ORIGINAL ARTICLE

Potential Diaphragm Muscle Weakness-related Dyspnea Persists 2 Years after COVID-19 and Could Be Improved by Inspiratory Muscle Training

Results of an Observational and an Interventional Clinical Trial

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Abstract

Rationale: Diaphragm muscle weakness might underlie persistent exertional dyspnea, despite normal lung and cardiac function in individuals who were previously hospitalized for acute coronavirus disease (COVID-19) illness.

Objectives: The authors sought, first, to determine the persistence and pathophysiological nature of diaphragm muscle weakness and its association with exertional dyspnea 2 years after hospitalization for COVID-19 and, second, to investigate the impact of inspiratory muscle training (IMT) on diaphragm and inspiratory muscle weakness and exertional dyspnea in individuals with long COVID.

Methods: Approximately 2 years after hospitalization for COVID-19, 30 individuals (11 women, 19 men; median age, 58 years; interquartile range [IQR] = 51–63) underwent comprehensive (invasive) respiratory muscle assessment and evaluation of dyspnea. Eighteen with persistent diaphragm muscle weakness and exertional dyspnea were randomized to 6 weeks of IMT or sham training; assessments were repeated immediately after and 6 weeks after IMT completion. The primary endpoint was change in inspiratory muscle fatiguability immediately after IMT.

Measurements and Main Results: At a median of 31 months (IQR = 23–32) after hospitalization, 21 of 30 individuals reported relevant persistent exertional dyspnea. Diaphragm muscle weakness on exertion and reduced diaphragm cortical activation were potentially related to exertional dyspnea. Compared with sham control, IMT improved diaphragm and inspiratory muscle function (sniff transdiaphragmatic pressure, 83 cm H_2O [IQR = 75–91] vs. 100 cm H_2O [IQR = 81–113], $P = 0.02$), inspiratory muscle fatiguability (time to task failure, 365 s [IQR = 284–701] vs. 983 s [IQR = 551–1,494], $P = 0.05$), diaphragm voluntary activation index (79% [IQR = 63–92] vs. 89% [IQR = 75–94], $P = 0.03$), and dyspnea (Borg score, 7 [IQR = 5.5–8] vs. 6 [IQR = 4–7], $P = 0.03$). Improvements persisted for 6 weeks after IMT completion.

Conclusions: To the best of the authors' knowledge, this study is the first to identify a potential treatment for persisting exertional dyspnea in long COVID and provide a possible pathophysiological explanation for the treatment benefit.

Clinical trial registered with www.clinicaltrials.gov (NCT 04854863, NCT 05582642).

Keywords: coronavirus; diaphragm muscle strength; inspiratory muscle strength training; pulmonary function; exertional dyspnea

(Received in original form September 7, 2023; accepted in final form May 19, 2024)

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Supported by the RWTH Aachen Faculty of Medicine (START grant supporting the junior research group around J.S.). This funding did not influence the design of the study; the data collection, analyses, or interpretation of data; the writing of the manuscript; or the decision to publish the results.

Am J Respir Crit Care Med Vol 210, Iss 5, pp 618–628, Sep 1, 2024

Originally Published in Press as DOI: 10.1164/rccm.202309-1572OC on May 19, 2024
Internet address: www.atsjournals.org

At a Glance Commentary

Scientific Knowledge on the Subject: The terms *long COVID* and *post-COVID-19 condition* are used to describe the persistence of otherwise unexplained symptoms and abnormalities for more than several months after recovery from acute COVID-19 illness. Dyspnea on exertion is a common symptom of long COVID, even when cardiac and pulmonary function are normal. Our group recently identified diaphragm muscle weakness as a potential correlate for persistent exertional dyspnea in patients after COVID-19 illness.

What This Study Adds to the Field: Persistent diaphragm muscle weakness was found at a median of 31 months after hospitalization for COVID-19. This, together with decreased diaphragm cortical activation, might underlie dyspnea on exertion in individuals with long COVID. To our knowledge, the present randomized, sham-controlled trial is the first to show that 6 weeks of inspiratory muscle strength training could improve inspiratory muscle endurance and cortical activation, and thereby exertional dyspnea, in individuals with long COVID. This represents a potential treatment for persisting long COVID–associated exertional dyspnea related to diaphragm muscle weakness.

It has been now more than 4 years since the beginning of the coronavirus disease (COVID-19) pandemic (1). Therefore, a substantial proportion of the population in many countries has recovered from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (2–4). Some of these individuals experience a range of otherwise unexplained symptoms and abnormalities that may persist for more than several months after recovery from acute illness (3–5).

The terms "long COVID" and "post–COVID-19 condition" have been used by the Centers for Disease Control and Prevention and the World Health Organization to describe these findings in presumably millions of patients worldwide. Dyspnea on exertion is one of the most common symptoms of long COVID, even when cardiac and pulmonary function are within normal limits, but in a substantial number of individuals, this symptom has not responded to any therapy to date (5, 6).

Our group recently documented the presence of diaphragm weakness (assessed using established invasive techniques) at 15 months after hospitalization for COVID-19 and its potential association with persistent dyspnea on exertion (7). These findings were consistent in individuals who were or were not treated with invasive mechanical ventilation during initial hospitalization and who were seen even when lung and cardiac function were normal (7). We and others have also emphasized the need to diagnose diaphragm muscle weakness using invasive measurement of transdiaphragmatic pressure (8, 9).

Therefore, in our opinion, there are three important points to be addressed. First, the persistence of diaphragm muscle weakness with potentially related exertional dyspnea needs to be determined over longer time periods. Second, the possibility of diaphragm muscle weakness mediating exertional dyspnea after COVID-19 can only be confirmed using an exercise protocol and by comparing individuals post–COVID-19 infection with exertional dyspnea and diaphragm impairment with those without exertional dyspnea or diaphragm dysfunction. Third, although inspiratory muscle training (IMT) has been shown to improve global inspiratory muscle weakness and related exercise endurance in different groups (10, 11), no clinical trial has yet investigated the use of IMT in individuals with confirmed diaphragm and/or global inspiratory muscle dysfunction and long COVID. This would help determine whether diaphragm muscle weakness is only peripheral or also has a central component, with potentially decreased cortical drive to the diaphragm contributing to exertional dyspnea, and would also facilitate understanding of the mechanisms underlying any favorable effects of IMT on dyspnea in this setting.

The aims of this study were *1*) to examine the persistence of respiratory muscle weakness and exertional dyspnea in patients hospitalized with acute COVID-19 illness 2 years previously and *2*) to evaluate the impact of inspiratory muscle training in a subset of these individuals who had inspiratory muscle weakness and otherwise unexplained exertional dyspnea.

