Potential Diaphragm Muscle Weakness-related Dyspnea Persists Two Years after COVID-19 and Could Be Improved by Inspiratory Muscle Training: Results of an Observational and an Interventional Trial

Short title: Inspiratory muscle training in long COVID

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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.

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. The current 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.

This article has an online data supplement, which is accessible at the Supplements tab.

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ABSTRACT

Rationale: Diaphragm muscle weakness might underly persistent exertional dyspnea despite normal lung/cardiac function in individuals previously hospitalized for acute COVID-19 illness.

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

Methods: ~2 years after hospitalization for COVID-19, 30 individuals (11 female, median age 58 [interquartile range (IQR) 51–63] years) 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.

Results: At median 31 [IQR 23-32] months after hospitalization, 21/30 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 [IQR 75–91] vs. 100 [IQR 81–113] cmH_2O; p=0.02), inspiratory muscle fatiguability (time to task failure 365 [IQR 284–701] vs. 983 [IQR 551–1494] sec; 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.

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Conclusions: 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.

Trial registration: ClinicalTrials.gov Identifier: NCT04854863, NCT05582642

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

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Introduction

It has been now more than three years since the beginning of the coronavirus disease 2019 (COVID-19) pandemic (1). Therefore, a substantial proportion of the population in many countries has recovered from severe acute respiratory syndrome coronavirus type 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 new 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 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).

In the opinion of the authors, there are therefore three important points to note and/or address. Firstly, the persistence of diaphragm muscle weakness with potentially related exertional dyspnea needs to be determined over longer time periods. Secondly, diaphragm muscle weakness possibly mediating exertional dyspnea after COVID-19 can only be confirmed using an exercise protocol and by comparing post-COVID individuals with exertional dyspnea and diaphragm impairment with those without exertional dyspnea or diaphragm

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dysfunction. Thirdly, although inspiratory muscle strength 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 also facilitate understanding of the mechanisms underlying any favorable effects of IMT on dyspnea in this setting.

The aims of this study were to (1) examine the persistence of respiratory muscle weakness and exertional dyspnea in patients hospitalized with acute COVID-19 illness two years previously, and (2) 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 (NCT04854863) included individuals hospitalized at RWTH University Hospital Aachen (Aachen, Germany) between February 2020 and April 2021 for the management of COVID-19 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 at approximately ~2 years after discharge (data that is reported in the present manuscript).

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A subset of the above individuals then participated in a prospective, open-label, sham-controlled randomized trial of IMT (NCT05582642) to determine the potential benefit of IMT on exertional dyspnea related to diaphragm weakness.

Both study protocols received ethical approval (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, required supplemental oxygen therapy and/or invasive mechanical ventilation, and some had persistent exertional dyspnea with diaphragm muscle weakness despite normal lung/cardiac function (7).

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Individuals with a marker of diaphragm muscle weakness (defined as twitch transdiaphragmatic pressure [twPdi] below the lower limit of normal [<16 cmH_2O] (12) or Sniff nasal pressure <60% of predicted (13) and exertional dyspnea (Borg Dyspnea Scale score ≥2) after a 6-minute walk test [6MWT]) at 2-year follow-up were recruited for the randomized trial from October 2022 to May 2023 (**Figure 1**). Individuals with comorbidities 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 Power breath KH2P device (Powerbreath, Oxford, UK) (14); a strength protocol was used in the treatment arm and a~~n~~ 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

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through the mouth while expanding the chest to achieve a full vital capacity. Full details of the IMT resistance settings are provided in the online data supplement.

Outcomes

2-Year Follow-up Study

The primary outcome for the evaluation of diaphragm muscle weakness over time (from median ~14 [interquartile range (IQR) 13; 19] months to ~31 [IQR 23; 32] months after initial hospitalization) was diaphragm muscle strength, expressed as twitch transdiaphragmatic pressure.

Inspiratory Muscle Training 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

Pre-training 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 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

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diaphragm (12, 17). The diaphragmatic voluntary activation index was calculated using the formula (18):

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

Further details are provided in the online data supplement.

Participants completed clinical questionnaires to determine dyspnea on exertion (modified Medical Research Council [mMRC] dyspnea scale, Borg dyspnea scale (19), clinical respiratory questionnaire (20)) and fatigue (modified Fatigue Severity Index [MFIS] (21)). Borg dyspnea scale scores were also determined before and after a 6-minute walk test without supplemental oxygen (22, 23). Exertional dyspnea severity during the 6-minute walk test was classified as mild/none (Borg dyspnea scale score 0–1), moderate (Borg score 2–5), or severe (Borg score ≥6) (7).

Whole-body plethysmography was performed according to current guidelines (22, 23) before and after bronchodilation (diffusion capacity of carbon monoxide 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 (VAS; 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 breath continuously through the IMT device until task failure, and twitch transdiaphragmatic pressure and the diaphragm voluntary activation index (DVAI) were determined every two minutes. Surface electrodes were used to continuously obtain electromyographic activity of the (left and right) diaphragm,

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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 side (**Figure 2**), both as previously described (26, 27). The parasternal intercostal thickening ratio (ITR) was calculated as thickness at total lung capacity divided by thickness at functional residual capacity (**Figure 2**) (27).

Inspiratory Muscle Training 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 above-mentioned clinical questionnaires, pulmonary function testing with respiratory muscle assessment including invasive volitional and non-volitional tests, inspiratory muscle fatigue protocol, and ultrasound and electromyography of the respiratory muscles. All raw data analysis and statistical assessments were performed by investigators unaware of participant characteristics and treatment group allocation, other members of the team including the principal investigators were aware of the randomization.

