intervention) and study`s outcomes (e.g., 6MWT) were investigated with the Two-way (2 x 2) Mixed ANOVA model. For those variables that did not meet the needed assumptions, log transformation was performed to achieve all assumptions before the analysis was carried out. Noteworthy, data was expressed in its natural values in the results, in order to

facilitate its interpretation. Additionally, data was checked to verify the presence of outliers, and a sensitivity analysis was run with and without the outliers to endorse the results. Chi-square test was also used where appropriated. A P value of 0.05 was considered as the threshold for statistical significance. Non-paired Student's T test or Mann-Whitney test were used for baseline comparisons between subjects who completed the ET versus those who dropped-out and also for comparison between subjects who were included versus those excluded from the study.

A sample size calculation was run (GPower 3.1) to verify the smallest number of subjects needed to achieve a power of 0.80, with a P value of 0.05, expecting an effect size of at least 0.4 in the 6MWT change after the intervention, whilst analyzed by the Two-way Mixed ANOVA test. Accordingly, a minimum sample size of 10 subjects per group was needed a significant group*time interaction.

3.4 RESULTS

A total of 164 subjects were screened. Ninety were not included in the analysis for different reasons, mostly (i.e., 79% of them) for not completing the ET program (figure 1). Therefore, 74 subjects were analyzed. There were no differences between the groups of subjects performing ET on UEL or UNOPAR, as well as no differences between the 74 analyzed subjects and those excluded from the analysis.

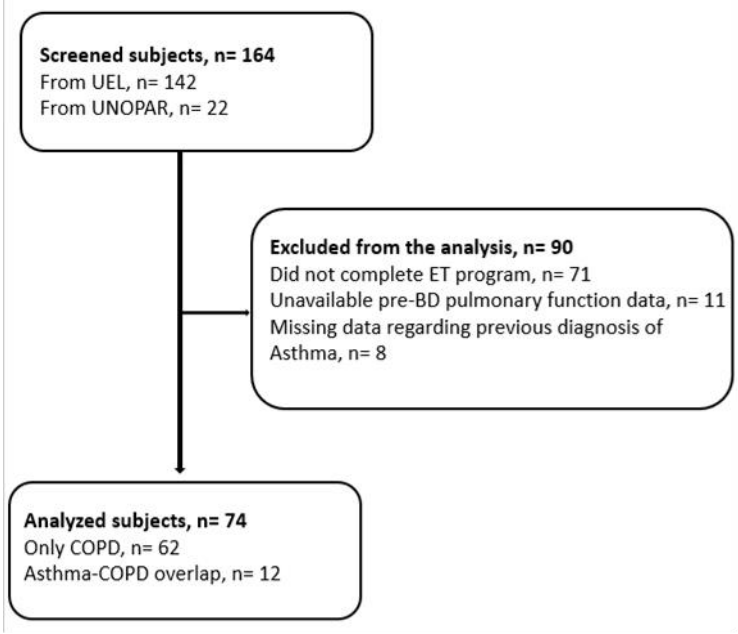

Figure 1. Flowchart of the study. UEL: State University of Londrina; UNOPAR: University of Northern Parana; ET: exercise training program; BD: bronchodilator; COPD: Chronic Obstructive Pulmonary Disease.

Table 1. Characteristics of the subjects in the groups COPD and ACO.

	COPD (n= 62)	ACO (n= 12)	*P*
Sex (female/male)	30 / 32	2 / 10	0.04
Age (yrs)	67 ± 8	63 ± 8	0.08
Body mass index (Kg/m²)	26 [21 – 32]	29 [25 – 32]	0.16

Data are presented as *n* of subjects, mean ± SD or median [25 – 75% quartiles]. COPD: Chronic Obstructive Pulmonary Disease; ACO: Asthma-COPD overlap

The sample had a well-balanced sex distribution (57% men), was aged 67±8 years, BMI 26 [21-32] kg/m² and was classified as having moderate to very severe COPD (FEV1 47±17

%predicted; GOLD I= 1; GOLD II= 35; GOLD III= 24; GOLD IV= 14). Twelve subjects (16%) of the final sample were classified as presenting Asthma-COPD overlap. The proportion of subjects with ACO among the group that did not complete the ET program was 23%, i.e., not different from the group of completers (P =0.40). Further, table 1 shows the only significant baseline difference between subjects with COPD presenting or not asthma overlap was a significantly higher proportion of male subjects in the ACO group. However, despite not significant, the ACO group showed a trend for being younger (P = 0.08; table 1) and having better preserved exercise capacity (i.e., 6MWT, m; P = 0.06) (table 2).

Table 2. Effects of Exercise Training in COPD and ACO.

	COPD		ACO		*P*		
	Pre ET	Post ET Δ	Pre ET	Post ET Δ	Group	Time	Group*time
FEV_1 (L)	1.22 (1.09–1.35)	-0.19 (-0.06–0.25)	1.59 (1.27–1.90)	-0.06 (-0.21–0.10)	0.43	0.13	0.25
FEV_1 (% pred)	46 (42–50)	6 (2–10)	52 (43–62)	7 (-2–15)	0.18	0.001	0.92
FEV_1/FVC	54 (50-57)	1.16 (-0.22–2.54)	52 (43–61)	4.39 (-0.03–8.75)	0.88	0.009	0.09
FFMI (kg/m^2)	16,6 (16.0–17.2)	0.05 (-0.12–0.22)	17.7 (16.7–18.6)	0.2 (-0.2–0.6)	0.40	0.40	0.57
FMI (kg/m^2)	9.7 (8.7–10.8)	0.13 (-0.34–0.07)	10.8 (8.4–13.2)	-0.34 (-0.1–0.31)	0.40	0.27	0.75
6MWD (m)	448 (427–469)	43 (33–52)	494 (468–529)	52 (31–73)	0.06	<0.001	0.84
6MWD (%pred)	84 (80-87)	8 (6–10)	90 (82–98)	10 (5–15)	0.10	<0.001	0.36
mMRC (0–5 pts)	3 (3–3)	-0.16 (0–0)	3 (3-4)	0 (-1–1)	0.56	0.31	0.96
LCADL (0–75 pts)	25 (22–27)	-3 (-5 - -1)	23 (17–29)	-4 (-6 - -1)	0.33	0.002	0.41
HADS anxiety (0–21 pts)	5.4 (4.0–6.8)	-1.5 (-2.7–0.3)	4.7 (0.6–8.7)	-0.3 (-3.3–2.6)	0.99	0.62	0.51
HADS depression (0–21 pts)	4.8 (3.6–6.1)	-0.4 (-1.8–1.0)	3.2 (0.7–5.6)	-0.3 (-2.4–1.7)	0.18	0.63	0.96
SGRQ (0–100 pts)	47 (40–53)	-4.3 (-10–1)	56 (39–73)	-12 (-23 - -1)	0.44	0.011	0.21
Strength QF (kg)	17 (15 - 18)	4 (3–5)	19 (15–23)	3 (1–6)	0.34	<0.001	0.70
Strength BB (kg)	12 (10–13)	3 (2–4)	12 (10–15)	4 (2–7)	0.31	<0.001	0.36
Strenght TB (kg)	13 (12–14)	3 (3–4)	14 (11–16)	4 (2–5)	0.65	<0.001	0.65
MIP (cmH_2O)	65 (58–71)	7 (3–11)	71 (53–90)	9 (-10–28)	0.36	0.009	0.95
MIP (% pred)	76 (66–79)	-1 (-5–4)	69 (56–83)	11 (-10–31)	0.67	0.007	0.98
MEP (cmH_2O)	92 (83–100)	8 (2–14)	121 (102–139)	-3 (-14–7)	0.015	0.41	0.08
MEP (% pred)	109 (100–117)	9 (3–15)	113 (95–131)	-3 (-13–6)	0.17	0.40	0.07

Data are presented as mean (95% CI).

COPD group, *n* = 62; ACO group, *n* = 12.

Table 2 shows a statistical significant main effect of time indicating improvements on exercise capacity (6MWT), peripheral and inspiratory muscle strength, functional status (assessed by the LCADL) and quality of life (SGRQ). However, no statistical significant group effect and, therefore, no time and group interaction was present (table 2) Noteworthy, figure 2 shows that, despite the majority of subjects in both groups achieving improvements higher than the minimum clinical important difference (MCID) for the 6MWT,17 only a small proportion of them reached the MCID for mMRC.28 There was no between-group difference in the proportion of subjects achieving the MCID neither for the 6MWT nor for the mMRC (figure 2).

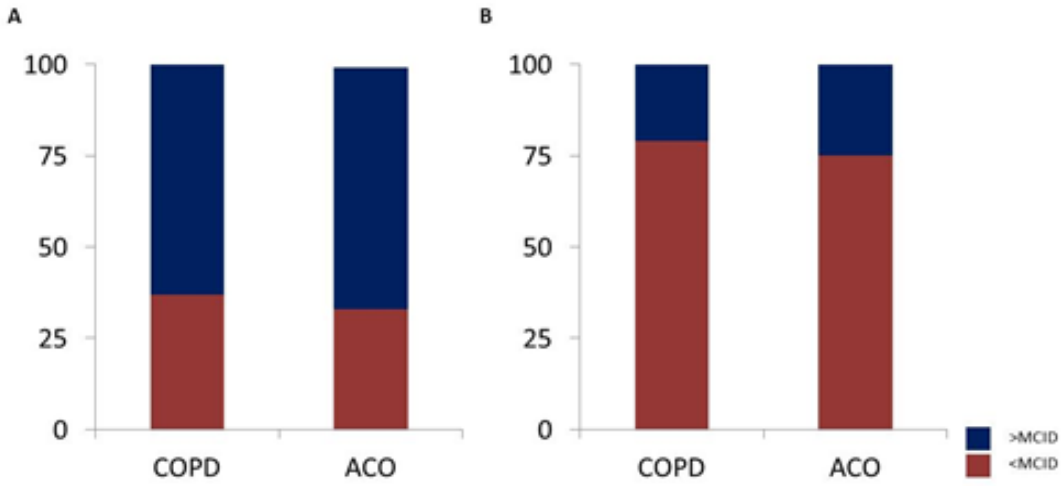

Figure 2. Percentage of patients who improved more than the minimal clinical important difference after the 12-week high-intensity exercise training program. COPD: Chronic Obstructive Pulmonary Disease; ACO: Asthma-COPD Overlap; MCID: minimal clinical important difference. Panel A: six-Minute Walk Test (6MWT) MCID: 30mts; Panel B: modified Medical Research Council (mMRC) scale MCID: 1point. Comparison of groups COPD and ACO showed P>0,05 both for 6MWT and mMRC.

3.5 DISCUSSION

Main findings

The main finding of this study is the absence of interactions between the overlap of asthma in subjects with COPD and the effects of a “classic” high-intensity exercise training program on exercise capacity, muscle strength, functional status and quality of life. Therefore, ET was equally effective in subjects with COPD independently of the presence of asthma overlap.

Exercise training effects

Exercise training has proven to be the most important intervention for reversing the impairments caused by an array of “pulmonary” diseases, including COPD and asthma.[1,4] Despite not being able to reverse lung impairments,[27] exercise training is effective in improving quality of life and exercise capacity, besides reversing morphological muscular harmful adaptations[1,4,29] and increasing survival in specific subjects.[30] Indeed, previous evidence showed ET as capable of enhancing quality of life and exercise capacity in subjects presenting bronchodilator responsiveness and fixed airflow obstruction; however, there were no differences in comparison to a control group receiving only usual care.[12] Likewise, there were no differences in the benefits promoted by exercise-training between subjects presenting

exclusively one of the two conditions, i.e., asthma or COPD.11,13 Therefore, these previous results corroborate the similar benefits found in the presence of asthma overlap in subjects with COPD in the present analysis.

Since subjects with COPD presenting or not asthma overlap need to cope with some similar limitations (e.g., airway obstruction, inactivity, dyspnea),1,4,6,7,14 interventions focused on reversing or minimizing these disease consequences are expected to be successful. However, as subjects with asthma are known to have increased inflammatory patterns, bronchial (hyper)responsiveness and remodeling compared to subjects with only COPD,6,14,31-34 one may hypothesize that exercise training could promote different clinical responses in subjects with COPD presenting asthma overlap, what was not the case herein. Therefore, further investigations might find out interventions that are able to increase exercise-related gains which are specific to the ACO population, differently than the ET protocol proposed in this study. Moreover, similarly to that found for subjects presenting only COPD, non-traditional interventions (e.g., one-leg cycling, non-linear ET)35,36 may be investigated concerning their feasibility and effectiveness for a wide spectrum of diseases, phenotypes and syndromes, including ACO. As a whole, the present results support “classic” ET programs as a rule of thumb also for subjects with COPD presenting asthma overlap.

General Considerations and Study Limitations

The absence of data on inflammatory markers could be considered as a limitation of the present study, since inflammatory status is known to differ in subjects with asthma or COPD

and has been considered to increase the likelihood of asthma overlap in subjects with COPD.6,14,31 In spite of this, the prevalence of asthma overlap in our sample was in accordance with previously published data,6 and the diagnosis criteria we used are known to differentiate between these two populations.6,14 Moreover, these criteria can be easily used in clinical practice. Additionally, despite the absence of statistical significance, it is noteworthy that also from the clinical point of view there were no differences in the responses evoked by ET regardless whether subjects with COPD presented or not asthma overlap, as can be noted by the confidence interval of the changes post ET (table 2).37; between-group differences in confidence did not exceeded clinical important differences for the outcomes measured in the study (table 2) despite changes from pre to post ET being higher than the MCID (e.g., 6MWT, table 2). Likewise, there were no between-group differences in the proportion of subjects exceeding MCID values (Figure 2). Of note, in both groups, the number of subjects exceeded the minimum sample size required according to an a priori sample size calculation for our statistical approach (i.e., identifying interactions of ACO and ET effects). Despite the fact that a relatively small number of subjects were required, the present sample size calculation was designed to uncover an effect size of at least 0.4. Thus, in case there was an interaction that would not be detected herein, this effect would be lower than 0.4 and, despite not trivial, classified as small.38 Moreover, the number of subjects included in the COPD-only group was higher than the minimum required sample, further improving the model's ability to identify a possible time-group interaction. Altogether, and simultaneously with the confirmation of absence of clinical differences seen in figure 2, these results seem to clearly yield that a

2248 “classic” high-intensity ET is equally effective for subjects with COPD independently of
2249 presenting or not asthma overlap.

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2251 ## 3.6 CONCLUSIONS

2252 The overlap of asthma in subjects with COPD did not affect the benefits evoked
2253 by a high-intensity exercise training program on exercise capacity, lung function, peripheral and
2254 inspiratory muscle force, functional status and quality of life.