Methods

Study Design

2-Year Follow-up Study. The initial prospective study (ClinicalTrials.gov ID: NCT 04854863) included individuals who were hospitalized at RWTH University Hospital Aachen between February 2020 and April 2021 for the management of COVID-19 and who required supplemental oxygen therapy and/or invasive mechanical ventilation. Participants consented to attend one research visit after approximately 14 months, with the respiratory muscle assessment data published previously (7), and another ∼2 years after discharge (data are reported in this article).

IMT Study. A subset of the aforementioned individuals then participated in a prospective, open-label, sham-controlled randomized trial of IMT (ClinicalTrials.gov ID: NCT05582642) to determine the

Author Contributions: J.S., B.J., B.R., and M.S. collected the data. J.S., B.R., R.G., A.G., and M.D. wrote the manuscript and contributed significantly to the study design. J.S. assumes responsibility for the accuracy and completeness of the analysis and for the fidelity of this report. All authors provided critical revision of the manuscript and approved the submission.

Data sharing statement: All data will be shared upon reasonable request.

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This article has a related editorial.

A data supplement for this article is available via the Supplements tab at the top of the online article.

potential benefit of IMT on exertional dyspnea related to diaphragm weakness.

Both study protocols received ethical approval (from Ethik-Kommission der Medizinischen Fakultät der RWTH Aachen), and written informed consent was obtained from all participants.

Participants

2-Year Follow-up Study. Eligible individuals had been hospitalized with acute COVID-19 illness between February 2020 and April 2021, they required supplemental oxygen therapy and/or invasive mechanical ventilation, and some had persistent exertional dyspnea with diaphragm muscle weakness despite normal lung and cardiac function (7).

IMT Study. Individuals with a marker of diaphragm muscle weakness—defined as twitch transdiaphragmatic pressure (twPdi) below the lower limit of normal (<16 cm H_2O) (12) or sniff nasal pressure less than 60% predicted value (13) and exertional dyspnea (Borg Dyspnea Scale score, ⩾2) after a 6-minute walk test (6MWT)—at the 2-year follow-up were recruited for the randomized trial from October 2022 to May 2023 (Figure 1). Individuals with comorbidities that are known to cause dyspnea on exertion were excluded (7). Eligible individuals were randomized (1:1) to undergo 6 weeks of IMT or sham IMT using the Powerbreath KH2P device (Powerbreath) (14); a strength protocol was used in the treatment arm, and a sham protocol was used in the sham arm. Participants were asked to complete one session of 30 breaths in the morning and one session of 30 breaths in the evening. Individuals in both the IMT and sham control groups were instructed to take a fast, maximal forceful breath in through the mouth while expanding the chest to achieve a full vital capacity. (For full details of the IMT resistance settings, *see* the online data supplement.)

Outcomes

2-Year Follow-up Study. The primary outcome for the evaluation of diaphragm muscle weakness over time—from a median of ∼14 months (interquartile range [IQR] = 13–19] to ∼31 months [IQR = 23–32] after initial hospitalization—was diaphragm muscle strength, expressed as twPdi.

IMT Study. The primary outcome for this part of the study was the change in inspiratory muscle fatiguability from before IMT to after 6 weeks of IMT.

Assessments

2-Year-Follow-up Study. Pretraining examinations included pulmonary function testing (including measurement of maximum sniff nasal inspiratory pressure [SNIP] as a measure of volitional inspiratory muscle strength), electrocardiography, and transthoracic echocardiography.

Changes in exertional dyspnea and respiratory muscle function over time were assessed by comparing data obtained at visits occurring at 14 months and 2 or more years after hospitalization for COVID-19; 14-month data have been reported previously (7), and 2-year follow-up data were available in 30/50 participants from the previous study (7) (Figure 1).

A comprehensive set of techniques were used to assess respiratory muscle function (Figure 2) (12, 15, 16), and twitch superimposition was determined to evaluate central drive to the diaphragm (12, 17). The diaphragmatic voluntary activation index (DVAI) was calculated using the following equation (18):

$$\mathrm{DVAI} = 1 - \frac{\text{twPdi at any given inspiratory effort}}{\text{max. twPdi at FRC}} \times 100.$$

(For further details, *see* the online supplement.)

Participants completed clinical questionnaires to determine dyspnea on exertion (the modified Medical Research Council dyspnea scale, the Borg dyspnea scale (19), and the Clinical Respiratory Questionnaire (20)) and fatigue (the modified Fatigue Severity Index (21)). The Borg dyspnea scale scores were also determined before and after a 6MWT without supplemental oxygen (22, 23). Exertional dyspnea severity during the

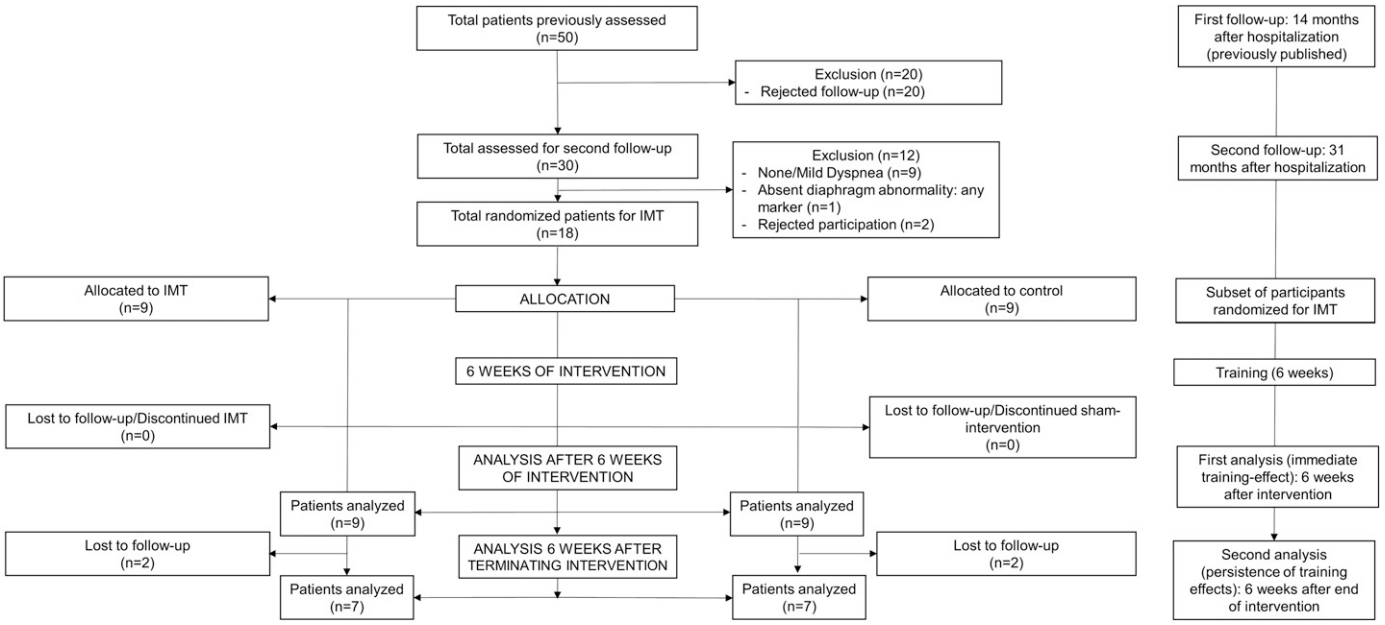

Figure 1. CONSORT flow diagram. IMT = inspiratory muscle training.