Statistical Analysis

Details of the sample size calculation are provided in the online data supplement. Statistical analyses were performed using Sigma Plot™ software (Version 13.0, Systat, Erkrath, Germany). Data are expressed as median values with interquartile range (IQR). The Shapiro–Wilk test was used to determine normality of distribution. For between-group comparisons, unpaired t-tests were used for normally distributed data and Mann–Whitney U test for non-normally distributed data. For within-group comparisons, paired t-tests were used for normally distributed data and the Kruskal-Wallis test for non-normally distributed data, with

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Tukey and Bonferroni post-hoc tests, respectively, for significant differences. $P \leq 0.05$ was considered statistically significant.

Results

Study Populations

2-Year Follow-up Study based on which the inspiratory muscle training study was performed

Thirty individuals were analyzed at a median of 31 [IQR 23; 32] months after hospitalization, of whom 21 reported relevant persistent dyspnea on exertion (Borg Dyspnea Scale score of ≥2 after a 6MWT) (**Table S2, Figure S1**). 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 S1**). The majority (90%) were able to return to their previous daily activities or work.

Inspiratory Muscle Training 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 male, median age 59 [IQR 52; 74] years) who had persistent exertional dyspnea (i.e. Borg Dyspnea Scale score ≥2 after a 6MWT) and markers of diaphragm muscle weakness (i.e. twPdi below the lower limit of normal [<16 cmH_2O] (12) or Sniff nasal pressure <60% of predicted) were included in the trial of IMT (9 per treatment arm) (**Figure 1**). At randomization, clinical characteristics were comparable between the IMT and sham control groups (**Table 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 (**Table S1**).

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Assessments and Outcomes in the 2 Year Follow-up study and the Inspiratory Muscle Training study

2-Year Follow-up Study

Phrenic nerve stimulation and inspiratory muscle strength

No significant change in twitch diaphragmatic pressure was seen from 14 [IQR 13; 19] months to 31 [IQR 23; 32] months after hospitalization for acute COVID-19 illness (*P*=0.08) (**Table S2, Figure S1**). There were significant subgroup differences in both twitch diaphragmatic pressure and sniff nasal pressure based on the severity of dyspnea on exertion (*P-ANOVA*=0.05 and <0.01 respectively) (**Table S2, Figure S1**). Sniff transdiaphragmatic and cough gastric pressure improved significantly over time (*P*=0.003 and *P*=0.01 respectively) (**Table S2, Figure S1**). In addition, the DVAI improved significantly over the time (*P*<0.001) and improvements were related to the severity of dyspnea on exertion (**Table S2, Figure S1**).

No significance differences or trends were observed in extra-diaphragmatic respiratory muscle ultrasound and electromyography findings across exertional dyspnea severity subgroups **(Table S3)**. The resistance set to perform an endurance test based on perceived dyspnea (Borg scale 4-6) differed significantly based on exertional dyspnea severity **(Table S2).**

Inspiratory Muscle Training 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 pre-training to the

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end of training (**Table 2, Figure 3, Table S4**). No harms or unintended effects occurred in either group.

Inspiratory muscle training study: Exertional Dyspnea

After training, the IMT treatment group showed significant improvements in median [IQR[Borg dyspnea scale score (7.0 [5.5; 8.0] to 6.0 [4.0; 7.0]; *P*=0.03), mMRC scale score (3.0 [2.0; 3.0] to 2.0 [1.0; 3.0]; *P*=0.02) and the Chronic Respiratory Questionnaire dyspnea domain score (2.4 [2.1; 2.8] to 3.2 [2.3; 3.7]; *P*=0.01); no changes were seen in the sham group (**Table 2, Figure 3**). Improvements in the treatment arm persisted until 6 weeks after the end of IMT (**Table S5**).

Inspiratory muscle training study: Phrenic nerve stimulation and inspiratory muscle strength

Median [IQR] maximal sniff nasal pressure (70 [55; 78] to 88 [60; 95] cmH_2O; *P*=0.02), sniff transdiaphragmatic pressure (83 [75; 91] to 100 [81; 113] cmH_2O; *P*=0.02), DVAI (79 [63; 92] to 89 [75; 94] %; *P*=0.03), and time to task failure (365 [284; 701] to 983 [551; 1494] sec; *P*=0.05) increased significantly from the 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] cmH_2O; *P*=0.18, sniff transdiaphragmatic pressure 96 [73; 127] to 102 [77; 132] cmH_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) (**Table 2, Figure 3**). No significant changes in twitch transdiaphragmatic pressure were seen in either the treatment or sham arm (**Table 2, Figure 3**). Results were maintained through to 6 weeks after the end of IMT (**Table S5**).

A decrease in twitch diaphragmatic pressure amplitude towards the end of the endurance test provided evidence for the induction of fatigue during the inspiratory muscle fatigue protocol (**Table S6**). Overall time to task failure improved in the treatment arm but not the sham arm (**Table 2, Figure 3**). EMG data showed no systematic change in the pattern of diaphragmatic

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and extra-diaphragmatic muscle activation during the acute fatigue protocol or after 6 weeks of IMT (**Table S6**). There was no change in diaphragm thickening ratio (DTR) or ITR during IMT in either the treatment or sham arm (**Table S7**).

Discussion

The present work shows for the first time 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 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 current study is that we also showed that inspiratory muscle fatiguability and decreased cortical diaphragm voluntary activation are further potential

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underlying mechanisms for exertional dyspnea in individuals with long COVID. This is important for three reasons. Firstly, 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. Secondly, the possible role of diaphragm muscle weakness in contributing to exertional dyspnea is now clearer based on the finding of potential association between exertional dyspnea and diaphragm muscle weakness and the detection of a possible cortical contribution to impaired inspiratory muscle strength and endurance. Thirdly, 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 fatiguability-related exertional dyspnea in individuals with long COVID.