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2256 ## 3.7 REFERENCES

2257 1. Nici L, Donner C, Wouters E, et al. American Thoracic Society/European
2258 Respiratory Society statement on pulmonary rehabilitation. American journal of respiratory and
2259 critical care medicine. 2006;173(12):1390-1413.

2260 2. McCarthy B, Casey D, Devane D, Murphy K, Murphy E, Lacasse Y. Pulmonary
2261 rehabilitation for chronic obstructive pulmonary disease. The Cochrane database of systematic
2262 reviews. 2015(2):CD003793.

2263 3. Lacasse Y, Cates CJ, McCarthy B, Welsh EJ. This Cochrane Review is closed:
2264 deciding what constitutes enough research and where next for pulmonary rehabilitation in
2265 COPD. The Cochrane database of systematic reviews. 2015(11):ED000107.

4. Spruit MA, Singh SJ, Garvey C, et al. An official American Thoracic Society/European Respiratory Society statement: key concepts and advances in pulmonary rehabilitation. American journal of respiratory and critical care medicine. 2013;188(8):e13-64.

5. Schols AM, Soeters PB, Mostert R, Pluymers RJ, Wouters EF. Physiologic effects of nutritional support and anabolic steroids in patients with chronic obstructive pulmonary disease. A placebo-controlled randomized trial. American journal of respiratory and critical care medicine. 1995;152(4 Pt 1):1268-1274.

6. "Global strategy for asthma management and prevention: GINA executive summary." E.D. Bateman, S.S. Hurd, P.J. Barnes, J. Bousquet, J.M. Drazen, J.M. FitzGerald, P. Gibson, K. Ohta, P. O'Byrne, S.E. Pedersen, E. Pizzichini, S.D. Sullivan, S.E. Wenzel and H.J. Zar. Eur Respir J 2008; 31: 143-178. Eur Respir J. 2018;51(2).

7. Tan WC, Bourbeau J, Aaron SD, et al. Global Initiative for Chronic Obstructive Lung Disease 2017 Classification and Lung Function Decline in Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med. 2018;197(5):670-673.

8. Hanania NA, Celli BR, Donohue JF, Martin UJ. Bronchodilator reversibility in COPD. Chest. 2011;140(4):1055-1063.

9. Gibson PG, Simpson JL. The overlap syndrome of asthma and COPD: what are its features and how important is it? Thorax. 2009;64(8):728-735.

10. Kauppi P, Kupiainen H, Lindqvist A, et al. Overlap syndrome of asthma and COPD predicts low quality of life. The Journal of asthma : official journal of the Association for the Care of Asthma. 2011;48(3):279-285.

11. Cambach W, Chadwick-Straver RV, Wagenaar RC, van Keimpema AR, Kemper HC. The effects of a community-based pulmonary rehabilitation programme on exercise tolerance and quality of life: a randomized controlled trial. The European respiratory journal. 1997;10(1):104-113.

12. Turner S, Eastwood P, Cook A, Jenkins S. Improvements in symptoms and quality of life following exercise training in older adults with moderate/severe persistent asthma. Respiration; international review of thoracic diseases. 2011;81(4):302-310.

13. Foglio K, Bianchi L, Bruletti G, Battista L, Pagani M, Ambrosino N. Long-term effectiveness of pulmonary rehabilitation in patients with chronic airway obstruction. The European respiratory journal. 1999;13(1):125-132.

14. Sin DD, Miravitlles M, Mannino DM, et al. What is asthma-COPD overlap syndrome? Towards a consensus definition from a round table discussion. Eur Respir J. 2016;48(3):664-673.

15. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. The European respiratory journal. 2005;26(2):319-338.

16. Pereira CA, Sato T, Rodrigues SC. New reference values for forced spirometry in white adults in Brazil. J Bras Pneumol. 2007;33(4):397-406.

17. Holland AE, Spruit MA, Troosters T, et al. An official European Respiratory Society/American Thoracic Society technical standard: field walking tests in chronic respiratory disease. Eur Respir J. 2014;44(6):1428-1446.

18. Britto RR, Probst VS, de Andrade AF, et al. Reference equations for the six-minute walk distance based on a Brazilian multicenter study. Brazilian journal of physical therapy. 2013;17(6):556-563.

19. Kovelis D, Segretti NO, Probst VS, Lareau SC, Brunetto AF, Pitta F. Validation of the Modified Pulmonary Functional Status and Dyspnea Questionnaire and the Medical Research Council scale for use in Brazilian patients with chronic obstructive pulmonary disease. J Bras Pneumol. 2008;34(12):1008-1018.

20. Camelier A, Rosa FW, Salim C, Nascimento OA, Cardoso F, Jardim JR. Using the Saint George's Respiratory Questionnaire to evaluate quality of life in patients with chronic obstructive pulmonary disease: validating a new version for use in Brazil. J Bras Pneumol. 2006;32(2):114-122.

21. Kyle UG, Pichard C, Rochat T, Slosman DO, Fitting JW, Thiebaud D. New bioelectrical impedance formula for patients with respiratory insufficiency: comparison to dual-energy X-ray absorptiometry. Eur Respir J. 1998;12(4):960-966.

22. Nyberg A, Saey D, Maltais F. Why and How Limb Muscle Mass and Function Should Be Measured in Patients with Chronic Obstructive Pulmonary Disease. Ann Am Thorac Soc. 2015;12(9):1269-1277.

23. Black LF, Hyatt RE. Maximal respiratory pressures: normal values and relationship to age and sex. The American review of respiratory disease. 1969;99(5):696-702.

24. Neder JA, Andreoni S, Lerario MC, Nery LE. Reference values for lung function tests. II. Maximal respiratory pressures and voluntary ventilation. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas. 1999;32(6):719-727.

25. Rodrigues A, Di Martino M, Nellessen AG, Hernandes NA, Neder JA, Pitta F. Is the six-minute walk test a useful tool to prescribe high-intensity exercise in patients with chronic obstructive pulmonary disease? Heart & lung : the journal of critical care. 2016;45(6):550-556.

26. Probst VS, Kovelis D, Hernandes NA, Camillo CA, Cavalheri V, Pitta F. Effects of 2 exercise training programs on physical activity in daily life in patients with COPD. Respir Care. 2011;56(11):1799-1807.

27. Pitta F, Troosters T, Probst VS, Langer D, Decramer M, Gosselink R. Are patients with COPD more active after pulmonary rehabilitation? Chest. 2008;134(2):273-280.

28. de Torres JP, Pinto-Plata V, Ingenito E, et al. Power of outcome measurements to detect clinically significant changes in pulmonary rehabilitation of patients with COPD. Chest. 2002;121(4):1092-1098.

29. Vogiatzis I, Terzis G, Stratakos G, et al. Effect of pulmonary rehabilitation on peripheral muscle fiber remodeling in patients with COPD in GOLD stages II to IV. Chest. 2011;140(3):744-752.

30. Camillo CA, Langer D, Osadnik CR, et al. Survival after pulmonary rehabilitation in patients with COPD: impact of functional exercise capacity and its changes. International journal of chronic obstructive pulmonary disease. 2016;11:2671-2679.

31. Ichinose M. Differences of inflammatory mechanisms in asthma and COPD. Allergology international : official journal of the Japanese Society of Allergology. 2009;58(3):307-313.

32. Barnes PJ. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. The Journal of allergy and clinical immunology. 2016;138(1):16-27.

33. Broide DH. Immunologic and inflammatory mechanisms that drive asthma progression to remodeling. The Journal of allergy and clinical immunology. 2008;121(3):560-570; quiz 571-562.

34. Slats A, Taube C. Asthma and chronic obstructive pulmonary disease overlap: asthmatic chronic obstructive pulmonary disease or chronic obstructive asthma? Therapeutic advances in respiratory disease. 2016;10(1):57-71.

35. Dolmage TE, Goldstein RS. Effects of one-legged exercise training of patients with COPD. Chest. 2008;133(2):370-376.

36. Klijn P, van Keimpema A, Legemaat M, Gosselink R, van Stel H. Nonlinear exercise training in advanced chronic obstructive pulmonary disease is superior to traditional exercise training. A randomized trial. American journal of respiratory and critical care medicine. 2013;188(2):193-200.

2364 37. Sedgwick P. Confidence intervals, P values, and statistical significance. Bmj.
2365 2015;350:h1113.

2366 38. Sullivan GM, Feinn R. Using Effect Size-or Why the P Value Is Not Enough. Journal
2367 of graduate medical education. 2012;4(3):279-282.

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4. CLUSTER ANALYSIS IDENTIFYING PATIENTS WITH COPD AT HIGH-RISK OF 2-YEAR ALL-CAUSE MORTALITY

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4.1 ABSTRACT

Objectives: To identify clusters of patients with COPD according to factors known to be associated with mortality; and to verify whether clusters' assignment is associated with 2-year mortality.

Methods: Patients (n=141) were evaluated by: bioelectrical impedance, maximal inspiratory pressure (MIP), one-repetition maximum test of the quadriceps femoris (1RMQF) and BODE index (body mass index; airflow obstruction [spirometry]; dyspnea [mMRC]; and exercise capacity [6MWD]). Vital status was retrospectively checked 2 years after the assessments and time to death was quantified for those deceased in this period.

Results: K-means analysis identified two clusters. Patients in cluster one (CL I, n=69) presented a impaired clinical status in comparison to cluster two (CL II, n=72). ROC curves identified the cutoffs discriminating patients composing CL I: FEV1 <44%pred; 6MWD <479m; 1RMQF <19kg; and MIP <73 cmH2O (AUC range 0.750–0.857). During the follow up, 19 (13%) patients deceased, 15 in CL I (224%) and four in CL II (0.06%) (P= 0.005). CL I was associated with a higher risk of 2-year mortality (HR [95%CI]: 4.3 [1.40–12.9], P= 0.01).

Conclusion: A cluster of patients with COPD highly associated with 2-year mortality was statistically identified, and cutoffs to identify these subjects were provided.

Keywords: Pulmonary Disease, Chronic Obstructive; Mortality.

4.2 INTRODUCTION

Chronic obstructive pulmonary disease (COPD)1 was accountable for more than three million deaths in 2002, representing five percent of all deaths worldwide2. Worrisome, despite the amount of financial investments2 and research development, it is estimated to be the third leading cause of death by 20303. Regardless of having its diagnosis based on lung function measurements, extra-pulmonary manifestations are undoubtedly involved in disease progression and prognosis1,4. Within this context, a substantial scientific effort has been devoted to understand which are the factors involved in disease's association with the ultimate outcome in health care, i.e., mortality (supporting information Table S1)5-13.

Single variables were shown to be associated with mortality in COPD6-9,12, and multidimensional indices have also been developed intending to increase the capacity to identify patients at high-risk of death5,10,11. Noteworthy, one of those indices which is based on the body mass index (BMI), airflow obstruction (forced expiratory volume in the first second [FEV1]), dyspnea (modified Medical Research Council scale [mMRC]) and exercise capacity (six-minute walk test [6MWT] distance), the BODE index5, has been widely used and recognized. Despite the fair capacity of these multidimensional indices to predict survival, their composition include outcomes based on expert opinion9, fact which is known for not providing the best scientific evidence level14, since expert opinions are, at least in part, influenced by the clinical characteristics of the patients routinely seen in practice. Thus, between-location differences in patients' characteristics could be a determinant of the expert opinion, and also of the accuracy of any index (or even a single variable) in predicting prognosis in COPD.

The variables composing the BODE index and each respective cut off were identified in a cohort of 207 patients and further validated in a distinct cohort of 625 COPD patients from Spain, Venezuela and United States of America. Noteworthy, differences across geographical locations are noted for clinical characteristics in the cohort, such as exercise capacity (i.e., 6MWT distance : 225 ±40 in Venezuela, 311 ± 121 in the United States and 446 ± 99 in Spain, P<0.001). Although this variability guarantees that between-location patient characteristics are taken into account, proper validation is required for extrapolation of the results (i.e., predicting responses to values outside the range of the predictor variables used in the model) in samples with characteristics out of the patterns from the populations included in any cohort.

Notwithstanding, this is the case of Brazilian patients with COPD, who have already been shown to have higher 6MWT distance than patients from Europe or North America (i.e., a shift in the regression line curve to the left). Hence, it was hypothesized that, by using a statistical method with the ability to identify clusters of patients according to well-known literature-based predictors of mortality previously identified in COPD, this would improve the capacity to identify which are the patients at high-risk of mortality. This would allow identifying patients characterized by different combinations of risk factors, instead of identifying a patient with worse/better prognosis according to each single variable and summing their single weights.

Thus, in order to prove this hypothesis, this study aimed to identify clusters of patients with COPD according to factors previously known to be associated with mortality, verifying whether these clusters' assignment is associated with 2-year mortality; and secondarily, to

compare the association of these clusters versus the association of the most recognized multidimensional index in COPD, i.e., the BODE index5.

4.3 MATERIAL AND METHODS

Study Participants

For this retrospective cohort study with an intention-to-treat analysis (see statistical analysis for more information), patients with diagnosis of COPD according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD)1, who were referred to our institutional exercise-training program and had data regarding mortality available at our database were included in this study. Whether any of the patients was evaluated more than once, only the first assessment was used for further analysis. Inclusion criteria were those for screening to the exercise program, i.e., clinical stability (i.e., absence of infections or exacerbations) in the previous three months, absence of severe and/or unstable cardiac diseases, and not having severe neurological or musculo-skeletal conditions which might interfere in the proposed protocol. Patients unable to perform the proposed assessments were excluded. For database and statistical analyses handling, patients were unnamed and identified by a unique study code. Approval for study execution was obtained from the ethics committee of the State University of Londrina, Brazil, approval 996.413 and all patients signed a written informed consent.

Vital Status

The Center for Information on Mortality (CIM), the agency responsible for registering all death events at Londrina, Brazil, was used for official date of death confirmation for the deceased patients. Formal authorization for using the CIM database was obtained from the Health Secretary of the city. Therefore, vital status was last checked, retrospectively, in January 2017, and quantified as time (i.e., months) from the last assessment to date of death for those who deceased. For those who were not at the CIM data base, telephone contact was done to guarantee whether they were alive, and time to last contact was quantified. The date of the preceding contact was used for quantification of the follow-up for those patients who were not located, neither by telephone contact nor at the CIM database, at the last attempt.

Measurements

A comprehensive initial questionnaire was used for characterization of the study sample, including information regarding anthropometrics, clinical history and comorbidities. Lung function was assessed by spirometry (Spirobank spirometer, version 3.6 MIR, Rome, Italy)15, inspiratory muscle strength by maximum inspiratory pressures (MIP; manovacuometry [Makil, Brazil])16, and functional exercise capacity by the six-minute walk test (6MWT)17. All the above cited tests followed specific international protocols and local reference values were used18-20. Body composition was assessed by bioelectrical impedance21. Peripheral muscle strength was measured by the one-repetition maximum test of the quadriceps femoris22, and

dyspnea sensation in daily life by the modified Medical Research Council scale (mMRC)23. Lastly, the BODE index (i.e., body mass index [BMI]; airflow obstruction [forced expiratory volume in the first second, [FEV1]; dyspnea [mMRC scale]; and exercise capacity [6MWT distance]) was calculated5.