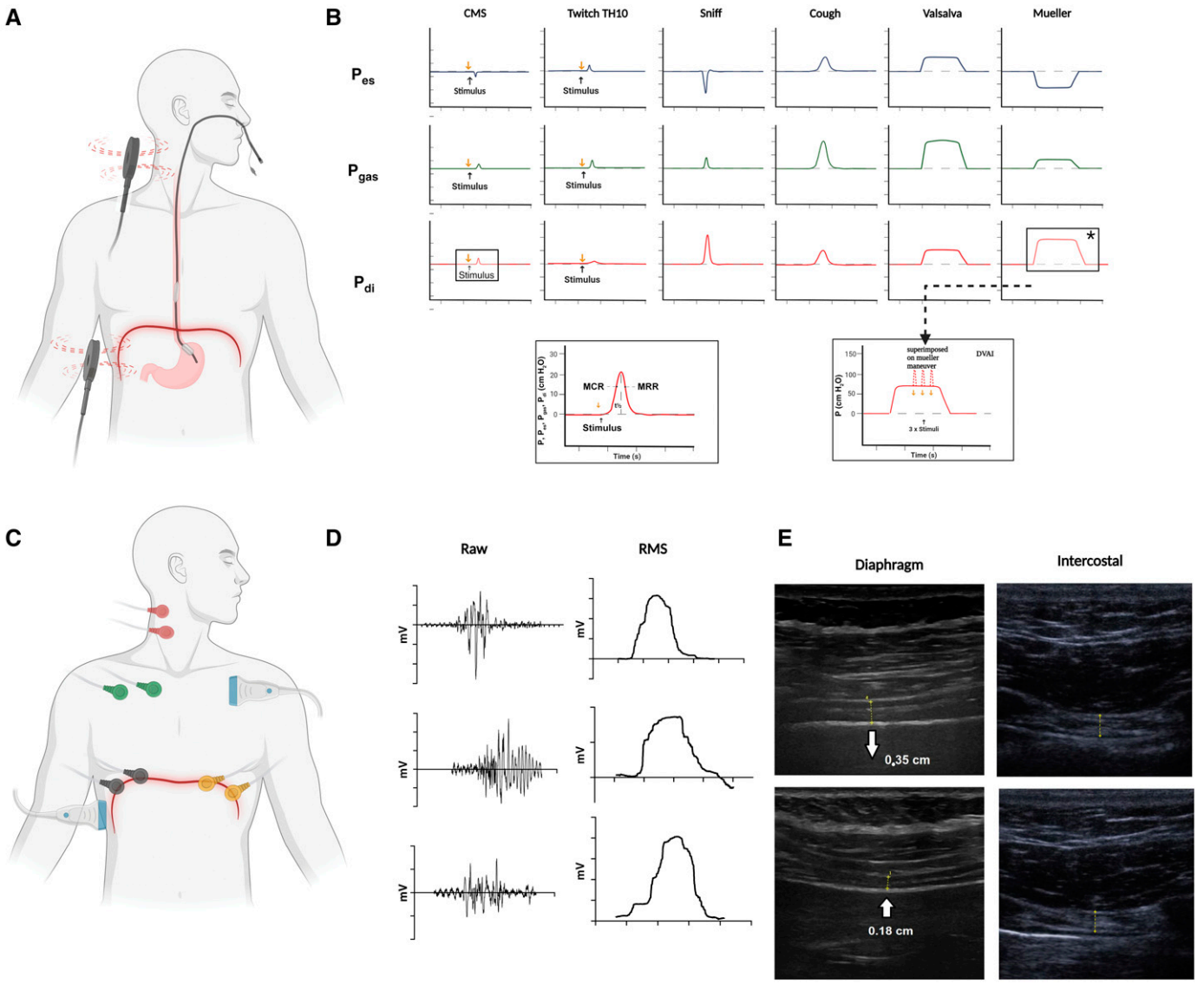

Figure 2. Experimental setup. (*A*) Subject with transnasal placement of double-balloon catheter measuring pressure from esophageal and gastric sensors for the calculation of transdiaphragmatic pressure (Pdi); magnetic coil placement for delivery of cervical magnetic stimulation (CMS) and 10th thoracic vertebrae (TH10) is shown for assessment of inspiratory and expiratory muscle strength, respectively. (*B*) Curves during different voluntary and nonvoluntary maneuvers. Readings from esophageal pressure (Pes) and gastric pressure (Pgas) sensors and calculated Pdi are shown. Representative twitch pressure recording after CMS and further in-depth analysis of a twitch curve; pressure amplitude, duration of the pressure deflection, maximum rate of contraction (MRC), and maximum rate of relaxation (MRR) were analyzed. MRC is defined as the positive peak of the pressure derivative as a function of time (i.e., the steepest slope of the inclining twitch Pdi [twPdi] curve) and reflects the maximum velocity of diaphragm contraction. MRR is defined as the negative peak of the pressure derivative over time and measures the initial part of the pressure decay, reflecting the maximum velocity of muscle relaxation. Both MRC and MRR were adjusted for twPdi. CMS twitches are superimposed on voluntary contraction and voluntary Pdi; performed on Mueller maneuver (negative Pes and positive Pgas). The diaphragm voluntary activation index (DVAI) reflects the percentage of diaphragmatic muscle mass activated by voluntary effort or the extent of diaphragmatic activation during any given inspiratory effort. *Means that (following the arrow) a zoomed in version of this part is provided here. (*C*) Placement of electromyogram (EMG) electrodes and ultrasound probe on both hemi diaphragms, parasternal intercostal and sternocleidomastoid muscle. (*D*) Raw EMG muscle signals with their respective root-mean-square (RMS) channel. (*E*) Representative examples of diaphragm (left) and parasternal intercostal (right) muscle ultrasound in inspiration (top) and expiration (bottom).

6MWT was classified as mild/none (Borg dyspnea scale score, 0–1), moderate (Borg score, 2–5), or severe (Borg score, 6 or higher) (7).

Whole-body plethysmography was performed according to current guidelines (22, 23) before and after bronchodilation (D_{LCO} was determined after bronchodilation only). Arterialized earlobe samples for capillary blood gas analysis were obtained during breathing of room air without supplemental oxygen.