Given that IMT therapy had a positive impact on exertional dyspnea in the current 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 was 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 extra-diaphragmatic (indicated by changes in Sniff Pdi and Sniff

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Pes) muscle strength, endurance, and cortical control without significantly impacting on static resting variables like twitch diaphragmatic pressure. While twitch diaphragmatic pressure 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 on diaphragm thickness or properties (such as twitch diaphragmatic pressure at rest) (30). It is also important to note that animal data indicate that use of too non-intermittent 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 based on 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 reduction in exertional dyspnea and improvements in SNIP and sniff transdiaphragmatic pressure during IMT in the current 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 extra-diaphragmatic respiratory muscles were not over recruited, either before or after 6 weeks of IMT, because such over-recruitment 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

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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 also 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, 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 current study, the beneficial effects of IMT were seen not only 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. Firstly, the small sample size reflects the difficulty in recruiting participants with

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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-group 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 intra-individual differences in the study parameters and not inter-group differences. However, even inter-individual changes (i.e. change in the treatment group vs. change in the control group following 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 fatiguability.

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 determing the strength of accessory inspiratory muscles and their performance on exertion. As stated above, our data indicates 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 extra-diaphragmatic 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.

Secondly, 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.

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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. IMT was found to improve global inspiratory muscle strength, potentially diaphragm voluntary activation, and inspiratory muscle endurance in the IMT-group for the first time, 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.

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Responsibility for data

JS assumes responsibility for the accuracy and completeness of the analysis and for the fidelity of this report.

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collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. Internal funding was provided by the RWTH Aachen Faculty of Medicine (START Grant supporting the junior research group around PD Dr. Dr. Jens Spiesshoefer). 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.

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FIGURE LEGENDS

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 TH10 is shown for assessment of inspiratory and expiratory muscle strength, respectively. **B**: Curves during different voluntary and non-voluntary maneuvers. Readings from esophageal (Pes), gastric (Pgas) pressure sensors and calculated Pdi are shown. Representative twitch pressure recording following a 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 [tw] Pdi 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 maximum velocity of muscle relaxation. Both MRC and MRR were adjusted for twPdi. CMS twitches 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. **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 and parasternal intercostal muscle ultrasound in inspiration (top row) and expiration (bottom row).

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Figure 3. Key study findings in the treatment (red) and sham (green) arms. *Indicates significant between-group-differences ($p<0.05$).

A: The impact of inspiratory muscle training (IMT) on exertional dyspnea. **B & C:** Effects of IMT on endurance time, volitional and non-volitional respiratory muscle parameters, six-minute walk distance, and pulmonary function parameters. **D:** Change in sniff nasal pressure and training parameters during 6 weeks of IMT.

6MWD, six-minute walk distance; Borg, Borg dyspnea scale score; CRQ Dyspnea, Chronic Respiratory Questionnaire dyspnea score; DVAI, diaphragm voluntary activation index; FEV_1, forced expiratory volume in 1 second; mMRC, modified Medical Research Council dyspnea scale score; Pdi, diaphragmatic pressure; Pgas, gastric pressure; SNIP, sniff nasal inspiratory pressure; Tw, twitch; VC, vital capacity.

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TABLES

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.

	Total patients followed up (n = 30)	**Intervention group**			***P*-value**
		Total (n = 18)	**Treatment-arm (n = 9)**	**Sham arm (n = 9)**	
Male sex, n (%)	19 (63)	11 (61)	6 (67)	5 (55)	0.65
Age, years	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
Post-discharge time, months	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	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	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	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, days	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					
Sniff nasal pressure, cmH_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	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, cmH_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

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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					
Time to task failure, sec	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	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

Values are median (interquartile range), or number of patients (%).

COPD, chronic obstructive pulmonary disease; ICU, intensive care unit; CMS, cervical magnetic stimulation (of the phrenic nerve roots); DTR, diaphragm thickening ratio; LLN, lower limit of normal; 6MWD, Six-minute walk distance.

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Table 2. Respiratory questionnaires, respiratory muscle strength, and endurance testing in both groups before and after IMT intervention.

	Treatment arm			Sham arm				
	Before training	**After training**	***P*-value**	**Before training**	**After training**	***P*-value**	**Mean between-group difference (95% CI)**	***P*-value for between-group difference**
Dyspnea								
mMRC dyspnea scale score	3.0 (2.0; 3.0)	2.0 (1.0; 3.0)	**0.02**	2.0 (2; 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
Non-volitional invasive RMS								
CMS twPDI, cmH_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, cmH_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, cmH_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, cmH_2O/msec	-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

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CMS MCR normalized, cmH_2O/msec	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, cmH_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, cmH_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	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								
Sniff Pdi, cmH_2O	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, cmH_2O	-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, cmH_2O	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, cmH_2O	-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, cmH_2O	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, cmH_2O	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, cmH_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, sec	365.0 (284.0; 700.5)	983.0 (551.0; 1494.0)	**0.05**	285.6 (279.8; 289.2.8)	573.5 (474.0; 610.8)	0.58	783.61 (33.70, 1533.01)	**0.05**
6 MWD, 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	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

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Values are median (interquartile range). Before IMT, none of the variables shown differed significantly between individuals randomized to the IMT or sham control group (all $P>0.05$).