Statistical Analysis

The Statistical Package of Social Science (SPSS) 20 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Normality in continuous data distribution was analyzed by the Shapiro-Wilk test. Continuous data following Gaussian distribution were expressed as means ± standard deviation. Otherwise, non-Gaussian distributed continuous data were expressed as median [interquartile range 25 – 75%]. For categorical data the number of cases and its respective percentages were chosen for data expression. Furthermore, previously known predictors of mortality in patients with COPD were identified by a non-systematic literature review. Among the identified factors, those which were available at our database, (i.e., FEV1 [% predicted]9, 6MWT [m]7, fat-free mass index [kg/m2]6, quadriceps femoris strength [kg]8, mMRC scale [0 – 4 points]12 and maximum inspiratory pressure [cmH2O]13; see table S1 for further information), were used to identify clusters of patients by K-means cluster analyses after data standardzation. Additionally, the receiver operating characteristics (ROC) curve was performed to identify which of these variables are able to discriminate patients composing each cluster; an AUC >0.500 was considered as the statistical cut off to assume that a unique variable has discriminatory capacity to identifying patients composing each cluster. The Youden's index

was used to identify the cut offs associated with better specificity and sensitivity. As patients were evaluated at the intake of an exercise-training program, an intention-to-treat analysis was carried out, by the last observation carried forward method, for those patients who did not finish the exercise-training program; for those patients who finished the program, the post-exercise training measurements were considered. A posteriori, clusters' validation was done by verifying its association with 2-year mortality by Kaplan-Meier approach with log-rank test. Cox's proportional-hazard regression was used for adjust estimates of potential confounders (i.e., BODE index, change in 6MWT (m) after exercise training, gender, comorbidities and pack years). Noteworthy, variables used to identify the clusters were not considered as confounders, as they are inherent to clusters composition. Non-paired t-test and chi-square test where also used when appropriated. A P value lower than 0.05 was adopted.

4.4 RESULTS

Out of the 162 patients registered in our exercise-training database, 153 had available data regarding mortality. Twelve out of these 153 patients were not able to be clustered because of missing data of at least one of the factors included in the K-means model. Accordingly, a sample of 141 COPD patients was used in the final analysis, mostly classified as having moderate to very severe COPD. Entire characteristics' description of the sample is shown in table 1. K-means cluster analysis was able to identify two clusters of patients, with 69 patients composing cluster one (CL I) and 72 cluster two (CL II). CL I patients had a more affected clinical status, having worse lung function, dyspnea sensation in daily life and exercise

capacity, lower peripheral muscle strength and FFMI. (table 2). Further analysis revealed all variables used for cluster composition as having powerful discriminatory capacity to identify patients with higher likelihood of being assigned to CL I (Table 3).

Table 1. Baseline characteristics of the study participants.

	n= 141
Age, yrs	70 ± 8
Gender, n (♀/♂)	62/79
BMI, kg/m^2	24.6 [21 – 29]
FVC, l	2.29 ± 0.78
FVC, %pred	66 ± 17
FEV_1, l	1.04 [0.70 -1.55]
FEV_1, %pred	42 ± 17
FEV_1/FVC	49 [36 – 65]
GOLD, I/II/III/IV	1/52/57/31
FFMI, kg/m^2	16.2 ± 2.3
6MWT, m	477 [424 – 531]
6MWT, %pred	88 [79 – 99]
1RM QF, kg	18 ± 8
mMRC, 0– 4 pts	3 [1 – 3]
MIP, cmH_2O	69 ± 23
MIP, %pred	76 ± 25
BODE index, I/II/III/IV	1 [1 – 1]
Comorbidities, n	2 [1 – 4]

Smoking history, pack years	46 [18 – 68]
Follow-up, months	24 [24 – 24]

Values are shown as mean ± standard deviation, median [interquartile 25 – 75%] or number of cases. CL I: cluster one; CL II: Cluster II; yrs: years; ♀: female; ♂: male; BMI: body mass index; FVC: forced vital capacity; FEV_1: forced expiratory volume in the first second; GOLD: global initiative for chronic obstructive pulmonary disease assignment; FFMI: fat-free mass index; 6MWT: six-minute walk test; 1RM QF: one-repetition maximum test of quadriceps femoris; mMRC: modified medical research council scale; MIP: maximum inspiratory pressure; BODE index: BMI, airflow obstruction (FEV_1); dyspnea (MRC), and exercise capacity (6MWT).

Table 2. Baseline characteristics and comparisons of the two identified clusters.

	CL I (n=69)	**CL II (n=72)**	***P***
Age, yrs	68 ± 8	64 ± 8	0.02
Gender, n (♀/♂)	36/33	26/46	0.06
BMI, kg/m²	23.1 [19.6 – 27.2]	28 [23.8 – 31.6]	<0.001
FVC, l	1.8 [1.4 – 2.2]	2.5 [2 – 3]	<0.001
FVC, %pred	58.6 [49 – 64.8]	70 [62.5 – 84.8]	<0.001
FEV_1, l	0.78 [0.65 – 1]	1.5 [1.1 – 1.8]	<0.001
FEV_1, %pred	33 ± 10	54 ± 15	<0.001
FEV_1/FVC	43 [35 – 55]	62 [51 – 67]	<0.001
GOLD, I/II/III/IV	0/8/36/25	1/447/21/6	0.000
FFMI, kg/m²	15 .4 ± 2.3	17.3 ± 1.8	<0.001
6MWT, m	435 [368 – 490]	524 [480 – 562]	<0.001
6MWT, %pred	80 [68 – 88]	96 [87 – 103]	<0.001
1RM QF, kg	13.8 ± 6.1	21.2 ± 8.1	<0.001
mMRC, 0 – 4 pts	3 [3 – 3]	2 [1 – 3]	<0.001
MIP, cmH_2O	57 ± 18	79 ± 23	<0.001
MIP, %pred	66 ± 20	86 ± 26	<0.001
BODE index, I/II/III/IV	1 [1 – 5]	1 [1 – 1]	<0.001

Comorbidities, n	3 [2–4]	2 [1–3]	0.08
Smoking history, pack years	42 [16–68]	42 [21–61]	0.80
Follow-up, months	24 [20–24]	24 [24–24]	0.09

Values are shown as mean ± standard deviation, median [interquartile 25 – 75%] or number of cases. CL I: cluster one; CL II: Cluster II; yrs: years; ♀: female; ♂: male; BMI: body mass index; FVC: forced vital capacity; FEV_1: forced expiratory volume in the first second; GOLD: global initiative for chronic obstructive pulmonary disease assignment; FFMI: fat-free mass index; 6MWT: six-minute walk test; 1RM QF: one-repetition maximum test of quadriceps femoris; mMRC: modified medical research council scale; MIP: maximum inspiratory pressure; BODE index: BMI, airflow obstruction (FEV_1); dyspnea (MRC), and exercise capacity (6MWT).

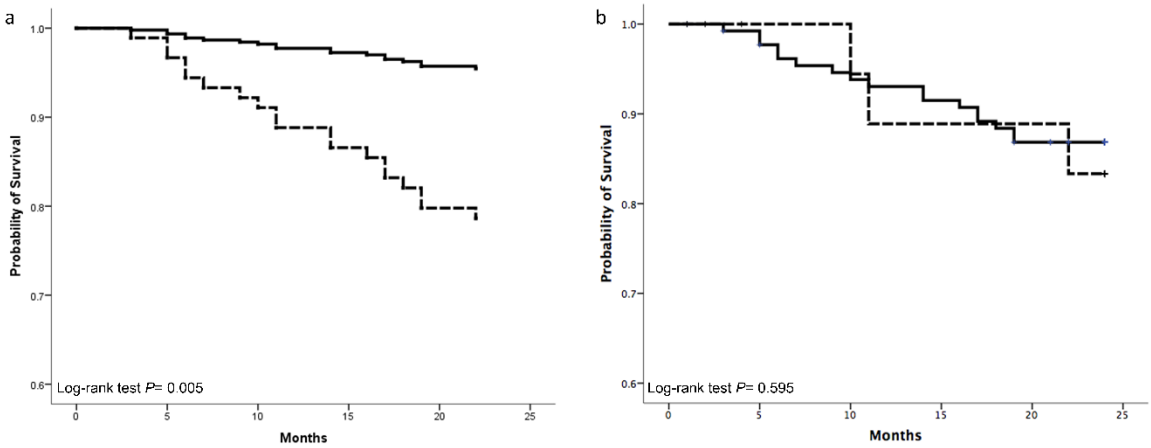
Table 3. ROC curve analyses identifying patients composing cluster two (CL II).

	FEV_1%pred	6MWT, m	FFMI	QF, kg	MIP, cmH_2O	mMRC
Cut off	44	479	16.7	18.5	73	3
Sensitivity	0.841	0.739	0.812	0.797	0.754	0.783
Specificity	0.764	0.764	0.653	0.667	0.681	0.653
AUC	0.857	0.796	0.750	0.768	0.784	0.782

6MWT: six-minute walk test; FEV_1: forced expiratory volume in first second; QF: quadriceps femoris strength; MIP: maximum inspiratory pressure; AUC: area under the curve.

The follow up period varied between 1 to 24 months. Within this period 19 (13%) patients deceased, fifteen in CL I (22%) and four in CL II (0.06%) (P= 0.005). Kaplan-Meier approach and univariate Cox's proportional-hazard regression analysis unveiled a statistically significant association between clusters' assignment and 2-year mortality, with patients assigned to CL I presenting more than four times increased chance to decease in two years

(Table 4 and Figure 1a). Noteworthy, in our sample, BODE index was not associated with survival (table 4 e Figure 1b). After correction for possible confounders in the multivariate model, clusters' assignment and gender were the only variables that remained in the model, with patients in CL I having more than 5.17 times increased chance to decease (Table 4).



	0-6 months	6-12 months	12-18 months	18-24 months	24 months
CL 1, n/death events/censured	69/3/3	63/5/0	58/4/0	54/3/0	51/0/-
CL 2, n/death events/censured	72/0/3	69/2/0	67/1/0	66/1/4	61/0/-
BODE index I/II, n/death events/censured	122/3/3	116/5/0	111/5/0	106/3/4	99/0/-
BODE index III/IV, n/death events/censured	19/0/3	16/2/0	14/0/0	14/1/0	13/0/-

Figure 1. Life table and Kaplan-Meier survival curves with logrank test. (a) For the identified clusters (shorter dashed plot indicate CL I patients; longer dashed plot indicate CL II patients); (b) For the BODE index (longer dashed plot indicate BODE I / II patients; shorter dashed plot indicate CL II patients) during the 24-month follow-up period.

Table 4. Risk of 2-year mortality due to any cause.

	Univariate		Multivariate*	
	Hazard ratio (95% CI)	***P* value**	**Hazard ratio (95% CI)**	***P* value**
Cluster, I vs. II	4.3 (1.4 – 12.9)	0.01	5.17 (1.7 – 15.7)	0.004
BODE index, pts	1.13 (0.92 – 1.39)	0.24	-	NS
Δ 6MWT, m	0.1 (0.98 – 1)	0.07	-	NS
Gender	2.95 (0.98 – 8.88)	0.06	3.77 (1.24 – 11.4)	0.019
Comorbidities	1.2 (0.79 – 5.07)	0.15	-	NS
Smoking history, pack years	0.1 (0.98 – 1.02)	0.95	-	NS

*Variables with a *P* value lower than 0.20 were considered as significant for adjustment at the multivariate level. 95% CI: 95% confidence interval; BODE index: BMI, airflow obstruction (FEV_1); dyspnea (MRC), and exercise capacity (6MWT); Δ 6MWT,m: change in the 6MWT distance after ET; Comorbidities: patients with more than two comorbidities versus those with less than two; NS: non-statistically significant.

4.5 DISCUSSION

Based on previously known predictors of mortality in COPD, two clusters were statistically identified, and further use of survival models unveil an outstanding association with 2-year mortality. Remarkably, the use of the BODE index as a comparator highlighted the promising potential of the present results for this population, since the BODE is the most scientifically used and recognized multidimensional prognostic index in COPD. However, it is anticipated that validation of these clusters in different samples involving worldwide

population-based studies is necessary before its full comprehension and acceptance. Thresholds enabling identification of patients with higher likelihood of being assigned to CL I is also valuable: as BODE index was not able to identify whose patients who deceased in our cohort (i.e., patients with less impaired/better preserved exercise capacity), these thresholds might proven as an valuable alternative to identify patients with a higher likelihood of composing CL I, and consequently increased chance to decease, in populations presenting such characteristics. Altogether, these findings make these results a promising tool for the evaluation of "short-term" mortality risk in patients with COPD.

Multidimensional indices have already proven to be better than a sole variable in identifying patients at high-risk of mortality, independently whether derived from measurements of lung function or from extrapulmonary manifestations of the disease5,10,11. In this study, the use of previously known predictors of mortality in COPD follows the same premise. The novelty herein, is the use of an advanced statistical method to cluster patients. The identified clusters presented a multitude of differences, being patients in CL I considerably more affected than those from CL II, and establishing a between-clusters known-groups validity (Table 2). This finding is in line with the original study proposing the BODE index, in which patients with worse scores were also more affected5. However, clusters' assignment in the present study was highly associated with mortality, but not the BODE index (Table 4 and Fig 1). In fact, a short follow up was investigated in the present study, instead of the relatively longer follow up investigated by Celli and colleagues5 as the main outcome (24 versus 52 months). This may, at least in part, help in the explanation of this finding. Furthermore, patients in the present cohort had a better preserved exercise capacity (Table 1), and even patients who

2619 deceased in the follow up period walked longer distances in the 6MWT than the patients who
2620 did not decease in the BODE cohort5 (mean 6MWT distance [m] of 384 ± 101 vs 264 ± 113,
2621 respectively). Corroborating the role of between-location differences in patients'
2622 characteristics, only 17 (12%) patients in our sample walked less than 350, the highest cut off
2623 for the 6MWT in the BODE index. This is not surprising, since it is already known that socio-
2624 educational-cultural differences (and consequently geographical location) may influence
2625 patients' level of exercise capacity24. Therefore, these differences should be taken into
2626 consideration when interpreting the present results.