An inspiratory muscle fatigue protocol evaluation was performed. Using the inspiratory muscle trainer device, initial resistance was set at maximum SNIP followed by five breaths through the device to demonstrate a maximal inspiratory effort of 10 on a visual analogue scale (0 = breathing without resistance; 10 = maximum effort). After a 10-minute rest period, resistance was decreased in 10% steps until self-reported effort after five breaths was rated as 4–7 on the VAS. Participants were then asked to breathe continuously through the IMT device until task failure, and twPdi and the diaphragm voluntary activation index (DVAI) were determined every 2 minutes. Surface electrodes were used to continuously obtain electromyographic activity of the left and right diaphragm, the parasternal intercostal muscle, and the sternocleidomastoidous (Figure 2), as described previously (24, 25).

After another 20-minute rest, diaphragm ultrasound was performed on the right hemidiaphragm and parasternal intercostal ultrasound was performed on the left and right sides (Figure 2), both as previously described (26, 27). The parasternal intercostal thickening ratio was calculated as thickness at total lung capacity divided by thickness at functional residual capacity (Figure 2) (27).

IMT Study. Randomized participants in the IMT phase had two additional visits: at the end of IMT and 6 weeks after the end of IMT, which included the aforementioned clinical questionnaires; pulmonary function testing with respiratory muscle assessment, including invasive volitional and nonvolitional tests; inspiratory muscle fatigue protocol; and ultrasound and electromyography of the respiratory muscles. All raw data analyses and statistical assessments were performed by investigators who were unaware of participant characteristics and treatment group allocation; other members of the team, including the principal investigators, were aware of the randomization.

Statistical Analysis

(For details of the sample size calculation, *see* the online supplement.) Statistical analyses were performed using Sigma Plot software (Version 13.0; Systat). Data are expressed as median values with IQR. The Shapiro–Wilk test was used to determine normality of distribution. For between-groups comparisons, unpaired *t* tests were used for normally distributed data and the Mann–Whitney *U* test was used for nonnormally distributed data. For within-group comparisons, paired *t* tests were used for normally distributed data, and the Kruskal–Wallis test was used for nonnormally distributed data, with Tukey and Bonferroni *post hoc* tests, respectively, for significant differences. $P \leqslant 0.05$ was considered statistically significant.

Results

Study Populations

2-Year Follow-up Study based on which IMT Study Was Performed. Thirty individuals were analyzed at a median of 31 months (IQR = 23–32) after hospitalization, of whom 21 reported relevant persistent dyspnea on exertion (Borg Dyspnea Scale score of 2 or higher after a 6MWT) (*see* Figure E1 in the online supplement and Table E2 discussed later). Over the period from 15 months after hospitalization to the next follow-up, only two individuals experienced an improvement in exertional dyspnea severity from severe/moderate to none/mild. Dyspnea status was stable over that period in the remaining 28 individuals (Figure E1). The majority (90%) were able to return to their previous daily activities or work.

IMT Study. Eighteen of the 30 individuals evaluated in the 2-year follow-up study (median follow-up, 31 [IQR = 24–32] months after discharge; 11 men; median age, 59 yr [IQR = 52–74]) who had persistent exertional dyspnea (i.e., Borg Dyspnea Scale score of 2 or higher after a 6MWT) and markers of diaphragm muscle weakness (i.e., twPdi below the lower limit of normal [<16 cm H_2O] (12) or sniff nasal pressure less than 60% predicted value) were included in the IMT trial (9 per treatment arm) (Figure 1). At randomization, clinical characteristics were comparable between the IMT and sham control groups (Tables 1 and 2), and none of the participants had any significant abnormalities in pulmonary function tests, capillary blood gas analysis, blood sampling, or transthoracic echocardiography (*see* Table E1).

Assessments and Outcomes in the 2-Year Follow-up Study and the IMT Study

2-Year Follow-up Study: Phrenic Nerve Stimulation and Inspiratory Muscle Strength. No significant change in twPdi was seen from 14 (IQR = 13–19) to 31 (IQR = 23–32) months after hospitalization for acute COVID-19 illness ($P = 0.08$) (Figure E1 and Table E2). There were significant subgroup differences in both twPdi and sniff nasal pressure on the basis of the severity of dyspnea on exertion (*P*-ANOVAs = 0.05 and <0.01, respectively) (Figure E1 and Table E2). Sniff transdiaphragmatic and cough gastric pressure improved significantly over time ($P = 0.003$ and $P = 0.01$, respectively) (Figure E1 and Table E2). In addition, the DVAI improved significantly over time ($P < 0.001$), and improvements were related to the severity of dyspnea on exertion (Figure E1 and Table E2).

No significance differences or trends were observed in extradiaphragmatic respiratory muscle ultrasound and electromyography findings across exertional dyspnea severity subgroups (*see* Table E3). The resistance set to perform an endurance test based on perceived dyspnea (Borg scale scores, 4–6) differed significantly on the basis of exertional dyspnea severity (Table E2).

IMT Study. All 18 participants completed IMT from October 2022 to May 2023 and underwent the 6-week follow-up assessment. Four individuals declined study measurements at 6 weeks after terminating IMT but consented to answer the questionnaires. Overall, participants completed 85% and 88% of IMT sessions in the treatment and sham arms, respectively; 91% of all weekly appointments were attended in both arms. In the treatment arm (but not in the sham arm), IMT-related resistance, load, and power significantly increased from pretraining to the end of training (Figure 3 and Tables 2 and E4). No harms or unintended effects occurred in either group.

IMT Study: Exertional Dyspnea. After training, the IMT treatment group showed significant improvements in median (IQR) Borg Dyspnea Scale scores, 7.0 (5.5–8.0) to 6.0 (4.0–7.0), $P = 0.03$; modified Medical Research Council dyspnea scale scores, 3.0 (2.0–3.0) to 2.0 (1.0–3.0), $P = 0.02$; and Chronic Respiratory Questionnaire dyspnea domain scores, 2.4 (2.1–2.8) to 3.2 (2.3–3.7), $P = 0.01$. No changes were seen in the sham group (Figure 3 and Table 2). Improvements in the treatment arm persisted until 6 weeks after the end of IMT (*see* Table E5).