CMS, cervical magnetic stimulation (of the phrenic nerve roots); CRQ, Chronic Respiratory Disease Questionnaire; DVAI, diaphragm voluntary activation index; MCR, maximum contraction rate; MFIS, Modified Fatigue Severity Index; mMRC, Modified Medical Research Council; MRR, maximum relaxation rate; Pdi, transdiaphragmatic pressure; Pes, esophageal pressure; Pgas, gastric pressure; RMS, respiratory muscle strength; RV, residual volume; TH10, tenth thoracic vertebrae; tw, twitch.

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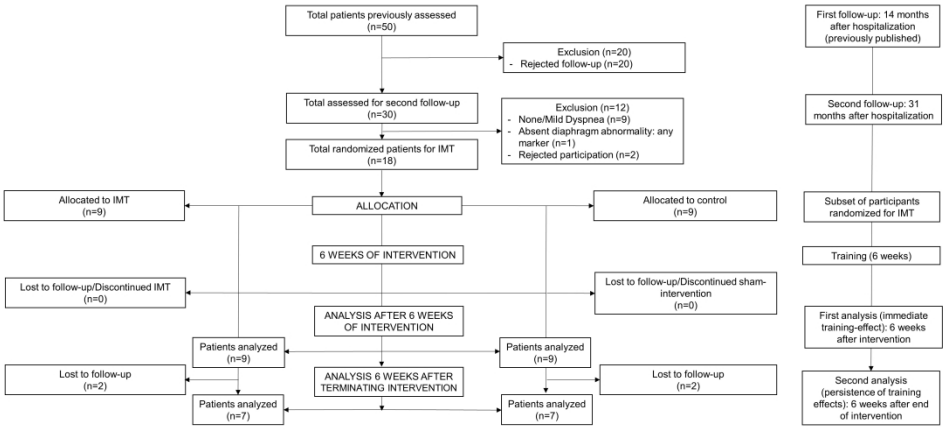

Figure 1. CONSORT flow diagram. IMT, inspiratory muscle training.

430x190mm (300 x 300 DPI)

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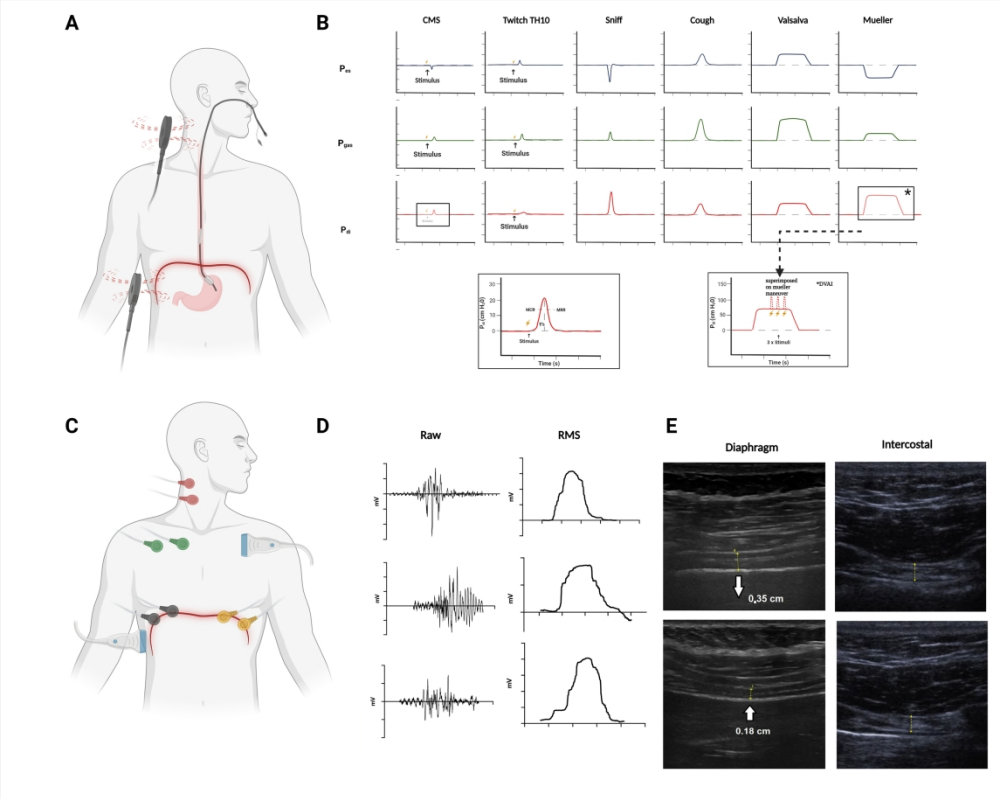

Figure 2. Experimental setup. A: Subject with transnasal placement of double-balloon catheter measuring pressure from esophageal and gastric sensors for the calculation of transdiaphragmatic pressure (Pdi); magnetic coil placement for delivery of cervical magnetic stimulation (CMS) and TH10 is shown for assessment of inspiratory and expiratory muscle strength, respectively. B: Curves during different voluntary and non-voluntary maneuvers. Readings from esophageal (Pes), gastric (Pgas) pressure sensors and calculated Pdi are shown. Representative twitch pressure recording following a 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 [tw] Pdi 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 maximum velocity of muscle relaxation. Both MRC and MRR were adjusted for twPdi. CMS twitches 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. 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 and parasternal intercostal muscle ultrasound in inspiration (top row) and expiration (bottom row).