2627 Despite the abovementioned differences in study design and sample, the survival
2628 curve of the BODE index's noticeably highlights patients in the "better" (or "milder")
2629 assignments (i.e., BODE I/II) having a steep death rate until approximately ten months of follow
2630 up (i.e., the survival curves cross approximately at this time point; Figure 1b). This indicated
2631 patients with a better clinical status according to the BODE index deceasing at shorter time, and
2632 also justified the higher 6MWT distance cut off identifying patients composing CL I (i.e., 479 m;
2633 Table 3) in comparison to the previously published value of 350 m7 (Table S1). Additionally, two
2634 other reasons may help explaining the fact that the present clusters' assignment achieved
2635 better short-term mortality associations than the BODE index: the substitution of variables/cut
2636 offs proposed by expert opinions by using a statistical analysis method to identity clusters of
2637 patients from variables previously known as predictors of mortality; and the variables with
2638 ability to identify the clusters (Table 3) having a bigger impact in short-term mortality than the
2639 variables used to classify patients according to the BODE index5 (i.e., BMI, FEV1, mMRC and
2640 6MWT).

Whereas an increased chance to decease in patients presenting worse clinical status is not surprising, how to identify these patients, however, is important clinical information. Thus, the use of simple and easy applicable cut offs presented in table 3, would enable the identification of patients with higher likelihood of short-term mortality. The level of obstruction, measured by the FEV1, was the cut off with higher discriminatory capacity (AUC 0.857); 6MWT, maximal inspiratory pressure, dyspnea in daily life, peripheral muscle strength and body composition also presented powerful discriminatory capacity to identifying patients composing CL I (AUC 0.750 – 0.796). Therefore, simple measurements, routinely performed in clinical practice, can be used with this purpose. Indeed, clusters being composed based on these aforementioned variables, all of which previously known to be associated with mortality, help in explaining the higher discriminatory capacity of then. Moreover, as these are measurements routinely seem in clinical practice, it easily allow the use any of the cut offs independently or in combination. For those cases where more than one or all of these variables are available, patients would be classified as having smaller or higher likelihood of short-term mortality according to the number of criteria meet (i.e., patients presenting values bellow the cut off of six variables having a higher likelihood to decease than patients presenting values bellow only one variable), despite external validation is needed to support or refute its aplicability.

Despite all the efforts applied in the development of the present study, some limitations should be pointed out. The retrospective design is known for not being the preferred design for cohort studies. To circumvent this limitation, corrections for comorbidities, improvement in 6MWT after the exercise training and other possible confounders were done

(table 4). However, other factors, such as the occurrence of acute exacerbations during the follow up period, were not possible to be adjusted for, as this information was not available in our database. Also, selection bias cannot be ruled out as a limitation due to the fact that the screening for inclusion was done at the initial assessment of a rehabilitation program. On the other hand, nowadays, not offering exercise-training for patients with COPD could even be considered unethical. Noteworthy, disease severity did not preclude the offer to take part in the exercise-training program and, consequently, participation in the present study. Moreover, to circumvent the influence of exercise-training in patients' survival, improvement in 6MWT after exercise-training was used as a confounder in the analysis (Table 4).

4.6 CONCLUSION

In summary, two clusters of patients were identified by using variables previously known as predictors of mortality in COPD, and clusters' assignment was proven to be highly associated with 2-year mortality. The cut offs allowing the identification of these clusters were provided and are easily applicable in clinical practice. However, further validation of these clusters and cut offs in different samples worldwide is welcomed in order to endorse or refute their applicability.

The authors have no conflict of interest to declare

4.7 REFERENCES

1. 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(5):557-582.

2. Ford ES, Murphy LB, Khavjou O, Giles WH, Holt JB, Croft JB. Total and state-specific medical and absenteeism costs of COPD among adults aged >/= 18 years in the United States for 2010 and projections through 2020. Chest. 2015;147(1):31-45.

3. World, Health, Organization. Chronic respiratory disease: Burden of COPD. 2017; http://www.who.int/respiratory/copd/burden/en/. Accessed 25/04/2017, 2017.

4. Spruit MA, Singh SJ, Garvey C, et al. An official American Thoracic Society/European Respiratory Society statement: key concepts and advances in pulmonary rehabilitation. American journal of respiratory and critical care medicine. 2013;188(8):e13-64.

5. Celli BR, Cote CG, Marin JM, et al. The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. N Engl J Med. 2004;350(10):1005-1012.

6. Vestbo J, Prescott E, Almdal T, et al. Body mass, fat-free body mass, and prognosis in patients with chronic obstructive pulmonary disease from a random population sample: findings from the Copenhagen City Heart Study. Am J Respir Crit Care Med. 2006;173(1):79-83.

7. Cote CG, Pinto-Plata V, Kasprzyk K, Dordelly LJ, Celli BR. The 6-min walk distance, peak oxygen uptake, and mortality in COPD. Chest. 2007;132(6):1778-1785.

8. Swallow EB, Reyes D, Hopkinson NS, et al. Quadriceps strength predicts mortality in patients with moderate to severe chronic obstructive pulmonary disease. Thorax. 2007;62(2):115-120.

9. Almagro P, Martinez-Camblor P, Soriano JB, et al. Finding the best thresholds of FEV1 and dyspnea to predict 5-year survival in COPD patients: the COCOMICS study. PLoS One. 2014;9(2):e89866.

10. Puhan MA, Garcia-Aymerich J, Frey M, et al. Expansion of the prognostic assessment of patients with chronic obstructive pulmonary disease: the updated BODE index and the ADO index. Lancet. 2009;374(9691):704-711.

11. Puhan MA, Hansel NN, Sobradillo P, et al. Large-scale international validation of the ADO index in subjects with COPD: an individual subject data analysis of 10 cohorts. BMJ Open. 2012;2(6).

12. Casanova C, Marin JM, Martinez-Gonzalez C, et al. Differential Effect of Modified Medical Research Council Dyspnea, COPD Assessment Test, and Clinical COPD Questionnaire for Symptoms Evaluation Within the New GOLD Staging and Mortality in COPD. Chest. 2015;148(1):159-168.

13. Gray-Donald K, Gibbons L, Shapiro SH, Macklem PT, Martin JG. Nutritional status and mortality in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 1996;153(3):961-966.

14. Centre, For, Evidence-based, Medicine. Oxford Centre for Evidence-based Medicine- Levels of Evidence 2009; http://www.cebm.net/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/. Accessed 25/04/2017, 2017.

15. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. The European respiratory journal. 2005;26(2):319-338.

16. Black LF, Hyatt RE. Maximal respiratory pressures: normal values and relationship to age and sex. The American review of respiratory disease. 1969;99(5):696-702.

17. Laboratories ATSCoPSfCPF. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med. 2002;166(1):111-117.

18. Pereira CA, Sato T, Rodrigues SC. New reference values for forced spirometry in white adults in Brazil. Jornal brasileiro de pneumologia : publicacao oficial da Sociedade Brasileira de Pneumologia e Tisilogia. 2007;33(4):397-406.

19. Neder JA, Andreoni S, Lerario MC, Nery LE. Reference values for lung function tests. II. Maximal respiratory pressures and voluntary ventilation. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas. 1999;32(6):719-727.

20. Britto RR, Probst VS, de Andrade AF, et al. Reference equations for the six-minute walk distance based on a Brazilian multicenter study. Brazilian journal of physical therapy. 2013;17(6):556-563.

21. Kyle UG, Pichard C, Rochat T, Slosman DO, Fitting JW, Thiebaud D. New bioelectrical impedance formula for patients with respiratory insufficiency: comparison to dual-energy X-ray absorptiometry. The European respiratory journal. 1998;12(4):960-966.

22. Robles PG, Mathur S, Janaudis-Fereira T, Dolmage TE, Goldstein RS, Brooks D. Measurement of peripheral muscle strength in individuals with chronic obstructive pulmonary disease: a systematic review. J Cardiopulm Rehabil Prev. 2011;31(1):11-24.

23. Kovelis D, Segretti NO, Probst VS, Lareau SC, Brunetto AF, Pitta F. Validation of the Modified Pulmonary Functional Status and Dyspnea Questionnaire and the Medical Research Council scale for use in Brazilian patients with chronic obstructive pulmonary disease. Jornal brasileiro de pneumologia : publicacao oficial da Sociedade Brasileira de Pneumologia e Tisilogia. 2008;34(12):1008-1018.

24. Pitta F, Breyer MK, Hernandes NA, et al. Comparison of daily physical activity between COPD patients from Central Europe and South America. Respir Med. 2009;103(3):421-426.

5. CONCLUSÃO GERAL DA TESE

Os artigos científicos apresentados nesta tese mostram que:

1) A suspeição/diagnóstico de fraqueza muscular inspiratória medida por meio da manuvacuometria pode ser impactada pela equação de referência utilizada para a interpretação dos resultados do exame; além disso, algumas equações de predição parecem ter maior associação com a alta probabilidade de fraqueza muscular inspiratória, segundo alterações clínicas e fisiológicas específicas que elevam a probabilidade do desenvolvimento de disfunção dos músculos inspiratórios.

2) Apesar de tanto o diafragma quanto os músculos inspiratórios "não-diafragmáticos" (e.g., escalenos, esternocleiomastoideo e intercostais) serem recrutados conforme acontece o aumento da demanda ventilatória, os músculos inspiratórios "não-diafragmáticos" parecem ser ativados como uma reserva, de maneira a sobrepor altas cargas impostas ao sistema respiratório.

3) Efeitos similares aos atingidos por pacientes com DPOC após um programa de treinamento físico de alta intensidade são também atingidos por pacientes com o diagnóstico de sobreposição de DPOC + asma.

4) Por fim, foi mostrado que por meio de uso da análise de *K-means* é possível identificar grupos de pacientes com DPOC com características específicas que se associam a um maior risco de óbito. Essas características foram: pior função pulmonar, dispneia na vida diária, força muscular periférica, capacidade de exercício e índice de massa magra. Também foi possível

determinar pontos de corte aplicáveis na prática clínica para esses desfechos que identificam o maior risco de mortalidade.

Esperamos que os resultados dos estudos contidos nessa tese contribuam para o aprofundamento científico nos tópicos nela abordados, de modo a avançar no entendimento da interpretação e caracterização da disfunção dos músculos inspiratórios, dos efeitos do treinamento físico de alta intensidade nos pacientes com associação entre DPOC e asma, e na identificação do perfil de pacientes com DPOC com maior risco de mortalidade.

6. REFERÊNCIAS DA TESE

1. Petty TL. The history of COPD. International journal of chronic obstructive pulmonary disease. 2006;1(1):3-14. Epub 2007/12/01. PubMed PMID: 18046898; PubMed Central PMCID: PMCPMC2706597.

2. Singh D, Agusti A, Anzueto A, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease: The GOLD Science Committee Report 2019. The European respiratory journal. 2019. doi: 10.1183/13993003.00164-2019. PubMed PMID: 30846476.

3. Pauwels RA, Buist AS, Calverley PM, Jenkins CR, Hurd SS, Committee GS. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary. American journal of respiratory and critical care medicine. 2001;163(5):1256-76. Epub 2001/04/24. doi: 10.1164/ajrccm.163.5.2101039. PubMed PMID: 11316667.

4. Spruit MA, Singh SJ, Garvey C, et al. An official American Thoracic Society/European Respiratory Society statement: key concepts and advances in pulmonary rehabilitation. American journal of respiratory and critical care medicine. 2013;188(8):e13-64. doi: 10.1164/rccm.201309-1634ST. PubMed PMID: 24127811.

5. Gea J, Pascual S, Casadevall C, Orozco-Levi M, Barreiro E. Muscle dysfunction in chronic obstructive pulmonary disease: update on causes and biological findings. J Thorac Dis. 2015;7(10):E418-38. Epub 2015/12/02. doi: 10.3978/j.issn.2072-1439.2015.08.04. PubMed PMID: 26623119; PubMed Central PMCID: PMCPMC4635259.

6. Gea J, Agusti A, Roca J. Pathophysiology of muscle dysfunction in COPD. Journal of applied physiology. 2013;114(9):1222-34. Epub 2013/03/23. doi: 10.1152/japplphysiol.00981.2012. PubMed PMID: 23519228.

7. Laveneziana P, Albuquerque A, Aliverti A, et al. ERS Statement on Respiratory Muscle Testing at Rest and during Exercise. The European respiratory journal. 2019. doi: 10.1183/13993003.01214-2018. PubMed PMID: 30956204.

8. Fisher KA, Stefan MS, Darling C, Lessard D, Goldberg RJ. Impact of COPD on the mortality and treatment of patients hospitalized with acute decompensated heart failure: the Worcester Heart Failure Study. Chest. 2015;147(3):637-45. Epub 2014/09/05. doi: 10.1378/chest.14-0607. PubMed PMID: 25188234; PubMed Central PMCID: PMCPMC4347532.

9. Chen W, Thomas J, Sadatsafavi M, FitzGerald JM. Risk of cardiovascular comorbidity in patients with chronic obstructive pulmonary disease: a systematic review and meta-analysis. Lancet Respir Med. 2015;3(8):631-9. Epub 2015/07/26. doi: 10.1016/S2213-2600(15)00241-6. PubMed PMID: 26208998.

10. Boulet LP, Reddel H, Bateman E, Pedersen S, FitzGerald JM, O'Byrne PM. The Global Initiative for Asthma (GINA): 25 years later. The European respiratory journal. 2019. Epub 2019/07/06. doi: 10.1183/13993003.00598-2019. PubMed PMID: 31273040.

11. "Global strategy for asthma management and prevention: GINA executive summary." E.D. Bateman, S.S. Hurd, P.J. Barnes, J. Bousquet, J.M. Drazen, J.M. FitzGerald, P. Gibson, K. Ohta, P. O'Byrne, S.E. Pedersen, E. Pizzichini, S.D. Sullivan, S.E. Wenzel and H.J. Zar. Eur Respir J 2008; 31: 143-178. The European respiratory journal. 2018;51(2). Epub 2018/02/02. doi: 10.1183/13993003.51387-2007. PubMed PMID: 29386342.

12. Akoumianaki E, Maggiore SM, Valenza F, et al. The application of esophageal pressure measurement in patients with respiratory failure. American journal of respiratory and critical care medicine. 2014;189(5):520-31. doi: 10.1164/rccm.201312-2193CI. PubMed PMID: 24467647.

13. Guenette JA, Chin RC, Cheng S, et al. Mechanisms of exercise intolerance in global initiative for chronic obstructive lung disease grade 1 COPD. The European respiratory journal. 2014;44(5):1177-87. doi: 10.1183/09031936.00034714. PubMed PMID: 25142487.

14. American Thoracic Society/European Respiratory S. ATS/ERS Statement on respiratory muscle testing. American journal of respiratory and critical care medicine. 2002;166(4):518-624. doi: 10.1164/rccm.166.4.518. PubMed PMID: 12186831.

15. Rodrigues A, Da Silva ML, Berton DC, et al. Maximal Inspiratory Pressure: Does the Choice of Reference Values Actually Matter? Chest. 2017;152(1):32-9. doi: 10.1016/j.chest.2016.11.045. PubMed PMID: 27940276.