IMT Study: Phrenic Nerve Stimulation and Inspiratory Muscle Strength. Median (IQR) maximal sniff nasal pressure of 70 (55–78) to 88 (60–95) cm H_2O, $P = 0.02$; sniff transdiaphragmatic pressure of 83

Table 1. Characteristics, Medical History, and Characteristics of the 2-Year Follow-up Population at Baseline and the Inspiratory Muscle Training Study Population before Inspiratory Muscle Training

Characteristic	Total Patients Followed Up ($n = 30$)	Intervention Group			
		Total ($n = 18$)	Treatment Arm ($n = 9$)	Sham Arm ($n = 9$)	*P* Value
Male sex, *n* (%)	19 (63)	11 (61)	6 (67)	5 (55)	0.65
Age, yr, median (IQR)	58.3 (51.2, 62.5)	59.0 (51.7, 73.8)	59.4 (51.6, 67.7)	58.8 (46.1, 75)	0.77
Postdischarge time, mo, median (IQR)	30.9 (23.4, 31.7)	31.2 (23.7, 31.7)	14 (10.6, 18.9)	31.5 (23.1, 31.8)	0.53
Height, m, median (IQR)	1.7 (1.7, 1.8)	1.7 (1.7, 1.8)	1.8 (1.7, 1.8)	1.7 (1.6, 1.8)	0.26
Weight, kg, median (IQR)	88.0 (78.5, 96.3)	88.5 (73, 99.2)	96 (77.5, 109.5)	86 (70, 93.5)	0.24
Body mass index, kg/m^2, median (IQR)	29.0 (26.1, 31.0)	29.5 (25.7, 31.5)	30.5 (27.1, 33.8)	28.4 (25, 30.8)	0.38
Comorbidities, *n* (%)					
COPD	0	0	0	0	1.00
Bronchial asthma	0	0	0	0	1.00
Hypertension	17 (51)	10 (55)	5 (55)	5 (55)	1.00
Systolic heart failure	0	0	0	0	1.00
Atrial fibrillation	1 (3)	1 (5)	0	1 (11)	0.33
Chronic kidney disease	4 (13)	4 (22)	2 (22)	2 (22)	1.00
Diabetes mellitus	4 (13)	4 (22)	2 (22)	2 (22)	1.00
In-hospital period					
Length of stay, d, median (IQR)	22.5 (13.8, 47.3)	33.5 (13.0, 60.3)	38.0 (14.0, 81.5)	24.0 (11.5, 54.0)	0.10
ICU stay, *n* (%)	15 (50)	9 (50)	5 (55)	4 (44)	0.66
Diaphragm/respiratory muscle function/strength, median (IQR)					
Sniff nasal pressure, cm H_2O	74.1 (67.7, 86.9)	70.0 (55.8, 77.7)	70.0 (54.6, 77.7)	71.4 (62.3, 77.7)	0.63
Sniff nasal pressure, % predicted value	79.7 (68.7, 90.9)	71.1 (66.2, 85.2)	68.9 (65.1, 84.3)	75.6 (62.9, 89.0)	0.56
CMS twPDI, cm H_2O	14.8 (9.1, 21.4)	13.4 (6.4, 19.2)	10.7 (6.7, 16.4)	15.0 (5.6, 21.8)	0.50
CMS twPDI, % LLN	92.2 (56.8, 133.6)	82.6 (39.9, 119.7)	66.9 (41.6, 102.2)	93.7 (35.2, 136.1)	0.50
DTR	1.6 (1.4, 1.8)	1.6 (1.4, 1.9)	1.6 (1.4, 2.1)	1.6 (1.4, 1.9)	0.47
DTR, % LLN	70.5 (61.8, 82.3)	74.4 (64.3, 85.6)	74.9 (64.8, 95.2)	84.1 (74.4, 19.6)	0.47
Endurance testing, median (IQR)					
Time to task failure, s	324.0 (241.0, 524.0)	296.0 (250.5, 478.5)	365.0 (284.0, 700.5)	285.6 (279.8, 289.2)	0.12
6MWD, m	480.0 (410.0, 552.0)	420.0 (350.0, 525.0)	420.0 (385.0, 570.0)	410.0 (290.0, 472.5)	0.56
6MWD, % predicted value	87.3 (76.5, 100.3)	83.0 (57.6, 100.9)	89.2 (78.0, 103.2)	77.9 (54.6, 93.8)	0.48

Definition of abbreviations: 6MWD = 6-minute walk distance; CMS = cervical magnetic stimulation (of the phrenic nerve roots); COPD = chronic obstructive pulmonary disease; DTR = diaphragm thickening ratio; IQR = interquartile range; LLN = lower limit of normal; twPdi = twitch transdiaphragmatic pressure.
Values are median (IQR) or number (%) of patients.

Table 2. Respiratory Questionnaires, RMS, and Endurance Testing in Both Groups Before and After IMT Intervention