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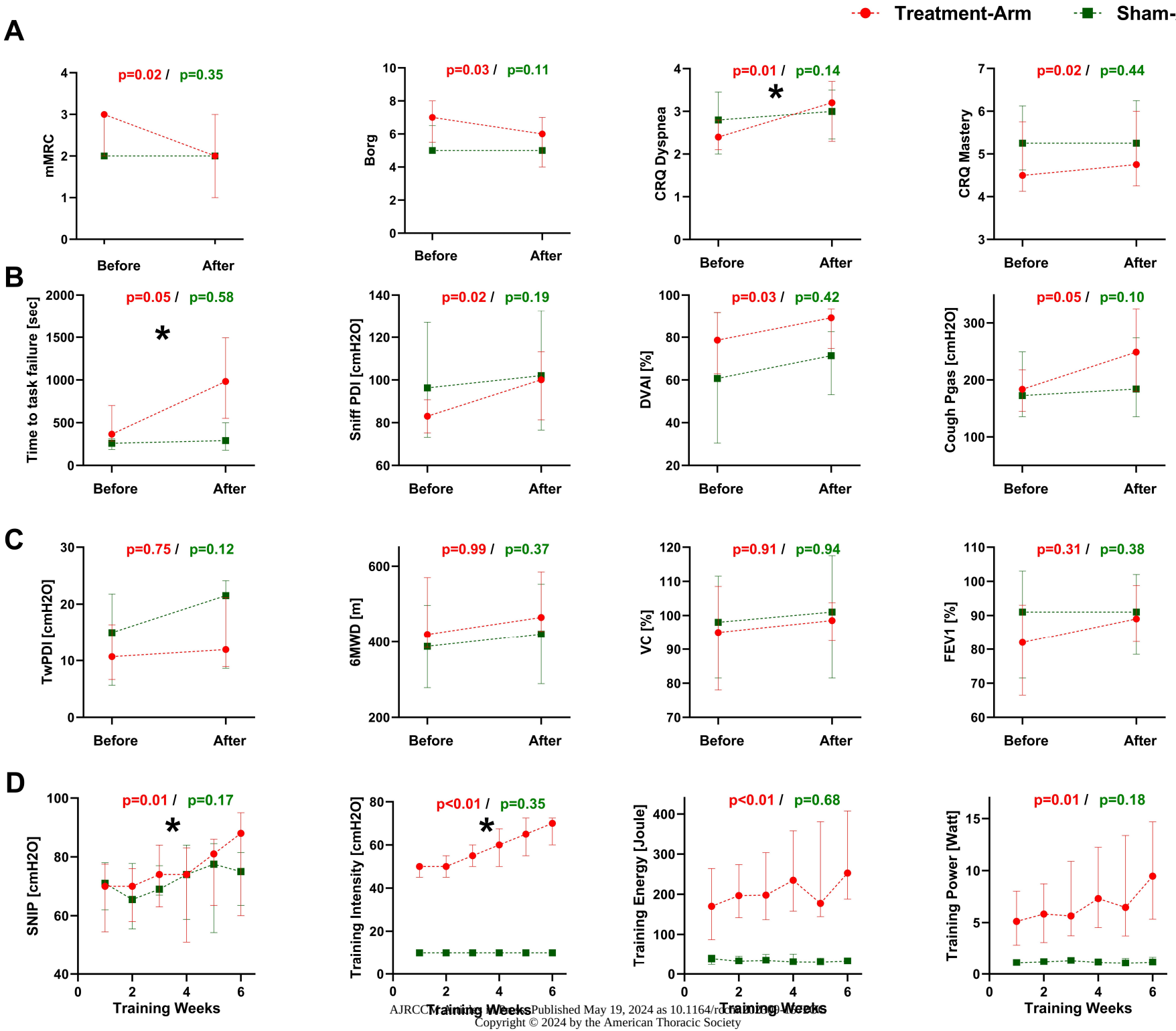

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Potential Diaphragm Muscle Weakness-related Dyspnea Persists Two Years after COVID-19 and Could Be Improved by Inspiratory Muscle Training: Results of an Observational and an Interventional Trial

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ONLINE DATA SUPPLEMENT

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Supplementary Methods

Inspiratory Muscle Training

In the IMT arm, IMT resistance was set at 40–50% of individual sniff nasal inspiratory pressure (SNIP), such that the strength of overcoming five of these breaths was rated 4-7 on a visual analog scale (VAS) from 0 [breathing air without resistance] to 10 [maximum effort required to overcome 100% of individual SNIP for five breaths]). When the VAS score for IMT was ≤4 in the previous week, resistance was increased by 5% and the individual was asked to complete five breaths to ensure that the perceived effort was rated as 4–7 on the VAS; resistance was increased (if needed) until the target VAS score was reached. If the targeted VAS was not reached, a new set of five breaths (with a resistance having been changed by 5%) were applied after a 20-seconds break.

In the sham arm, IMT resistance was set at 10% of the individual maximal SNIP for the duration of IMT. To improve adherence to the training protocol, participants in the sham arm were told that they were pursuing "endurance training". Participants were contacted weekly to determine whether they were having any problems with IMT. At these visits, IMT device data were downloaded (sessions completed, average load of breathing expressed as watt and joule and volume achieved per session) and a print version of the patient diary that included VAS scoring for each session was sighted and saved.

Phrenic Nerve Stimulation Studies

Posterior cervical magnetic stimulation (CMS) was performed with the subject in a seated position. Stimuli were delivered using a MagPro Compact^{TM} magnetic stimulator equipped with a 2 Tesla 12 cm C–100 circular coil (MagVenture, Willich, Germany). For posterior CMS, the coil was placed at C7 and then moved up towards C6 until the highest reproducible twitch transdiaphragmatic pressure (twPdi) was obtained. At least five stimuli were delivered to achieve the highest possible twPdi that showed <10% variation from the preceding two stimulations. Supramaximality of magnetic stimuli (with 0.1 msec duration each and 2.0 Tesla maximum magnetic field output) was achieved by judging the relationship between stimulation intensity and the amplitude of twitch transdiaphragmatic pressure (see **Figure 2** in main manuscript). There was a ≥30-second resting period between twitches. Stimulation at functional residual capacity (FRC) was determined by visual observation of abdominal movements combined with visualization of pressure curves on a large flat screen to reproducibly generate a state of FRC.