16. Neder JA, Andreoni S, Lerario MC, Nery LE. Reference values for lung function tests. II. Maximal respiratory pressures and voluntary ventilation. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas. 1999;32(6):719-27. PubMed PMID: 10412550.

17. Culver BH. How should the lower limit of the normal range be defined? Respiratory care. 2012;57(1):136-45; discussion 43-5. doi: 10.4187/respcare.01427. PubMed PMID: 22222132.

18. Culver BH, Graham BL, Coates AL, et al. Recommendations for a Standardized Pulmonary Function Report. An Official American Thoracic Society Technical Statement.

American journal of respiratory and critical care medicine. 2017;196(11):1463-72. Epub 2017/12/02. doi: 10.1164/rccm.201710-1981ST. PubMed PMID: 29192835.

19. Sclauser Pessoa IM, Franco Parreira V, Fregonezi GA, Sheel AW, Chung F, Reid WD. Reference values for maximal inspiratory pressure: a systematic review. Can Respir J. 2014;21(1):43-50. Epub 2013/10/19. doi: 10.1155/2014/982374. PubMed PMID: 24137574; PubMed Central PMCID: PMCPMC3938239.

20. Cooper BG, Stocks J, Hall GL, et al. The Global Lung Function Initiative (GLI) Network: bringing the world's respiratory reference values together. Breathe (Sheff). 2017;13(3):e56-e64. Epub 2017/09/29. doi: 10.1183/20734735.012717. PubMed PMID: 28955406; PubMed Central PMCID: PMCPMC5607614.

21. Rochester DF, Braun NM, Arora NS. Respiratory muscle strength in chronic obstructive pulmonary disease. The American review of respiratory disease. 1979;119(2 Pt 2):151-4. Epub 1979/02/01. doi: 10.1164/arrd.1979.119.2P2.151. PubMed PMID: 426343.

22. Similowski T, Yan S, Gauthier AP, Macklem PT, Bellemare F. Contractile properties of the human diaphragm during chronic hyperinflation. The New England journal of medicine. 1991;325(13):917-23. Epub 1991/09/26. doi: 10.1056/NEJM199109263251304. PubMed PMID: 1881417.

23. De Troyer A, Boriek AM. Mechanics of the respiratory muscles. Comprehensive Physiology. 2011;1(3):1273-300. doi: 10.1002/cphy.c100009. PubMed PMID: 23733642.

24. Maltais F, Decramer M, Casaburi R, et al. An official American Thoracic Society/European Respiratory Society statement: update on limb muscle dysfunction in chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine.

2014;189(9):e15-62. Epub 2014/05/03. doi: 10.1164/rccm.201402-0373ST. PubMed PMID: 24787074; PubMed Central PMCID: PMCPMC4098112.

25. Ramirez-Sarmiento A, Orozco-Levi M, Guell R, et al. Inspiratory muscle training in patients with chronic obstructive pulmonary disease: structural adaptation and physiologic outcomes. American journal of respiratory and critical care medicine. 2002;166(11):1491-7. doi: 10.1164/rccm.200202-075OC. PubMed PMID: 12406842.

26. Kraemer WJ, Ratamess NA. Fundamentals of resistance training: progression and exercise prescription. Medicine and science in sports and exercise. 2004;36(4):674-88. PubMed PMID: 15064596.

27. Orozco-Levi M, Gea J, Lloreta JL, et al. Subcellular adaptation of the human diaphragm in chronic obstructive pulmonary disease. The European respiratory journal. 1999;13(2):371-8. Epub 1999/03/05. PubMed PMID: 10065684.

28. Ribera F, N'Guessan B, Zoll J, et al. Mitochondrial electron transport chain function is enhanced in inspiratory muscles of patients with chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine. 2003;167(6):873-9. Epub 2002/12/21. doi: 10.1164/rccm.200206-519OC. PubMed PMID: 12493645.

29. Wijnhoven JH, Janssen AJ, van Kuppevelt TH, Rodenburg RJ, Dekhuijzen PN. Metabolic capacity of the diaphragm in patients with COPD. Respir Med. 2006;100(6):1064-71. Epub 2005/11/01. doi: 10.1016/j.rmed.2005.09.029. PubMed PMID: 16257195.

30. Doucet M, Debigare R, Joanisse DR, et al. Adaptation of the diaphragm and the vastus lateralis in mild-to-moderate COPD. The European respiratory journal. 2004;24(6):971-9. Epub 2004/12/02. doi: 10.1183/09031936.04.00020204. PubMed PMID: 15572541.

31. Levine S, Kaiser L, Leferovich J, Tikunov B. Cellular adaptations in the diaphragm in chronic obstructive pulmonary disease. The New England journal of medicine. 1997;337(25):1799-806. Epub 1997/12/18. doi: 10.1056/NEJM199712183372503. PubMed PMID: 9400036.

32. Zhu E, Petrof BJ, Gea J, Comtois N, Grassino AE. Diaphragm muscle fiber injury after inspiratory resistive breathing. American journal of respiratory and critical care medicine. 1997;155(3):1110-6. Epub 1997/03/01. doi: 10.1164/ajrccm.155.3.9116995. PubMed PMID: 9116995.

33. Orozco-Levi M, Lloreta J, Minguella J, Serrano S, Broquetas JM, Gea J. Injury of the human diaphragm associated with exertion and chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine. 2001;164(9):1734-9. Epub 2001/11/24. doi: 10.1164/ajrccm.164.9.2011150. PubMed PMID: 11719318.

34. Lloreta J, Orozco M, Gea J, Corominas JM, Serrano S. Selective diaphragmatic mitochondrial abnormalities in a patient with marked air flow obstruction. Ultrastruct Pathol. 1996;20(1):67-71. Epub 1996/01/01. PubMed PMID: 8789212.

35. Polkey MI, Kyroussis D, Hamnegard CH, et al. Diaphragm performance during maximal voluntary ventilation in chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine. 1997;155(2):642-8. doi: 10.1164/ajrccm.155.2.9032207. PubMed PMID: 9032207.

36. Polkey MI, Kyroussis D, Keilty SE, et al. Exhaustive treadmill exercise does not reduce twitch transdiaphragmatic pressure in patients with COPD. American journal of respiratory and

critical care medicine. 1995;152(3):959-64. doi: 10.1164/ajrccm.152.3.7663810. PubMed PMID: 7663810.

37. Bellemare F, Wight D, Lavigne CM, Grassino A. Effect of tension and timing of contraction on the blood flow of the diaphragm. Journal of applied physiology: respiratory, environmental and exercise physiology. 1983;54(6):1597-606. doi: 10.1152/jappl.1983.54.6.1597. PubMed PMID: 6874482.

38. Gosselink R, De Vos J, van den Heuvel SP, Segers J, Decramer M, Kwakkel G. Impact of inspiratory muscle training in patients with COPD: what is the evidence? The European respiratory journal. 2011;37(2):416-25. doi: 10.1183/09031936.00031810. PubMed PMID: 21282809.

39. Whipp BJ. Ventilatory control during exercise in humans. Annu Rev Physiol. 1983;45:393-413. Epub 1983/01/01. doi: 10.1146/annurev.ph.45.030183.002141. PubMed PMID: 6405676.

40. O'Donnell DE, James MD, Milne KM, Neder JA. The Pathophysiology of Dyspnea and Exercise Intolerance in Chronic Obstructive Pulmonary Disease. Clin Chest Med. 2019;40(2):343-66. Epub 2019/05/13. doi: 10.1016/j.ccm.2019.02.007. PubMed PMID: 31078214.

41. Neder JA, Berton DC, Muller PT, et al. Ventilatory Inefficiency and Exertional Dyspnea in Early Chronic Obstructive Pulmonary Disease. Ann Am Thorac Soc. 2017;14(Supplement_1):S22-S9. Epub 2017/03/28. doi: 10.1513/AnnalsATS.201612-1033FR. PubMed PMID: 28345959.

42. Langer D, Charususin N, Jacome C, et al. Efficacy of a Novel Method for Inspiratory Muscle Training in People With Chronic Obstructive Pulmonary Disease. Physical therapy. 2015;95(9):1264-73. doi: 10.2522/ptj.20140245. PubMed PMID: 25858974.

43. Charususin N, Dacha S, Gosselink R, et al. Respiratory muscle function and exercise limitation in patients with chronic obstructive pulmonary disease: a review. Expert Rev Respir Med. 2018;12(1):67-79. Epub 2017/10/27. doi: 10.1080/17476348.2018.1398084. PubMed PMID: 29072087.

44. Illi SK, Held U, Frank I, Spengler CM. Effect of respiratory muscle training on exercise performance in healthy individuals: a systematic review and meta-analysis. Sports Med. 2012;42(8):707-24. Epub 2012/07/07. doi: 10.2165/11631670-000000000-00000. PubMed PMID: 22765281.

45. Sin DD, Miravitlles M, Mannino DM, et al. What is asthma-COPD overlap syndrome? Towards a consensus definition from a round table discussion. The European respiratory journal. 2016;48(3):664-73. Epub 2016/06/25. doi: 10.1183/13993003.00436-2016. PubMed PMID: 27338195.

46. Tan WC, Bourbeau J, Aaron SD, et al. Global Initiative for Chronic Obstructive Lung Disease 2017 Classification and Lung Function Decline in Chronic Obstructive Pulmonary Disease. American journal of respiratory and critical care medicine. 2018;197(5):670-3. Epub 2017/09/01. doi: 10.1164/rccm.201706-1154LE. PubMed PMID: 28858570.

47. Belman MJ, Mittman C. Ventilatory muscle training improves exercise capacity in chronic obstructive pulmonary disease patients. The American review of respiratory disease. 1980;121(2):273-80. doi: 10.1164/arrd.1980.121.2.273. PubMed PMID: 7362134.

48. 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. The American review of respiratory disease. 1991;143(1):9-18. Epub 1991/01/01. doi: 10.1164/ajrccm/143.1.9. PubMed PMID: 1986689.

49. McCarthy B, Casey D, Devane D, Murphy K, Murphy E, Lacasse Y. Pulmonary rehabilitation for chronic obstructive pulmonary disease. Cochrane Database Syst Rev. 2015(2):CD003793. Epub 2015/02/24. doi: 10.1002/14651858.CD003793.pub3. PubMed PMID: 25705944.

50. Felcar JM, Probst VS, de Carvalho DR, et al. Effects of exercise training in water and on land in patients with COPD: a randomised clinical trial. Physiotherapy. 2018;104(4):408-16. Epub 2018/11/28. doi: 10.1016/j.physio.2017.10.009. PubMed PMID: 30477678.

51. Vogiatzis I, Nanas S, Roussos C. Interval training as an alternative modality to continuous exercise in patients with COPD. The European respiratory journal. 2002;20(1):12-9. Epub 2002/08/09. PubMed PMID: 12166558.

52. Breyer MK, Breyer-Kohansal R, Funk GC, et al. Nordic walking improves daily physical activities in COPD: a randomised controlled trial. Respir Res. 2010;11:112. Epub 2010/08/24. doi: 10.1186/1465-9921-11-112. PubMed PMID: 20727209; PubMed Central PMCID: PMCPMC2933683.

53. Camillo CA, Langer D, Osadnik CR, et al. Survival after pulmonary rehabilitation in patients with COPD: impact of functional exercise capacity and its changes. International journal of chronic obstructive pulmonary disease. 2016;11:2671-9. Epub 2016/11/09. doi: 10.2147/COPD.S113450. PubMed PMID: 27822029; PubMed Central PMCID: PMCPMC5087703.

54. Wedzicha JAEC-C, Miravitlles M, Hurst JR, et al. Management of COPD exacerbations: a European Respiratory Society/American Thoracic Society guideline. The European respiratory journal. 2017;49(3). Epub 2017/03/17. doi: 10.1183/13993003.00791-2016. PubMed PMID: 28298398.

55. Spruit MA, Singh SJ, Rochester CL, et al. Pulmonary rehabilitation for patients with COPD during and after an exacerbation-related hospitalisation: back to the future? The European respiratory journal. 2018;51(1). Epub 2018/01/13. doi: 10.1183/13993003.01312-2017. PubMed PMID: 29326328.

56. Ryrso CK, Godtfredsen NS, Kofod LM, et al. Lower mortality after early supervised pulmonary rehabilitation following COPD-exacerbations: a systematic review and meta-analysis. BMC Pulm Med. 2018;18(1):154. Epub 2018/09/17. doi: 10.1186/s12890-018-0718-1. PubMed PMID: 30219047; PubMed Central PMCID: PMCPMC6139159.

57. Jones SE, Barker RE, Nolan CM, Patel S, Maddocks M, Man WDC. Pulmonary rehabilitation in patients with an acute exacerbation of chronic obstructive pulmonary disease. J Thorac Dis. 2018;10(Suppl 12):S1390-S9. Epub 2018/06/22. doi: 10.21037/jtd.2018.03.18. PubMed PMID: 29928521; PubMed Central PMCID: PMCPMC5989101.

58. Schols AM, Soeters PB, Mostert R, Pluymers RJ, Wouters EF. Physiologic effects of nutritional support and anabolic steroids in patients with chronic obstructive pulmonary disease. A placebo-controlled randomized trial. American journal of respiratory and critical care medicine. 1995;152(4 Pt 1):1268-74. Epub 1995/10/01. doi: 10.1164/ajrccm.152.4.7551381. PubMed PMID: 7551381.

59. Rocha A, Arbex FF, Sperandio PA, et al. Excess Ventilation in Chronic Obstructive Pulmonary Disease-Heart Failure Overlap. Implications for Dyspnea and Exercise Intolerance. American journal of respiratory and critical care medicine. 2017;196(10):1264-74. Epub 2017/07/01. doi: 10.1164/rccm.201704-0675OC. PubMed PMID: 28665692.

60. Ma J, Ward EM, Siegel RL, Jemal A. Temporal Trends in Mortality in the United States, 1969-2013. JAMA. 2015;314(16):1731-9. Epub 2015/10/28. doi: 10.1001/jama.2015.12319. PubMed PMID: 26505597.

61. Berry CE, Wise RA. Mortality in COPD: causes, risk factors, and prevention. COPD. 2010;7(5):375-82. Epub 2010/09/22. doi: 10.3109/15412555.2010.510160. PubMed PMID: 20854053.

62. Sin DD, Anthonisen NR, Soriano JB, Agusti AG. Mortality in COPD: Role of comorbidities. The European respiratory journal. 2006;28(6):1245-57. Epub 2006/12/02. doi: 10.1183/09031936.00133805. PubMed PMID: 17138679.

63. Quaderi SA, Hurst JR. The unmet global burden of COPD. Glob Health Epidemiol Genom. 2018;3:e4. Epub 2018/06/06. doi: 10.1017/gheg.2018.1. PubMed PMID: 29868229; PubMed Central PMCID: PMCPMC5921960.