Variable	Treatment Arm			Sham Arm			Mean Between-Groups Difference (95% CI)	*P* Value for Between-Groups Difference
	Before Training	After Training	*P* Value	Before Training	After Training	*P* Value		
Dyspnea								
mMRC Dyspnea Scale score	3.0 (2.0, 3.0)	2.0 (1.0, 3.0)	**0.02**	2.0 (2.0, 3.0)	2.0 (2.0, 2.0)	0.35	−0.40 (−1.13, 0.33)	0.18
Borg Dyspnea Scale score	7.0 (5.5, 8.0)	6.0 (4.0, 7.0)	**0.03**	5.0 (5.0, 6.5)	5.0 (4.0, 6.0)	0.11	−0.56 (−1.79, 0.66)	0.24
MFIS	43.0 (20.0, 57.0)	27.0 (23.0, 52.0)	0.15	35.0 (27.0, 43.0)	29.0 (15.5, 46.0)	0.10	−0.33 (−8.30, 7.64)	0.93
CRQ dyspnea domain	2.4 (2.1, 2.8)	3.2 (2.3, 3.7)	**0.01**	2.8 (2.0, 3.5)	3.0 (2.4, 3.5)	0.14	0.39 (−0.01, 0.80)	**0.05**
CRQ fatigue domain	4.3 (2.9, 5.1)	4.3 (3.5, 5.0)	0.8	3.8 (2.9, 4.5)	3.5 (2.6, 4.6)	0.32	0.25 (−0.49, 0.99)	0.48
CRQ emotional function	4.1 (3.1, 5.6)	4.7 (3.7, 5.3)	0.37	4.4 (3.6, 5.9)	5.3 (3.9, 5.9)	0.14	−0.13 (−0.76, 0.50)	0.66
CRQ mastery	4.5 (4.1, 5.8)	4.8 (4.3, 6.0)	**0.02**	5.3 (4.6, 6.1)	5.3 (4.8, 6.3)	0.44	0.14 (−0.13, 0.41)	0.29
Nonvolitional invasive RMS								
CMS twPdi, cm H_2O	10.7 (6.7, 16.4)	12.0 (8.9, 21.2)	0.75	15.0 (5.6, 21.8)	21.5 (8.6, 24.1)	0.12	−1.31 (−7.98, 5.34)	0.49
CMS twPes, cm H_2O	−8.9 (−13.6, −6.3)	−7.8 (−14.6, −6.2)	0.69	−10.9 (−12.9, −5.0)	−8.1 (−12.6, −4.0)	0.22	−3.23 (−10.42, 3.95)	0.24
CMS twPgas, cm H_2O	1.1 (0.2, 7.6)	3.5 (2.4, 5.0)	0.45	2.4 (0.7, 7.6)	8.0 (3.1, 12.7)	0.07	−1.70 (−7.16, 3.77)	0.27
CMS MRR normalized, cm H_2O/ms	−16.5 (−20.4, −11.5)	−12.4 (−17.4, −8.6)	**0.01**	−14.8 (−29.5, −11.1)	−10.3 (−21.8, −9.0)	0.22	−0.45 (−6.78, 5.89)	0.71
CMS MCR normalized, cm H_2O/ms	45.6 (18.3, 115.9)	50.0 (33.2, 110.9)	0.40	25.6 (23.2, 47.1)	38.4 (32.8, 70.2)	**0.0098**	−4.47 (−23.76, 14.82)	0.27
TH10 twPgas, cm H_2O	3.7 (2.1, 12.5)	10.8 (10.0, 18.1)	0.10	4.1 (3.2, 13.9)	16.4 (10.2, 25.0)	0.07	−2.42 (−13.03, 8.19)	0.47
Sniff nasal pressure, cm H_2O	70.0 (54.6, 77.7)	88.2 (60.2, 95.2)	**0.02**	71.4 (62.3, 77.7)	74.9 (63.7, 81.2)	0.17	9.54 (−0.42, 19.51)	**0.03**
Sniff nasal pressure, % predicted value	68.9 (65.1, 84.3)	89.4 (68.6, 95.1)	**0.01**	75.6 (62.9, 89.0)	77.1 (66.6, −90.0)	0.18	10.80 (0.60, 20.73)	**0.04**
Volitional invasive RMS, cm H_2O								
Sniff Pdi	83.0 (75.1, 90.8)	100.1 (81.4, 113.2)	**0.02**	96.3 (73.2, 127.0)	102.0 (76.6, 132.4)	0.19	12.26 (−0.93, 25.46)	0.92
Sniff Pes	−57.0 (−72.3, −49.0)	−79.5 (−105.5, −60.0)	**0.03**	−68.5 (−82.6, −42.5)	−67.7 (−90.4, −50.6)	0.35	−15.79 (−35.21, 3.63)	0.51
Mueller Pdi	85.7 (57.4, 98.2)	96.0 (69.5, 123.2)	0.88	64.0 (23.2, 100.7)	104.4 (44.4, 114.2)	0.12	−20.38 (−70.84, 30.09)	0.26
Mueller Pes	−58.0 (−73.6, −46.2)	−80.2 (−102.3, −65.6)	0.09	−52.0 (−78.6, −38.0)	−95.7 (−107.1, −48.9)	0.06	4.06 (−31.67, 39.79)	0.50
Valsalva Pgas	187.0 (100.5, 260.3)	230.0 (157.5, 309.6)	0.10	101.2 (72.9, 155.2)	116.0 (108.0, 226.4)	0.07	22.00 (−41.59, 85.60)	0.76
Cough Pgas	183.6 (145.0, 217.9)	248.9 (180.2, 325.5)	**0.05**	172.7 (135.6, 249.4)	184.0 (135.7, 274.0)	0.10	47.44 (−11.53, 106.41)	0.67
Neural control								
DVAI, %	78.6 (62.9, 91.5)	89.1 (74.8, 93.5)	**0.03**	60.7 (30.4, 91.5)	71.4 (53.1, 82.7)	0.42	3.03 (−17.37, 23.44)	0.56
Endurance testing								
Resistance set, cm H_2O	50.0 (45.0, 50.0)	70.0 (60.0, 72.5)	**<0.0001**	10.0 (10.0, 11.0)	10.0 (10.0, 11.0)	0.35	18.42 (15.19, 21.64)	**0.03**
Time to task failure, s	365.0 (284.0, 700.5)	983.0 (551.0, 1,494.0)	**0.05**	285.6 (279.8, 290.0)	573.5 (474.0, 611.0)	0.58	783.61 (33.70, 1,533.01)	**0.05**
6MWD, m	420.0 (385.0, 570.0)	465.0 (427.5, 585.0)	0.99	410.0 (290.0, 472.5)	367.5 (292.5, 570.0)	0.37	−30.12 (−56.20, 2.51)	0.73
6MWD, % predicted value	89.2 (78.0, 103.2)	88.0 (68.3, 111.4)	0.96	77.9 (54.6, 93.8)	74.2 (63.1, 97.5)	0.24	−32.2 (−109.9, 45.41)	0.39

Definition of abbreviations: 6MWD = 6-minute walk distance; CI = confidence interval; CMS = cervical magnetic stimulation (of the phrenic nerve roots); CRQ = Chronic Respiratory Questionnaire dyspnea domain; DVAI = diaphragm voluntary activation index; IMT = inspiratory muscle training; MCR = maximum contraction rate; MFIS = Modified Fatigue Severity Index; MRR = maximum relaxation rate; RMS = respiratory muscle strength; twPdi = twitch transdiaphragmatic pressure; twPes = twitch esophageal pressure; twPgas = twitch gastric pressure.

Values are presented as median (interquartile range). Before IMT, none of the variables shown differed significantly between individuals randomized to the IMT or the sham control group (all *P*s > 0.05). *P* values below 0.05 are highlighted bold.

(75–91) to 100 (81–113) cm H_2O, $P = 0.02$; DVAI of 79% (63–92) to 89% (75–94), $P = 0.03$); and time to task failure of 365 (284–701) to 983 (551–1,494) seconds, $P = 0.05$; increased significantly from before IMT to the end of IMT in the treatment arm but not in the sham arm: maximal sniff nasal pressure, 71 (62–78) to 75 (64–81) cm H_2O, $P = 0.18$; sniff transdiaphragmatic pressure, 96 (73–127) to 102 (77–132) cm H_2O, $P = 0.19$; DVAI, 61% (30–92) to 71% (53–83), $P = 0.42$; and time to task failure, 286% (280–290) to 573.5% (474–610), $P = 0.58$ (Figure 3 and Table 2). No significant changes in twPdi were seen in either the treatment or the sham arm (Figure 3 and Table 2). Results were maintained through to 6 weeks after the end of IMT (Table E5).

A decrease in twPdi amplitude toward the end of the endurance test provided evidence for the induction of fatigue during the inspiratory muscle fatigue protocol (*see* Table E6). Overall time to task failure improved in the treatment arm but not in the sham arm (Figure 3 and Table 2). EMG data showed no systematic change in the pattern of diaphragmatic and extradiaphragmatic muscle activation during the acute fatigue protocol or after 6 weeks of IMT (Table E6). There was no change in diaphragm thickening ratio or intercostal thickening ratio during IMT in either the treatment or sham arm (*see* Table E7).

Discussion

The present work shows for the first time, to the best of our knowledge, that diaphragm muscle weakness persists at a mean of 28 months after hospitalization for COVID-19, that this weakness (along with decreased diaphragm cortical activation) was potentially associated with exertional dyspnea, and that IMT improved dyspnea on exertion and its probable pathophysiological correlate (i.e., diaphragm/inspiratory muscle weakness) in individuals with long COVID. These improvements persisted for 6 weeks after the end of therapy. The effects of IMT on global inspiratory muscle strength (as reflected by SNIP), potentially improved diaphragm voluntary activation (as reflected by the DVAI), and greater diaphragm endurance (as reflected by improved diaphragm muscle endurance time, load, and energy, and as seen using a sophisticated inspiratory muscle and diaphragm fatigue protocol in our lab before and after IMT) might underlie the improvement of exertional dyspnea.