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Twitch Superimposition

First, maximum twPdi at FRC and maximum voluntary transdiaphragmatic pressure were determined, the latter by encouraging the subject to perform a maximum inspiratory effort against an occluded airway at FRC. Repetitive increasing stimuli were then deployed during voluntary inspiration (still with the airway occluded). During isovolumetric activation of the diaphragm, twitch interpolation was specifically timed by visual determination of 100% of the individual maximum voluntary transdiaphragmatic pressure (see **Figure 2** in main manuscript). Next, the diaphragmatic voluntary activation index was calculated using the formula (1):

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

The DVAI reflects the percentage of diaphragmatic muscle mass activated by voluntary effort or the extent of diaphragmatic activation during any given inspiratory effort. This parameter has been proposed for assessment of central drive to the diaphragm. For example, if no twPdi could be observed at individual maximum voluntary transdiaphragmatic pressure (i.e. if it was not possible to superimpose a twPdi on individual maximum transdiaphragmatic pressure), then the DVAI would be 100%.

Diaphragm/Parasternal Intercostal Ultrasound

Diaphragm ultrasound was performed on the right hemidiaphragm as previously described (2). Briefly, a portable ultrasound device (LOGIQ S8-XD, GEHealthcare) with a 10-MHz linear transducer was used for evaluation of diaphragm thickness in the zone of apposition. The diaphragm thickening ratio (DTR) was calculated as thickness at total lung capacity (TLC) divided by thickness at FRC (2).

To perform the parasternal intercostal ultrasound, a 10 -MHz linear transducer was placed at second intercostal space around 6-8 cm lateral of the sternal edge, perpendicular to the ventral thorax as previously described (3). Both left and right sides were assessed. The intercostal thickening ratio was calculated as the ratio of parasternal intercostal muscle thickness at TLC divided by thickness at FRC.

Sample Size Calculation

Assuming a two-sided significance level of 0.05 (alpha) and 80% power (beta), a sample size of 8 subjects per group was calculated to allow detection of a 25% difference in diaphragm

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fatiguability (defined as endurance time, relative to baseline) within the IMT treatment group. Nine participants per arm were recruited to account for a potential loss of one patient to follow-up after 6 weeks of IMT.

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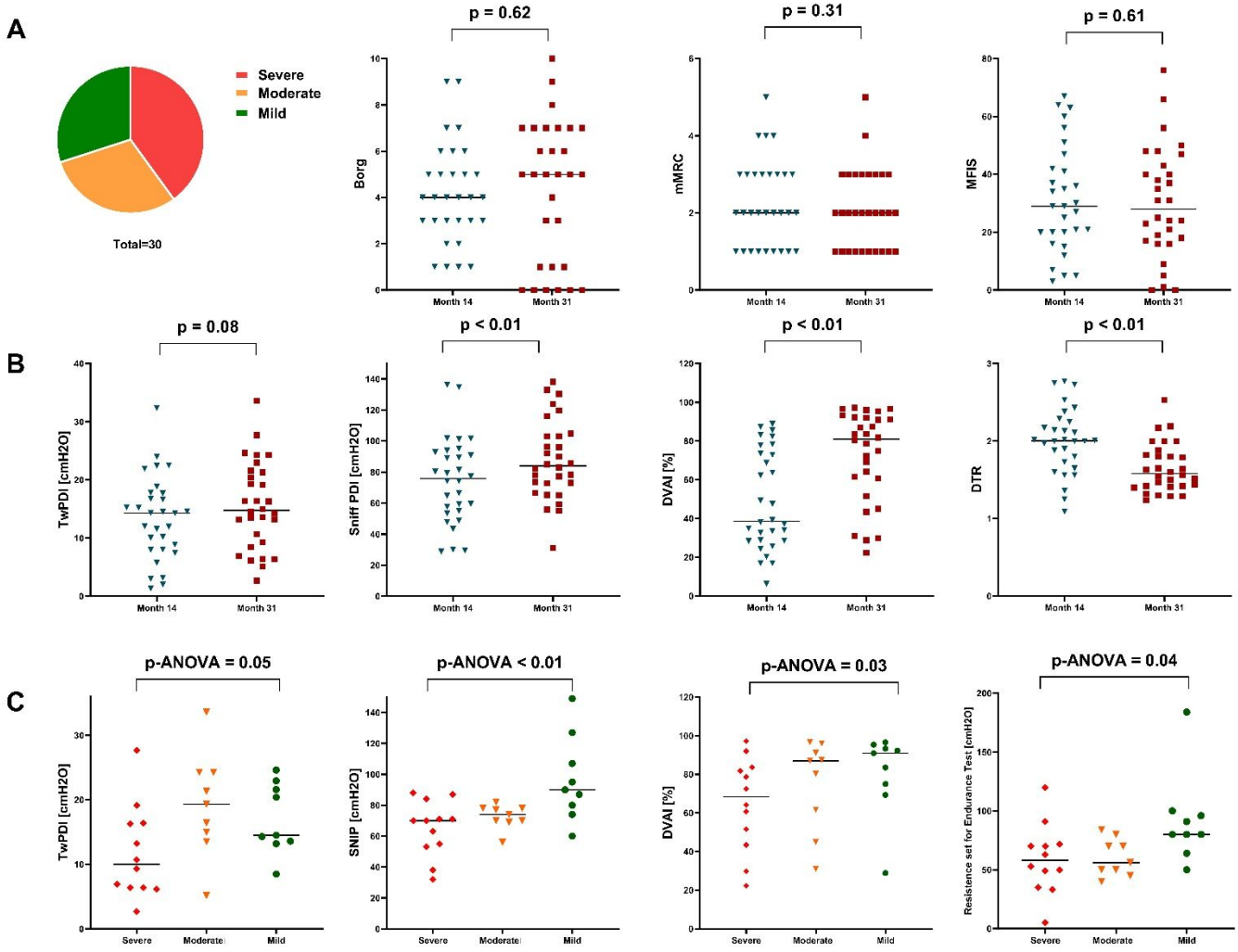
SUPPLEMENTARY FIGURES