64. Soler-Cataluna JJ, Martinez-Garcia MA, Roman Sanchez P, Salcedo E, Navarro M, Ochando R. Severe acute exacerbations and mortality in patients with chronic obstructive pulmonary disease. Thorax. 2005;60(11):925-31. Epub 2005/08/02. doi: 10.1136/thx.2005.040527. PubMed PMID: 16055622; PubMed Central PMCID: PMCPMC1747235.

65. Groenewegen KH, Schols AM, Wouters EF. Mortality and mortality-related factors after hospitalization for acute exacerbation of COPD. Chest. 2003;124(2):459-67. Epub 2003/08/09. doi: 10.1378/chest.124.2.459. PubMed PMID: 12907529.

66. Chung LP, Winship P, Phung S, Lake F, Waterer G. Five-year outcome in COPD patients after their first episode of acute exacerbation treated with non-invasive ventilation. Respirology. 2010;15(7):1084-91. Epub 2010/06/25. doi: 10.1111/j.1440-1843.2010.01795.x. PubMed PMID: 20573056.

67. Flattet Y, Garin N, Serratrice J, Perrier A, Stirnemann J, Carballo S. Determining prognosis in acute exacerbation of COPD. International journal of chronic obstructive pulmonary disease. 2017;12:467-75. Epub 2017/02/17. doi: 10.2147/COPD.S122382. PubMed PMID: 28203070; PubMed Central PMCID: PMCPMC5293360.

68. Rodrigues A, Camillo CA, Furlanetto KC, et al. Cluster analysis identifying patients with COPD at high risk of 2-year all-cause mortality. Chron Respir Dis. 2019;16:1479972318809452. Epub 2018/11/16. doi: 10.1177/1479972318809452. PubMed PMID: 30428721; PubMed Central PMCID: PMCPMC6301836.

69. Celli BR. Predictors of mortality in COPD. Respir Med. 2010;104(6):773-9. Epub 2010/04/27. doi: 10.1016/j.rmed.2009.12.017. PubMed PMID: 20417082.

70. Celli BR, Cote CG, Marin JM, et al. The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. The New England journal of medicine. 2004;350(10):1005-12. Epub 2004/03/05. doi: 10.1056/NEJMoa021322. PubMed PMID: 14999112.

71. Abu Hussein N, Ter Riet G, Schoenenberger L, et al. The ADO index as a predictor of two-year mortality in general practice-based chronic obstructive pulmonary disease cohorts. Respiration. 2014;88(3):208-14. Epub 2014/08/15. doi: 10.1159/000363770. PubMed PMID: 25115178.

7. RESUMO DOS ESTUDOS APRESENTADOS NA TESE

Quadro 2.

	Objetivos do estudo	**A mostra estudada**	**Principais resultados**	**Implicações clínicas**	**Limitações**
Estudo 1	Investigar o impacto do uso de diferentes equações de predição para força muscular inspiratória na suspeição/diagnóstico de fraqueza muscular inspiratória	1,729 sujeitos com idade entre 20 – 94 anos de idade, 50% do sexo masculino e 47% apresentando alta probabilidade de fraqueza muscular inspiratória	O uso de diferentes equações de referência para os valores de pressão inspiratória máxima (MIP) impactou de maneira significativa na prevalência de fraqueza muscular inspiratória	Valores absolutos e relativos (i.e., %predito) que mostraram alta capacidade de identificar sujeitos com características clínicas e fisiológicas associadas a presença de fraqueza muscular inspiratória são fornecidos com base nos resultados geradas pelo uso associado de equações de predição que mostraram ser particularmente uteis na identificação	Estudo realizado somente com pacientes Canadenses; ausência de testes adicionais como Sniff ou pressões respiratórias (i.e., Pes e Pdi) em associação ao uso de valores de MIP.

				de indivíduos que requerem avaliação neuromuscular mais avançada.	
Estudo 2	Foi investigada a ativação e a oxigenação dos músculos respiratórios e o gasto energético e oferta sistêmica de oxigênio de sujeitos com DPOC durante dois tipos de sobrecarga.	16 sujeitos com diagnóstico de DPOC, idade de 65±13 anos, 56% do sexo masculino, obstrução de moderada a grave (VEF_1 60±6% predito) e Pimax de 82±5% do predito.	Uma sessão de treinamento de força muscular inspiratória resultou em esforço respiratório aumentado em comparação com a demanda ventilatória similar à demanda causada por uma sessão de exercício físico de alta intensidade (i.e., ≈70% da ventilação voluntária máxima). Além disso, durante a sessão de treinamento de força muscular inspiratória houve um menor	O treinamento de força dos músculos inspiratórios elicita diferentes estímulos em comparação a uma sessão de treinamento físico de alta intensidade; o treinamento de força muscular inspiratória parece ser um estimulo adicional ao evocado durante uma sessão de treinamento físico de alta intensidade (i.e., ≈70% da ventilação voluntária máxima.	O número de pacientes limita o poder estatístico para a realização de análises de subgrupos; o desenho transversal não permite que sejam identificados os efeitos crônicos da exposição aos dois tipos de estímulos na ativação na oxigenação muscular após um período de exposição crônica aos dois tipos de estímulos utilizados no estudo.

			aumento tanto do consumo energético quanto da oferta de oxigênio a nível sistêmico. Os músculos inspiratórios não diafragmáticos, porém, experienciaram uma menor saturação tecidual de oxigênio em conjunto com uma oferta de oxigênio inadequada para suprir as demandas do tecido muscular.		
Estudo 3	Os efeitos de um programa de treinamento físico de alta intensidade (>60% da capacidade máxima de	74 pacientes com DPOC, 16% deles apresentando sobreposição de	A sobreposição de asma em indivíduos com DPOC não afetou os benefícios de um programa de treinamento físico	O treinamento físico de alta intensidade (i.e., >60% da capacidade máxima de exercício) deve ser realizado em pacientes com DPOC	Ausência de marcadores sanguíneos inflamatórios; ausência de dados sobre efeitos adversos ou intercorrências como crises de broncoespasmo.

	exercício) na qualidade de vida, estado funcional, sintomas de ansiedade e depressão, impacto da dispneia durante atividades de vida diária e capacidade de exercício foram comparados em sujeitos apresentando somente DPOC e pacientes com ACO	DPOC e asma.	de alta intensidade na composição corporal, capacidade de exercício, estado funcional, sintomas de ansiedade e depressão, sintomas de dispneia na vida diária, qualidade de vida e força muscular periférica e respiratória.	independentemente da sobreposição ou não de asma.	
Estudo 4	Fatores que previamente demonstraram ser associados com mortalidade em pacientes com DPOC foram utilizados	141 pacientes com DPOC, com idade de 70±8 anos, 62% do sexo masculino e obstrução ao fluxo	Grupos de pacientes foram identificados usando variáveis previamente conhecidas como preditores de mortalidade na DPOC, e a atribuição a um dos	A interação entre diferentes variáveis associadas à mortalidade em pacientes com DPOC pode ser utilizada como método para identificação de pacientes com maior	O menor número de sujeitos incluído em comparação a estudos previamente publicados; a ausência de validação em diferentes populações.

	para agrupar pacientes com DPOC por meio da análise de *k-means*, e a associação entre ser alocado nos diferentes grupos e o óbito em dois anos foi analisada.	aéreo de moderada a muito grave (VEF_1 42±17%pred).	grupos mostrou-se altamente associada à mortalidade em 2 anos. Os pontos de corte que permitem a identificação desses grupos de pacientes foram identificados e descritos, e são facilmente aplicáveis na prática clínica.	risco de mortalidade.	

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8. CURRICULIM DO CANDIDATO

Antenor Luiz Lima Rodrigues, Fisioterapeuta, MSc.

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Atualmente, Antenor Rodrigues é doutorando em Ciências da Reabilitação na Universidade Estadual de Londrina, Brasil. É formado em Fisioterapia (UNIFIL, 2011), possui Especialização (Residência) em Fisioterapia Respiratória (Universidade Estadual de Londrina, 2014) e Mestrado em Ciências da Reabilitação (Universidade Estadual de Londrina, 2016). Ele tem experiência na área de Reabilitação Pulmonar, DPOC e Exercício Físico, e é pesquisador formalmente vinculado ao Laboratório de Pesquisa em Fisioterapia Respiratória – LFIP - (da Universidade Estadual de Londrina) desde 2012. Participou como *Research Fellow* no Laboratory of Clinical Exercise Physiology, Queen's University, Canadá e na Katholieke Universiteit Leuven, Bélgica, tendo passado por períodos de intercâmbio nessas duas instituições.

1. FORMAÇÃO ACADEMICA

2016 - ATUAL Doutorando em Ciências da Reabilitação

Universidade Estadual de Londrina

Orientador: Fabio Pitta

Suporte financeiro: Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq.

2018 - 2019 Research Fellow (International Scholar)

Katholieke Universiteit Leuven

Projeto: Differences in respiratory muscle responses to hyperpnea or loaded breathing in COPD

Supervisor: Thierry Trooster

Co-supervisor: Daniel Langer

Co-supervisor: Rik Gosselink

Suporte financeiro: Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq.

2015 - 2015 Research Fellow.

Laboratory of Clinical Exercise Physiology, Queen's University, Canada

Projeto: Maximal Inspiratory Pressure: Does the Choice of Reference Values Actually Matter?

Supervisor: J. Alberto Neder

Suporte financeiro: N.A.

2014 - 2016 Mestrado em Ciências da Reabilitação.

Universidade Estadual de Londrina, Brasil

Dissertação: Is the Six-Minute Walk test a useful tool to prescribe high-intensity exercise in patients with Chronic Obstructive Pulmonary Disease?

Orientador: Fabio Pitta

Suporte financeiro: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

2012 - 2014 Especialização (Residência) em Fisioterapia Pulmonar.

Universidade Estadual de Londrina

Trabalho de conclusão de curso: Which factors determine the need of oxygen supply during exercise training in patients with COPD?

Orientador: Fabio Pitta

Suporte financeiro: Governo do Estado do Paraná

2008 - 2011 Graduação em Fisioterapia.

UNIFIL, Londrina, Brazil

Trabalho de conclusão de curso: Influência do alongamento dos músculos isquiotibiais na performance muscular do quadríceps: estudo transversal.

Orientador: Fernando Kenji Nampo

2. EXPERIÊNCIA PROFISSIONAL

2016 – 2018 Coordenador de Pesquisa Clínica

Laboratório de Pesquisa em Fisioterapia Pulmonar (LFIP) Universidade Estadual de Londrina, Brasil

2017 – 2018 Professor

Faculdade Pitágoras de Londrina, Brasil

3. CONHECIMENTO DE LÍNGUAS

Inglês: boa escrita, leitura e fala (TOEFL ITP pontuação de 570)

Lingua mãe: Português Brasileiro

4. PREMIAÇÕES

2017- ERS Young Scientist Sponsorship, European Respiratory Society

2017 - Trabalho premiado em 3º lugar na categoria pôster, IX Congresso Sulbrasileiro de Fisioterapia Respiratória, Cardiovascular e em Terapia Intensiva.

2017 - Trabalho premiado em 2º Lugar na categoria pôster-, IX Congresso Sulbrasileiro de Fisioterapia Respiratória, Cardiovascular e em Terapia Intensiva.

2013 - Segundo lugar na categoria Apresentação Oral do XII Congresso Londrinense de Fisioterapia, UEL.

LISTA DE PUBLICAÇÕES

Publicações em periódicos científicos

1. DACHA, S.; JANSSENS, L.; **RODRIGUES, A.**; LOUVARIS, Z.; JANSSENS, L.; GOSSELINK, R.; LANGER, D.. Comparison between Manual and (Semi-)automated Analyses of Esophageal Diaphragm Electromyography during Endurance Cycling in Patients with COPD. Frontiers of Physiology (accepted).

2. BELO, L.F.; **RODRIGUES, A.**; PAES, T.; MACHADO, F. V. C.; SCHNEIDER, L. PA.; VICENTIN, A. P.; PROBST, V. S.; PITTA, F.; HERNANDES, N. A.. Functional Status of Patients with COPD Assessed by London Chest Activity of Daily Living Scale: Gender Association and Validity of a Cutoff Point. LUNG. , 2019.

3. BELO, L.F., **RODRIGUES, A.,** VICENTIN, A. P., PAES, T., CASTRO, L.A., HERNANDES, N.A., PITTA, F. A breath of a fresh air: validit and reliability of a portuguese version of the Multidimensional Dyspnea Profile for patients with COPD. PlosONE. (2019).

4. SCHNEIDER, L.P., FURLANETTO, K.C., **RODRIGUES, A.**, LOPES, J.R., HERNANDES, N.A., PITTA, F. Sedentary behaviour and physical inactivity in patients with Chronic Obstructive Pulmonary Disease: Two Sides of the Same Coin? COPD: Journal of Chronic Obstructive Pulmonary Disease. v.15(5), 432 – 438, 2019.

5. MACHADO, V.F.C., SCHNEIDER, L.P., FONSECA, J., BELO, L.F., BONOMO, C., MORITA, A.A., FURLANETTO, K.C., FELCAR, J.M., **RODRIGUES, A.**, FRANSSEN, F.M.E., SPRUIT, M.A., PITTA, F., HERNANDES, N.A. Clinical impact of body composition phenotypes in patients with COPD: a retrospective analysis. European Journal of Clinical Nutrition (Epub ahead of print)

6. **RODRIGUES, A.;** CAMILLO, C. A.; FURLANETTO, K. C.; PAES, T.; MORITA, A. A.; SILVA, T. S.; DONARIA, L.; RIBEIRO, M.; PROBST, V. S.; HERNANDES, N. A. PITTA, F. Cluster Analysis identifying patients with COPD at high –risk of 2-year all-cause mortality. Chronic Respiratory Disease, 2018 (accepted)

7. **RODRIGUES, A.**; SCHEINDER, L. P.; MACHADO, F. V. C.; BRITO, I. L.; Pitta F. Increasing physical activity in daily life in COPD: each piece counts to solve the puzzle. American Journal of Respiratory and Critical Care Medicine, v.197(8), p. 1088 – 1089, 2017.

8. TRAVASSOS A; **RODRIGUES, A.**; FURLANETTO, KARINA C; DONARIA, L.; BISCA, G. W.; HERNANDES, NIDIA A; PITTA F. Fat-free mass depletion in patients with COPD in Brazil: Development of a new cut-off point and its relation with mortality and extrapulmonary manifestations. European Journal of Clinical Nutrition, v.71(11), p. 1285 – 1290, 2017

9. SANT' ANNA, T.; DONÁRIA, L.; FURLANETTO, K. C.; MORAKAMI, F; **RODRIGUES, A**; GROSSKREUTZ, T; HERNANDES, N. A.; GOSSELINK, R.; PITTA, FABIO. Development, Validity and Reliability of the Londrina Activities of Daily Living Protocol for Subjects with COPD. Respiratory Care, v.62, p.288 - 297, 2017.