Recent data suggest that the symptoms of long COVID persist for at least 12 to 18 months (28, 29). Our study also documented persistent diaphragm muscle weakness in individuals with long COVID, and this was still present a median of 31 months after acute COVID-19 illness. This extends the findings of our previous study showing diaphragm muscle weakness in people with long COVID at 15 months after hospitalization for acute illness (7). Persistence of symptoms is clinically relevant, because we have also clearly shown that diaphragm muscle weakness might be associated with dyspnea, which is one of the major symptoms of long COVID (7).

A novel finding of the present study is that we also showed that inspiratory muscle fatigability and decreased cortical diaphragm voluntary activation are further potential underlying mechanisms for exertional dyspnea in individuals with long COVID. This is important for three reasons. First, it now appears that there is not much natural recovery of diaphragm muscle weakness–related dyspnea over time in people with long COVID. Some measures of diaphragm muscle strength and its cortical activation improved slightly over time, but not consistently, and therefore did little to improve dyspnea. Second, the possible role of diaphragm muscle weakness in contributing to exertional dyspnea is now clearer on the basis of the finding of a potential association between exertional dyspnea and diaphragm muscle weakness and the detection of a possible cortical contribution to impaired inspiratory muscle strength and endurance. Third, standard lung function tests are not a suitable approach for detecting exertional dyspnea related to diaphragm and other inspiratory muscle weakness, because they do not predict inspiratory muscle behavior on exertion. These factors provided the rationale for the randomized controlled trial component of this study, which investigated the impact of IMT on persistent diaphragm/inspiratory muscle weakness and fatigability-related exertional dyspnea in individuals with long COVID.

Given that IMT therapy had a positive impact on exertional dyspnea in the present study, and the fact that adherence to IMT therapy was good, it is possible that IMT could be used successfully in clinical practice if a strict protocol with careful supervision and training adjustments is used. It is possible that the gradual increases in inspiratory resistance over time in individuals who underwent IMT were associated with gradual increases in inspiratory muscle strength, resulting in the significant improvements in global inspiratory muscle strength and exercise endurance seen, compared with the individuals in the sham control group, as demonstrated mechanistically in a previous study (10).

To the best of our knowledge, this is the first study to use a set of established techniques to determine diaphragm and global inspiratory muscle strength, function, control, and endurance after IMT in people with long COVID. As a result, our data showed that IMT possibly improved diaphragm and extradiaphragmatic (indicated by changes in sniff Pdi and sniff Pes) muscle strength, endurance, and cortical control without significantly impacting static resting variables such as twPdi. Although twPdi represents the established standard for diagnosing diaphragm muscle weakness, it is not a dynamic metric that would reflect the performance of the diaphragm and other inspiratory muscles on exertion and thus mirror improvements after IMT. It is, therefore, likely that the sophisticated inspiratory muscle strength training protocol used in the present study was associated with improvements in inspiratory muscle performance that are in line with the improvements in exertional dyspnea. This is consistent with animal data showing that IMT induces a fiber twitch that likely increases diaphragm and overall inspiratory muscle endurance over time without necessarily impacting diaphragm thickness or properties (e.g., twPdi at rest) (30). It is also important to note that animal data indicate that use of too nonintermittent loading for too long can lead to diaphragm injury (30). This highlights the appropriateness of our experimental protocol for IMT, whereby resistances in the treatment arm were dynamically increased each week on the basis of perceived respiratory effort and actual diaphragm strength. This approach appears optimal because it allowed improvements in exertional dyspnea and the endurance of the inspiratory muscles to be achieved. The