Figure S1. A-B: Proportion of individuals with different degrees of exertional dyspnea severity at median 14 months after hospitalization for coronavirus disease (COVID-19) and changes in dyspnea, fatigue and respiratory muscle function over time (first follow-up was at 14 months and the second follow-up was at 31 months). **C:** Differences in different respiratory muscle function variables between subgroups based on the severity of exertional dyspnea at 28 months after hospitalization.

ANOVA, analysis of variance; Borg, Borg dyspnea scale score; DTR, diaphragm thickening ratio; DVAI, diaphragm voluntary activation index;MFIS, modified fatigue impact score; mMRC, modified Medical Research Council dyspnea scale score; SNIP, sniff nasal inspiratory pressure; Tw, twitch; Pdi, diaphragmatic pressure**.**



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SUPPLEMENTARY TABLES

Table S1. Diffusion, capillary blood analysis, echocardiography and laboratory findings for the overall study population, and in patient subgroups based on intervention.

	Total 2-year follow-up group (n=30)	Total IMT study group (n=18)	IMT study treatment arm (n=9)	IMT study sham arm (n=9)	*P*-value	Normal values, if applicable
Diffusion parameters and capillary blood gases						
DLCO, % predicted	69.0 (58.0; 81.0)	67.0 (56.0; 75.0)	71.5 (57.3; 80.0)	63.0 (52.0; 70.0)	0.47	
DLCO/VA, % predicted	89.0 (78.0; 97.0)	83.0 (76.0; 94.0)	92.5 (85.8; 99.3)	79.0 (73.5; 82.0)	0.27	
PaO_2, mmHg	77.6 (70.0; 92.3)	77.2 (71.3; 96.3)	81.7 (72.9; 96.9)	75.9 (69.4; 95.4)	0.96	
$PaCO_2$, mmHg	38.4 (36.2; 39.9)	38.1 (35.1; 39.5)	37.7 (36.5; 39.1)	38.1 (34.2; 39.9)	0.96	
pH	7.4 (7.4; 7.4)	7.4 (7.4; 7.4)	7.4 (7.4; 7.4)	7.4 (7.4; 7.4)	0.92	
Base excess, mmol/L	0.3 (-1.3; 1.9)	0.3 (-1.5; 2.1)	-0.6 (-2.2; 2.1)	0.7 (-0.2; 2.2)	0.70	
Echocardiography						
LVEF, %	55.0 (51.8; 55.8)	55.0 (52.0; 55.0)	55.0 (50.0; 55.0)	55.0 (54.5; 55.0)	0.21	
TAPSE, mm	2.2 (1.9; 2.4)	2.0 (1.8; 2.4)	2.3 (2.0; 2.6)	1.8 (1.7; 2.2)	0.23	
Hema-logy						
White blood cells, 1/nL	6.9 (6.1; 8.4)	6.6 (6.1; 7.9)	6.6 (5.7; 8.6)	6.6 (6.2; 7.4)	0.86	4–10
Hemoglobin, g/dL	14.3 (13.2; 15.9)	14.3 (13.2; 16.0)	14.5 (13.7; 16.2)	13.6 (13.0; 16.0)	0.27	11.2–15.7
Platelets, 1/nL	250.0 (200.0; 273.0)	244.0 (203.5; 264.0)	257.5 (198.3; 272.3)	219.0 (203.5; 246.5)	0.46	150–400
Clinical chemistry						
Bilirubin-direct, mg/dL	0.5 (0.3; 0.7)	0.5 (0.3; 0.7)	0.5 (0.4; 0.6)	0.6 (0.2; 0.8)	0.79	<1.2
ALT, U/L	21.0 (17.0; 29.0)	23.0 (17.5; 40.0)	31.5 (16.3; 47.0)	21.0 (17.5; 24.5)	0.89	<35
Lactate dehydrogenase, U/L	204.0 (181.0; 225.0)	200.0 (168.0; 223.5)	202.0 (180.8; 232.0)	184.0 (147.5; 221.5)	0.14	<250
NT–proBNP, pg/mL	87.9 (32.8; 182.8)	73.7 (32.4; 122.3)	47.8 (31.0; 116.8)	97.9 (34.0; 217.5)	0.22	<486
Creatinine, mg/dL	1.1 (0.9; 1.3)	1.1 (0.8; 1.4)	1.1 (0.8; 1.3)	1.1 (0.9; 1.7)	0.40	<1.0
C-reactive protein, mg/L	1.7 (1.0; 5.3)	1.7 (0.9; 4.3)	1.4 (0.7; 3.1)	1.8 (1.0; 14.4)	0.44	<5

Values are median and first and third quartile.