10. MACHADO, F. V. C.; BISCA, G. W.; MORITA, A. A.; **RODRIGUES, A.**; PROBST, V. S.; FURLANETTO, K. C.; PITTA, F.; HERNANDES, N. A. Agreement of different reference equations to classify patients with COPD as having poor 6MWD. Revista Portuguesa de Pneumologia, 2017.

11. **RODRIGUES, A**; DA SILVA, M L.; BERTON, D C.; CIPRIANO, G; PITTA, F; O'DONNELL, D E.; NEDER, J A. Maximal Inspiratory Pressure: Does the Choice of Reference Values Actually Matter? CHEST, v.152(1), p.32 - 39, 2016.

12. **RODRIGUES, A**; DI MARTINO, M; NELLESSEN, A G.; HERNANDES, N A.; NEDER, J. A; PITTA, F. Is the six-minute walk test a useful tool to prescribe high-intensity exercise in patients with chronic obstructive pulmonary disease? Heart & Lung, v.45, p.550 - 556, 2016.

13. **RODRIGUES, A.**; NELLESSEN, A. G.; IKEZAKI, F.I.; di MARTINO, M.; SANTANNA, T.; HERNANDES, N. A.; PITTA, F. Quais fatores determinam uso de oxigênio no treinamento físico de pacientes com DPOC?. ASSOBRAFIR Ciência, v.5, p.11 - 22, 2014.

Livros publicados

1. **RODRIGUES, A.** Fisioterapia na Saúde do Adulto na Atenção Terciária. Londrina: Editora e Distribuidora Educacional S.A., 2018, v.1. p.200.

Resumos em conferências internacionais

1. **RODRIGUES, ANTENOR**; CAMILLO, CARLOS AUGUSTO; CERQUEIRA, PEDRO; MACHADO, FELIPE VILAÇA CAVALARI; BELO, LETICIA; SCHNEIDER, LORENA PALTAIN; FONSECA, JESSICA; PAES, THAIS; HERNANDES, NIDIA APARECIDA; PITTA, FABIO

A network analysis of clinical characteristics of patients with COPD: partial results In: ERS International Congress 2018 abstracts

Physiotherapists. European Respiratory Society, 2018. p.PA1486 -

2. **RODRIGUES, ANTENOR**; RODRIGUES, LUIZ ANTONIO LIMA; SCHNEIDER, LORENA PALTAIN; MACHADO, FELIPE VILAÇA CAVALARI; BELO, LETICIA; PAES, THAIS; FURLANETTO, KARINA COUTO; CAMILLO, CARLOS AUGUSTO; HERNANDES, NIDIA APARECIDA; PITTA, FABIO

Classification Decision Tree models to understanding subjects with COPD physical activity profiles: Preliminary results In: ERS International Congress 2018 abstracts

Physiotherapists. , 2018. p.PA5425 -

3. MACHADO, FELIPE; SCHNEIDER, LORENA; FONSECA, JÉSSICA; FERNADES, LETÍCIA; BONOMO, CAMILA; MORITA, ANDREA; **RODRIGUES, ANTENOR**; FURLANETTO, KARINA; FELCAR, JOSIANE; FRANSSEN, FRITS; SPRUIT, MARTIJN; PITTA, FABIO; HERNANDES, NIDIA

Clinical characteristics, physical function, physical activity and their associations with body composition phenotypes in patients with COPD In: ERS International Congress 2018 abstracts

Physiotherapists. European Respiratory Society, 2018. p.PA5426 -

4. **RODRIGUES, ANTENOR**; MACHADO, FELIPE VILAÇA CAVALARI; BELO, LETICIA; SCHNEIDER, LORENA PALTAIN; FONSECA, JULIANA; SPOSITON, THAMYRES; CALEFFI, FERNANDO; MORITA, ANDREA; BRITO, IGOR LOPES; ANDRELLO, ANA CAROLINA; PAES, THAIS; CAMILLO, CARLOS AUGUSTO; HERNANDES, NIDIA APARECIDA; PITTA, FABIO

Effects of high-intensity exercise training: what about the asthma-COPD overlap syndrome? In: ERS International Congress 2018 abstracts

Physiotherapists. European Respiratory Society, 2018. p.PA3833 -

5. HIRATA, RAQUEL PASTRELLO; SCHNEIDER, LORENA PALTANIN; LOPES, JOSÉ ROBERTO; BERTOCHE, MARIANA PEREIRA; OLIVEIRA, JOICE MARA; BONOMO, CAMILA; MACHADO, FELIPE; BELO, LETÍCIA FERNANDES; PAES, THAIS; **RODRIGUES, ANTENOR**; HERNANDES, NIDIA APARECIDA; PITTA, FABIO; FURLANETTO, KARINA COUTO

Heterogeneity of physical activity and its relationship with clinical outcomes in patients with COPD In: ERS International Congress 2018 abstracts

Physiotherapists. European Respiratory Society, 2018. p.OA1983 -

6. SILVA, HUMBERTO; AGUIAR, WAGNER F; GONÇALVES, ALINE F L; SILVA, THATIELLE G; LOYOLA, WALTER A S; PEREIRA, PAULO S; **RODRIGUES, ANTENOR**; PROBST, VANESSA S; PITTA, FABIO; CAMILLO, CARLOS A

Is physical activity equally reduced in patients with respiratory disease? - Preliminary results In: ERS International Congress 2018 abstracts

Physiotherapists. European Respiratory Society, 2018. p.PA5427 -

7. MORITA, ANDREA; MACHADO, FELIPE; **RODRIGUES, ANTENOR**; SCHNEIDER, LORENA; HERNANDES, NIDIA; PITTA, FABIO; PROBST, VANESSA

Predictors of exercise-induced oxygen desaturation in subjects with chronic obstructive pulmonary disease In: ERS International Congress 2018 abstracts

Physiotherapists. , 2018. p.PA1488 -

8. **RODRIGUES, A.**; CAMILLO, C. A.; FURLANETTO, K. C.; PAES, T. R.; MORITA, A. A.; SILVA, T. S.; DONARIA, L.; HERNANDES, N. A.; PITTA F

Cluster analysis identifying patients with COPD at high-risk of 2-year mortality: preliminary results In: European Respiratory Society (ERS) International Congress, 2017, Milão.

Physiotherapists. European Respiratory Society. , 2017.

9. BRITO, I. L.; **RODRIGUES, A.**; SCHEINDER, L. P.; FURLANETTO, K. C.; HERNANDES, N. A.; PITTA, F.; PROBST, V. S.

Maximal exercise capacity as a discriminatory factor to identify subjects with COPD as physical active-inactive: preliminary results In: European Respiratory Society (ERS) International Congress, 2017, Milão.

Physiotherapists. European Respiratory Society. , 2017.

10. SCHEINDER, L. P.; FURLANETTO, K. C.; **Rodrigues, A.**; LOPES, J. R. R.; BERTOCHE, M.; OLIVEIRA, L.; HERNANDES, N. A.; PITTA, F.

Sedentary behavior and physical inactivity in patients with COPD: two sides of the same coin In: European Respiratory Society (ERS) International Congress, 2017, Milão.

Physiotherapists. European Respiratory Society. , 2017.

11. BELO LF; **RODRIGUES, A.**; PAES, T. R.; BONOMO, C.; VOLPE, R.; PROBST, V. S.; PITTA, F.; HERNANDES, N. A.

Validity of a cutoff point for the London Chest Activity Daily Living scale in patients with COPD In: European Respiratory Society (ERS) International Congress, 2017, Milão.

Physiotherapists. European Respiratory Society. , 2017.

12. MACHADO, F. V. C.; BISCA, G. W.; MORITA, A. A.; **RODRIGUES, A.**; FURLANETTO, K. C.; PITTA, F.; HERNANDES, N. A.

Which reference equation should be used to classify Brazilian patients with COPD as having poor 6-minute walking distance? In: European Respiratory Society (ERS) International Congress, 2017, Milão.

Physiotherapists. European Respiratory Society. , 2017.

13. **RODRIGUES, A.**; FURLANETTO, K. C.; SIANI, L; MORITA, A. A.; MORAKAMI, F.; PAES, T. R.; BISCA, G. W.; NELLESSEN, A.; DONARIA, L.; HERNANDES, N. A.; PITTA, F.

ATS/ERS criteria for oxygen supplementation and progression of work rate during exercise training session in patients with COPD In: European Respiratory Society Congress, 2015, Amsterdã.

Physiotherapists. European Respiratory Society. , 2015.

14. MORAKAMI, F.; **RODRIGUES, A.**; BRUSTOLIN, J.; COIMBA, F.; ESCOBAR, V.; HERNANDES, N. A.; PITTA, F.

Comparison of two maximal exercise tests on treadmill in patients with COPD-Preliminary results In: European Respiratory Congress, 2015, Amsterdã.

Physiotherapists. European Respiratory Society. , 2015.

15. BISCA, G. W.; MORITA, A. A.; BRITO, I. L.; FURLANETTO, K. C.; DONARIA, L.; NELLESSEN, A.; **RODRIGUES, A.**; SANTANNA, T.; HERNANDES, N. A.; PITTA, F.

Gender-related differences after two exercise training programs in patients with COPD In: ERS International Congress, 2014, Munich.

Physiotherapists. European Respiratory Society. , 2014.

16. QUESSADA, A.; NELLESSEN, A. G.; BISCA, G. W.; SILVA, J. V.; LONGO, T.; **RODRIGUES, A.**; DONARIA, L.; FURLANETTO, K. C.; SANTANNA, T.; HERNANDES, N. A.; PITTA, F.

Minimal important difference of quadriceps femoris muscle strength after high-intensity exercise training in patients with COPD: Preliminary results In: ERS International Congress, 2014, Munich.

Physiotherapists. European Respiratory Society. , 2014.

17. **RODRIGUES, A.**; NELLESSEN, A.; IKEZAKI, F. I.; DI MARTINO, M.; DONARIA, L.; FURLANETTO, K. C.; SANTANNA, T.; HERNANDES, N. A.; PITTA, F.

Which Factors determine the need for oxygen supplementation during exercise training in patients with COPD In: ERS international Congress, 2014, Munich.

Physiotherapists. European Respiratory Society. , 2014.

9. ANEXOS

9.1 PARECER DO COMITÊ DE ÉTICA ESTUDO: MAXIMAL INSPIRATORY PRESSURE: DOES THE CHOICE OF REFERENCE VALUES ACTUALLY MATTER?

9.2 PARECER DO COMITE DE ÉTICA ESTUDO DIFFERENCES IN RESPIRATORY MUSCLE RESPONSES TO HYPERPNEA OR LOADED BREATHING IN COPD

UZ LEUVEN

Leuven, 1 augustus 2017

Commissie Medische Ethiek
UZ KU Leuven / Onderzoek
U.Z. Gasthuisberg
Herestraat 49
B 3000 Leuven (Belgium)

prof. Rik Gosselink
LIAISON KINE GHB - KRD

Ons kenmerk:	EudraCT-nr:	Belg. Regnr:
S58513		B322201526399

Effecten van ademspiertraining op symptomen van kortademigheid (dyspnoe) en posturale controle bij patiënten met COPD.

AMENDEMENT/BIJKOMENDE STUDIEDOCUMENTEN
DEFINITIEF GUNSTIG ADVIES AMEND-Id: 0003

Geachte Collega,

De Commissie Medische Ethiek van UZ KU Leuven / Onderzoek heeft vermeld protocol initieel goedgekeurd op 19 november 2015.

Met betrekking tot vermeld protocol werden bijkomende documenten ingediend bij de Commissie Medische Ethiek van UZ KU Leuven / Onderzoek.

Bij het beoordelen van dit amendement werd rekening gehouden met alle aan dit amendement gerelateerde documenten die ingediend werden op 19 juni 2017, 11 juli 2017 en 23 juli 2017.

Het amendement werd goedgekeurd op 31 juli 2017.

Dit gunstig advies betreft:

Protocol
Protocol version 6 dd. 19-06-2017
Informatie en toestemmingsformulier
Patiëntinformatie versie 6 dd. 19-06-2017 (NL)

De wijzigingen in het informatie- en toestemmingsformulier dienen duidelijk meegedeeld te worden aan patiënt, hetzij door middel van highlights, hetzij door middel van tracked changes of, indien nodig voor de duidelijkheid, door middel van een apart document waarin de wijzigingen worden opgelijst.

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Volgende documenten werden ter notificatie ingediend :

Andere
attest afdeling pneumologie ontvangen 31/07/2017
attest afdeling LAG ontvangen 31/07/2017

De Commissie bevestigt dat ze werkt in overeenstemming met de ICH-GCP principes (International Conference on Harmonization Guidelines on Good Clinical Practice), met de meest recente versie van de Verklaring van Helsinki en met de van toepassing zijnde wetten en regelgeving.

De Commissie bevestigt dat in geval van belangenconflict, de betrokken leden niet deelnemen aan de besluitvorming omtrent het amendement.

Een ledenlijst wordt bijgevoegd.

Aandachtspunten: (indien van toepassing)

De opdrachtgever is verantwoordelijk voor de conformiteit van de anderstalige documenten met de Nederlandstalige documenten.

Indien dit amendement een protocol en/of een clinical trial agreement voor UZ Leuven betreft, dan moet dit amendement ook ingediend worden bij CTC UZ Leuven.

Studies met geneesmiddelen en sommige studies met "medische hulpmiddelen" dienen door de opdrachtgever aangemeld te worden bij het FAGG.
Studies met geneesmiddelen mogen slechts aanvangen op voorwaarde dat de minister (FAGG) geen bezwaren heeft kenbaar gemaakt binnen de wettelijke termijnen zoals beschreven in art.13 van de Belgische wet van 7/5/2004 inzake experimenten op de menselijke persoon.
Voor bepaalde studies met medische hulpmiddelen gelden eveneens wettelijke termijnen (zie KB van 17/3/2009). Voor meer informatie hieromtrent verwijzen we naar de website van het FAGG www.fagg-afmps.be.

Onderzoek op embryo's in vitro valt onder de wet van 11 mei 2003. Voor dergelijk onderzoek is er naast een positief advies van het Ethisch Comité ook een goedkeuring van de Federale Commissie voor medisch en wetenschappelijk onderzoek op embryo's in vitro noodzakelijk vooraleer dit onderzoeksproject kan doorgaan.

Gelieve ook rekening te houden met de regelgeving van het ziekenhuis betreffende weefselbeheer en met de beschikkingen van de wet van 19 december 2008.

Dit gunstig advies van de Commissie houdt niet in dat zij de verantwoordelijkheid voor de geplande studie op zich neemt. U blijft hiervoor dus zelf verantwoordelijk. Bovendien dient U erover te waken dat uw mening als betrokken onderzoeker wordt weergegeven in publicaties, rapporten voor de overheid enz., die het resultaat zijn van dit onderzoek.
U wordt eraan herinnerd dat bij klinische studies iedere door U waargenomen ernstige verwikkeling onmiddellijk zowel aan de opdrachtgever (desgevallend de producent) als aan de commissie medische ethiek moet worden gemeld, ook al is het oorzakelijke verband met de studie onduidelijk.