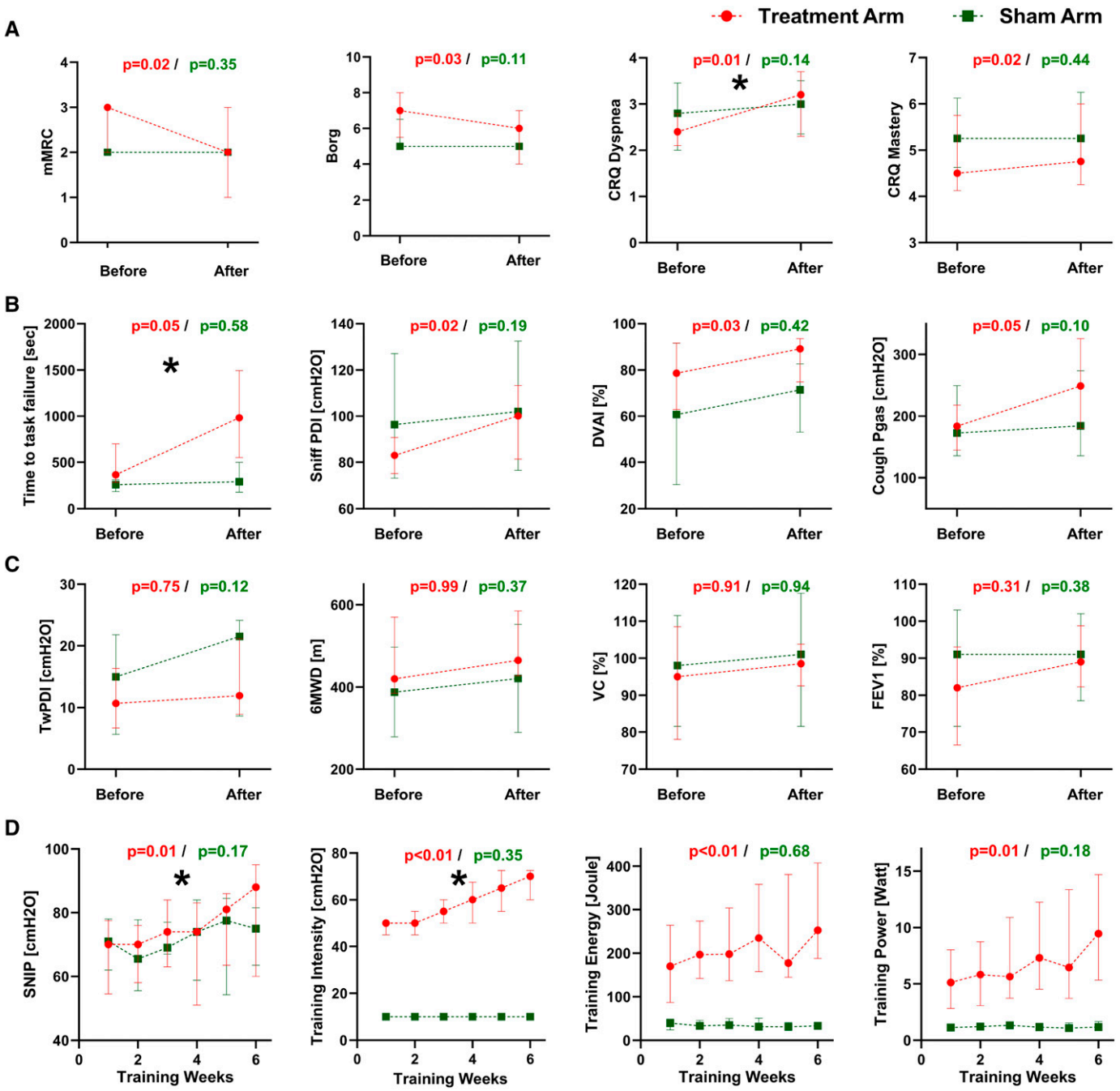

Figure 3. Key study findings in the treatment (red) and sham (green) arms. *Significant between-groups differences ($P < 0.05$). (*A*) The impact of inspiratory muscle training (IMT) on exertional dyspnea. (*B* and *C*) Effects of IMT on endurance time, volitional and nonvolitional respiratory muscle parameters, 6MWD, and pulmonary function parameters. (*D*) Change in sniff nasal pressure and training parameters during 6 weeks of IMT. 6MWD = 6-minute walk distance; Borg = Borg dyspnea scale score; CRQ Dyspnea = Chronic Respiratory Questionnaire dyspnea domain score; DVAI = diaphragm voluntary activation index; mMRC = modified Medical Research Council dyspnea scale score; Pdi = diaphragmatic pressure; Pgas = gastric pressure; SNIP = sniff nasal inspiratory pressure; tw = twitch; VC = vital capacity.

reduction in exertional dyspnea and improvements in SNIP and sniff transdiaphragmatic pressure during IMT in the present study are consistent with previous data obtained in studies of IMT in people without COVID (10, 31, 32).

Appropriate improvement in inspiratory muscle endurance during IMT in this study is also supported by the fact that extradiaphragmatic respiratory muscles were not overrecruited, either before or after 6 weeks of IMT, because such

overrecruitment would have been indicative of training at a resistance that was too high, with the risk of inspiratory muscle injury as previously reported (33).

Our study also is the first to show that inspiratory muscle endurance and strength after IMT in individuals with long COVID is probably the result not only of adaptation at the muscular level of the diaphragm and potentially other inspiratory muscles but also of changes in the cortical excitability of the diaphragm. These central neural adaptions to training that likely contribute to an increase in maximal muscle force output have been increasingly described for other muscles (34). In addition to showing that DVAI increased after 6 weeks of IMT in the IMT arm (supporting the contribution of neural adaptation to overall improved muscle force output of the diaphragm, and probably other inspiratory muscles, and the observed improvements after IMT), this study also found another potential link between the DVAI and the presence/severity of exertional dyspnea. Therefore, to the best of our knowledge, we have shown for the first time that the potential mechanism underlying exertional dyspnea related to diaphragm muscle weakness might have a cortical component as well as a peripheral component. IMT has activity that targets both of these mechanisms to improve inspiratory muscle endurance and their cortical drive, which, in turn, might reduce exertional dyspnea. Nevertheless, it must be emphasized that improvements in inspiratory muscle endurance after IMT may also be related not only to improvements in diaphragm muscle strength but also to improvements in other inspiratory muscles, because sniff Pdi and sniff Pes are also influenced by inspiratory muscles other than the diaphragm, and these parameters showed significant improvement after IMT in the present trial.

In the present study, the beneficial effects of IMT not only were seen during therapy but also persisted for at least 6 weeks after the end of IMT. These results suggest that the possible positive effects of IMT on exertional dyspnea in individuals with long COVID may be durable without the need for continued training. Additional studies are needed to determine how long after IMT these beneficial effects persist.

Key strengths of our study include the comprehensive methodology applied, the use of IMT in long COVID and comparison with a sham control group, and the novel findings regarding exertional dyspnea and diaphragm muscle weakness. However, there are also some limitations. First, the small sample size reflects the difficulty in recruiting participants with exertional dyspnea for long-term follow-up and to undertake demanding and extensive physiological testing over several hours on multiple occasions. Nevertheless, the sample size was sufficient to demonstrate a significant between-groups difference in the primary outcome and to provide information on the potential pathophysiological basis of how diaphragm muscle weakness mediates exertional dyspnea and why IMT improved dyspnea on exertion in the training arm. Specifically, statistically, it should be reiterated that our study was powered to detect intraindividual differences in the study parameters and not intergroup differences. However, even interindividual changes (i.e., change in the treatment group vs. change in the control group after IMT) in CRQ dyspnea domain score, SNIP, and endurance time (Table 2) were statistically significant, (further) indicating that IMT potentially improves exertional dyspnea and inspiratory muscle fatigability.

Additionally, we cannot exclude the possibility that the strength of the other accessory inspiratory muscles might have also improved during IMT (and, therefore, contributed to the improvement in exertional dyspnea after IMT), but there is no current gold-standard technique for determining the strength of accessory inspiratory muscles and their performance on exertion. As stated earlier, our data indicate that improvements in inspiratory muscle endurance after IMT may be related not only to improvements in diaphragm strength but also to improvements in the strength of the extradiaphragmatic inspiratory muscles, as pressures generated by compound effort of global inspiratory muscles, such as sniff Pdi and sniff Pes, showed significant improvement after IMT in our study.

Second, the selection of a study population without any underlying cardiac or pulmonary disease (to eliminate potential confounders) means that the current data cannot be extrapolated to individuals with long COVID who have other causes of exertional dyspnea or do not have diaphragm muscle weakness. Finally, the largely open-label nature of the IMT study means that the possibility of unknown biases cannot be excluded.

Conclusions

Diaphragm muscle weakness persisted at a median of 31 months after hospitalization for COVID-19 and, together with potentially decreased cortical activation of the diaphragm, may be a potential underlying mechanism for exertional dyspnea in individuals with long COVID. For the first time, to our knowledge, IMT was found to improve global inspiratory muscle strength, potentially diaphragm voluntary activation, and inspiratory muscle endurance in the IMT group, and these improvements were possibly associated with an improvement in exertional dyspnea that persisted for 6 weeks after the completion of IMT. Overall, IMT could be a potential therapy for persistent exertional dyspnea that is related to diaphragm muscle weakness in people with long COVID. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: The authors thank all the individuals with COVID-19 whose cooperation made this study possible. The authors acknowledge the help of Ms. Faniry Ratsimba in analyzing patient-related data. English-language editing assistance was provided by Nicola Ryan, an independent medical writer. Thanks also to Dr. Gerold Kierstein (AD Instruments) for help in performing an analysis of twitch transdiaphragmatic pressure gradients after cervical stimulation of the phrenic nerve roots.

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