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Gelieve ons mee te delen indien een studie niet wordt aangevat of wanneer ze wordt afgesloten of vroegtijdig onderbroken (met opgave van reden).

*Indien de studie niet binnen het jaar beëindigd is, vereist de ICH-GCP dat een **jaarlijks vorderingsrapport** aan de commissie wordt bezorgd.*

*Gelieve tenslotte het (vroegtijdige of geplande) stopzetten van een studie binnen de door de wet vastgestelde termijnen mee te delen en een **Clinical Study Report** aan de Commissie te bezorgen.*

Met vriendelijke groet,

Prof. dr. Minne Casteels
Voorzitter Commissie Medische Ethiek
UZ KU Leuven / Onderzoek

Prof. Dr. Minne Casteels
Voorzitter
Commissie Medische Ethiek UZ KU Leuven / Onderzoek

Cc:
FAGG (Federaal Agentschap voor Geneesmiddelen en Gezondheidsproducten)

CTC (Clinical Trial Center UZ Leuven)

Deelnemende centra

Lokale Commissie *Onderzoeker*

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Ledenlijst/Samenstelling van de Commissie op 31 juli 2017:

prof. dr. Maria-Reinhilde Casteels	Clinical Pharmacology
prof. dr. em. Guido Verhoeven	Experimental Medicine
De heer Jean-Jacques Derèze	Medica Legislation
Mevr. Els Raets	Nurse
Mevr. Godelieve Goossens	Nurse
Mevr. Hélène De Somer	Nurse
Mevr. Joke De Vocht	Psychologist
Mevr. Stefanie Goris	Pharmacist
apr. J.R. Thomas	Clinical Pharmacology
dr. Anne Smits	Paediatrics
dr. José Thomas	Medica Oncology
dr. Lut De Groote	General Practitioner
dr. Sabine Graux	Physician
prof. Ben Van Calster	Statistics
prof. dr. Dominique Bullens	Paediatrics
prof. dr. Gregor Verhoef	Haematology
prof. dr. Jan Van Hemelrijck	Anesthesiology
prof. dr. Jan de Hoon	Clinical Pharmacology
prof. dr. Xavier Bossuyt	Immunology
prof. dr. em. Raymond Verhaeghe	Cardiology
prof. dr. em. Willem Daenen	Cardiac Surgery

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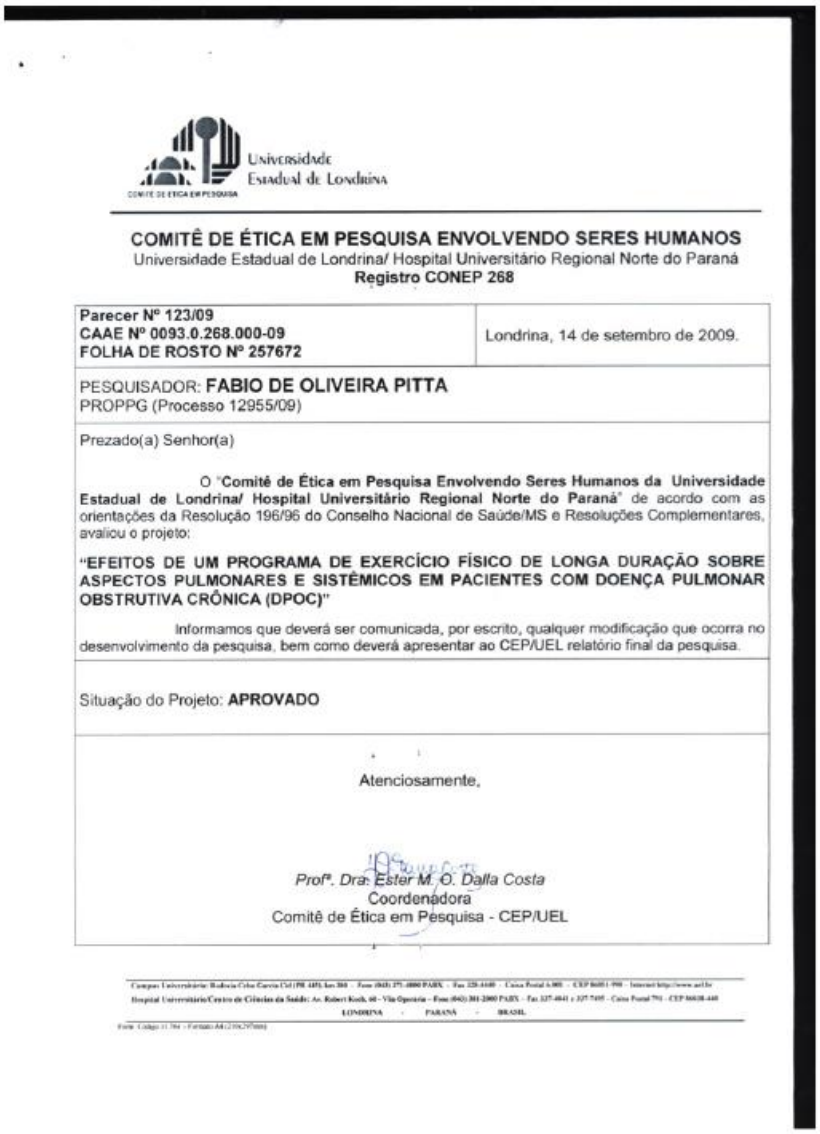
9.3 PARECER DO COMITE DE ÉTICA ESTUDO: WHAT ABOUT THE EFFECTS OF HIGH-INTENSITY EXERCISE TRAINING IN PATIENTS WITH COPD AND ASTHMA OVERLAP?



Universidade Estadual de Londrina

COMITÊ DE ÉTICA EM PESQUISA

COMITÊ DE ÉTICA EM PESQUISA ENVOLVENDO SERES HUMANOS
Universidade Estadual de Londrina/ Hospital Universitário Regional Norte do Paraná
Registro CONEP 268

Parecer Nº 123/09 **CAAE Nº 0093.0.268.000-09** **FOLHA DE ROSTO Nº 257672**	Londrina, 14 de setembro de 2009.

PESQUISADOR: **FABIO DE OLIVEIRA PITTA**
PROPPG (Processo 12955/09)

Prezado(a) Senhor(a)

O "**Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina/ Hospital Universitário Regional Norte do Paraná**" de acordo com as orientações da Resolução 196/96 do Conselho Nacional de Saúde/MS e Resoluções Complementares, avaliou o projeto:

"EFEITOS DE UM PROGRAMA DE EXERCÍCIO FÍSICO DE LONGA DURAÇÃO SOBRE ASPECTOS PULMONARES E SISTÊMICOS EM PACIENTES COM DOENÇA PULMONAR OBSTRUTIVA CRÔNICA (DPOC)"

Informamos que deverá ser comunicada, por escrito, qualquer modificação que ocorra no desenvolvimento da pesquisa, bem como deverá apresentar ao CEP/UEL relatório final da pesquisa.

Situação do Projeto: **APROVADO**

Atenciosamente,

Profª. Dra. Ester M. O. Dalla Costa
Coordenadora
Comitê de Ética em Pesquisa - CEP/UEL

LONDRINA - PARANÁ - BRASIL

9.4 PARECER DO COMITE DE ÉTICA ESTUDO: CLUSTER ANALYSIS IDENTIFYING PATIENTS WITH COPD AT HIGH-RISK OF 2-YEAR ALL-CAUSE MORTALITY

ANEXO A

Parecer do Comitê de Ética em Pesquisa

UNIVERSIDADE ESTADUAL DE LONDRINA - UEL/ HOSPITAL REGIONAL DO NORTE DO

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Titulo da Pesquisa: ESTUDO SOBRE (IN)ATIVIDADE FÍSICA DA VIDA DIÁRIA E MORTALIDADE EM PACIENTES COM DPOC

Pesquisador: KARINA COUTO FURLANETTO

Área Temática:

Versão: 2

CAAE: 41437014.0.0000.5231

Instituição Proponente: CCS - Departamento de Fisioterapia

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 996.413

Data da Relatoria: 20/03/2015

Apresentação do Projeto:

Trata-se de um projeto de pesquisa sob coordenação da Prof. Karina Couto Furlanetto, do Departamento de Fisioterapia da Universidade Estadual de Londrina (UEL). É um "estudo de coorte com análise retrospectiva de prontuários de pacientes com DPOC [doença pulmonar obstrutiva crônica], incluídos em um programa de reabilitação pulmonar nos anos de 2006 a 2014. Na avaliação inicial, os pacientes foram submetidos à avaliação do nível de atividade física na vida diária (AFVD) por meio de acelerômetros, além de avaliações da função pulmonar, força muscular respiratória, capacidade de exercício, força muscular periférica, qualidade de vida, estado funcional e sensação de dispneia. Os dados atuais referentes ao estado vital serão coletados por meio do acesso ao banco de dados do Núcleo de Informação de Mortalidade (NIM) da Autarquia Municipal de Saúde do Estado do Paraná. Caso o paciente tenha ido a óbito, serão coletadas informações sobre a data de morte e a etiologia no referido banco de dados. Após coletadas essas informações, os dados serão analisados levando-se em consideração dois grupos: grupo sobrevivente e grupo não sobrevivente". O estudo parte da seguinte hipótese: "[...] o tempo gasto em sedentarismo ou em atividade física seja um importante fator de predição de mortalidade em pacientes com DPOC".

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Bairro: Campus Universitário CEP: 86.057-970
UF: PR Município: LONDRINA
Telefone: (43)3371-5455 E-mail: cep268@uel.br

Objetivo da Pesquisa:

Objetivo Primário:

Definir um ponto de corte para tempo gasto em sedentarismo em pacientes com DPOC, e investigar sua associação com a mortalidade, comparando este ponto de corte a outros fatores preditores de mortalidade já estabelecidos na literatura.

Objetivo Secundário:

• Identificar um ponto de corte para tempo gasto em sedentarismo em pacientes com DPOC a partir da recomendação de 30 minutos de atividade física de intensidade moderada a vigorosa (AFMV).

• Investigar a associação desse novo ponto de corte com a mortalidade nesses pacientes.

• Avaliar o poder de predição de mortalidade de diferentes desfechos de (in)atividade física de vida diária medida objetivamente em pacientes com DPOC e compará-los com o poder de predição de desfechos previamente conhecidos como preditores de mortalidade da doença.

• Determinar a variável de (in)atividade física de vida diária com maior poder de predição de mortalidade em pacientes com DPOC.

Avaliação dos Riscos e Benefícios:

Como não há a previsão de participantes no projeto, apenas a consulta de prontuários, não há riscos para participantes. Com relação aos benefícios, são para a área de estudos.

Comentários e Considerações sobre a Pesquisa:

A pesquisadora sanou todas as pendências, como indicado a seguir, in verbis:

1) TCLE para os participantes da pesquisa prospectiva finalizada, que figurarão como participantes na nova pesquisa;

R: A nova pesquisa é um estudo retrospectivo que dispensa o TCLE, devido à metodologia proposta. Os dados sobre mortalidade dos participantes da nova pesquisa NÃO serão coletados por meio de contato telefônico ou visita domiciliar, e sim em prontuários, por meio do acesso ao banco de dados do Núcleo de Informação de Mortalidade (NIM) da Autarquia Municipal de Saúde do Estado do Paraná (adequações no sistema da Plataforma Brasil foram realizadas).

2) TCLE para os responsáveis por informações sobre a morte dos participantes da pesquisa prospectiva finalizada, que figurarão como participantes na nova pesquisa;

R: A nova pesquisa não realizará ligações telefônicas ou visitas domiciliares. Os dados atuais referentes ao estado vital serão coletados por meio do acesso ao Núcleo de Informação em Mortalidade (NIM) da Autarquia Municipal de Saúde.

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3) Folha de rosto assinada pela coordenação do Programa de Doutorado em Ciências da Reabilitação UEL/UNOPAR;
R: Acredito que houve um mal entendido quanto à instituição proponente do projeto de pesquisa devido ao anexo da autorização emitido pela Autarquia. Este projeto não foi cadastrado na UEL como projeto de pós-graduação e sim no meu nome, como docente do departamento de Fisioterapia. Eu também sou aluna de doutorado do programa de Ciências da Reabilitação orientada pelo professor Fabio Pitta, que é colaborador desse projeto, e quando a solicitei a autorização na Autarquia Municipal de Saúde do Estado do Paraná, também forneci essa informação. Os dados coletados neste projeto de pesquisa provavelmente renderão um segundo artigo durante o período do meu doutorado e talvez um segundo artigo da aluna Leila Donária (citado na autorização da Autarquia), por isso achei pertinente informá-los, mas o ESTUDO SOBRE (IN)ATIVIDADE FÍSICA DA VIDA DIÁRIA E MORTALIDADE EM PACIENTES COM DPOC, não é projeto de doutorado ou mestrado e sim do departamento de Fisioterapia. Gostaria gentilmente de solicitar que fosse mantida a folha de rosto previamente enviada para que fosse levado adiante como está, pois este projeto contabiliza carga horária docente na UEL e já está até na pauta da próxima reunião de departamento.

4) Apresentação do "Termo de Sigilo e Confidencialidade";
R: Inclui o "Termo de Sigilo e Confidencialidade" (em anexo no sistema).

5) Indicação de um membro da equipe que propiciará acolhimento ou indicação do tipo de encaminhamento a ser dado no caso dos participantes se sentirem emocionalmente abalados por conta da abordagem da questão da morte.
6) Roteiro das perguntas que serão feitas aos participantes, especialmente aquelas que tratarão sobre óbitos.
R (questões 5 e 6): Visto que os dados serão coletados em prontuários, as questões 5 e 6 também não se aplicam ao presente estudo.

Considerações sobre os Termos de apresentação obrigatória:
Foram contemplados todos os termos de apresentação obrigatória.

Recomendações:
Não há.

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UNIVERSIDADE ESTADUAL DE LONDRINA - UEL/ HOSPITAL REGIONAL DO NORTE DO

Continuação do Parecer: 996.413

Conclusões ou Pendências e Lista de Inadequações:

Não há.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

Prezado (a) Pesquisador (a),

Este é seu parecer final de aprovação, vinculado ao Comitê de Ética em Pesquisas Envolvendo Seres Humanos da Universidade Estadual de Londrina. É sua responsabilidade imprimi-lo para apresentação aos órgãos e/ou instituições pertinentes.

Coordenação CEP/UEL.

LONDRINA, 24 de Março de 2015

Assinado por:
Alexandrina Aparecida Maciel Cardelli
(Coordenador)

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