ALT, alanine aminotransferase; DLCO, diffusing capacity for carbon monoxide; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro B–type natriuretic peptide; $PaCO_2$, partial pressure of carbon dioxide; PaO_2, partial pressure of oxygen; pH, potential of hydrogen; TAPSE, tricuspid annular plane systolic excursion; VA, alveolar volume.

P-values relate to the difference between the treatment and the sham-arm.

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Table S2. Diaphragm muscle strength at a median of 14 and 31 months after hospitalization for coronavirus disease, overall and in patient subgroups based on the severity of dyspnea on exertion at median 31-month follow-up.

	Respiratory muscle strength at follow-up			**Severity of dyspnea at second follow-up (Month 31)**			
	First follow-up (median 14 months)	**Second follow-up (median 31 months)**	***P*-value**	**Severe**	**Moderate**	**Mild**	***P*-ANOVA**
Dyspnea							
mMRC dyspnea scale score	2.0 (1.0; 3.0)	2.0 (1.0; 3.0)	0.31	2.5 (2.0; 3.0)	2.0 (2.0; 3.0)	1.0 (1.0; 1.0)	**<0.001**§‡
Borg dyspnea scale score	4.0 (3.0; 5.3)	5.0 (1.0; 7.0)	0.62	7.0 (6.3; 7.8)	5.0 (3.5; 5.0)	0.0 (0.0; 1.0)	**<0.001**†§‡
MFIS score	29.0 (19.0; 43.3)	28.0 (16.8; 44.0)	0.61	45.0 (32.0; 54.0)	23.0 (12.5; 38.5)	17.0 (3.0; 23.0)	**<0.001**†§
CRQ dyspnea domain score	-	2.8 (2.3; 4.1)	-	2.3 (2.0; 2.6)	3.3 (2.8; 3.8)	6.0 (3.0; 6.5)	**<0.001**§‡
CRQ fatigue domain score	-	4.8 (3.7; 5.6)	-	3.9 (2.8; 4.7)	4.3 (3.4; 5.3)	5.8 (5.4; 5.8)	**<0.001**§‡
CRQ emotional function score	-	5.3 (4.0; 6.0)	-	4.1 (3.5; 5.1)	5.6 (4.1; 6.1)	5.9 (5.4; 6.1)	**0.007**§
CRQ mastery score	-	5.3 (4.5; 6.5)	-	4.5 (4.3; 5.3)	5.8 (4.9; 6.8)	6.3 (5.4; 7.0)	**0.002**†§
Non-volitional invasive RMS							
CMS twPdi, cmH_2O	14.3 (8.0; 17.7)	14.8 (9.1; 21.4)	0.08	10.0 (6.4; 16.4)	19.3 (14.2; 24.3)	14.5 (13.4; 22.3)	**0.05**
CMS twPes, cmH_2O	-5.9 (-8.5; -3.2)	-10.6 (-13.4; -6.6)	**<0.001**	-8.4 (-10.7; -5.6)	-13.5 (-17.4; -11.4)	-10.1 (-14.4; -7.0)	**0.02**†
CMS twPgas, cmH_2O	6.1 (2.7; 9.4)	2.9 (0.7; 8.3)	0.064	2.5 (0.5; 8.1)	1.8 (0.9; 10.1)	7.2 (3.1; 10.2)	0.80
CMS MRR normalized, cmH_2O/msec	-11.0 (-14.6; -7.2)	-14.4 (-21.7; -9.7)	**0.009**	-17.4 (-23.0; -14.3)	-10.4 (-16.7; -8.7)	-10.3 (-30.9; -6.3)	0.55
CMS MCR normalized, cmH_2O/msec	24.0 (19.9; 29.4)	29.5 (23.2; 45.1)	**0.012**	51.6 (25.1; 107.0)	25.6 (20.9; 32.1)	28.2 (21.0; 36.0)	**0.02**†§
TH10 twPgas, cmH_2O	18.1 (10.6; 23.3)	6.8 (3.3; 16.2)	**<0.001**	3.8 (2.3; 9.3)	7.2 (3.7; 19.9)	14.8 (4.3; 18.0)	0.11
Sniff nasal pressure, cmH_2O	-	74.1 (67.7; 86.9)	**-**	70.0 (53.7; 80.9)	74.2 (69.7; 77.7)	90.0 (77.0; 117.0)	**0.003**§‡
Volitional invasive RMS							
Sniff Pdi, cmH_2O	75.8 (54.6; 93.0)	84.0 (71.2; 103.5)	**0.003**	80.6 (68.1; 96.2)	85.6 (73.2; 125.0)	90.0 (65.2; 110.5)	0.43
Sniff Pes, cmH_2O	-59.9 (-77.5; -33.1)	-54.0 (-73.7; -41.0)	0.64	-53.5 (-68.4; -37.3)	-57.0 (-78.3; -51.9)	-44.0 (-73.8; -32.3)	0.43
Mueller Pdi, cmH_2O	62.3 (34.3; 101.5)	75.5 (53.6; 100.0)	0.32	66.8 (29.5; 96.0)	64.0 (45.1; 96.2)	87.7 (59.9; 109.7)	0.45
Mueller Pes, cmH_2O	-47.2 (-69.4; -26.9)	-52.0 (-71.1; -40.6)	0.48	-55.0 (-71.1; -45.7)	-42.9 (-76.2; -25.2)	-54.0 (-73.0; -39.5)	0.81
Valsalva Pgas, cmH_2O	151.8 (87.1; 200.0)	141.1 (101.2; 205.2)	0.41	110.0 (82.6; 238.2)	117.4 (94.2; 185.6)	158.2 (141.1; 211.4)	0.85
Cough Pgas, cmH_2O	158.8 (97.1; 193.4)	173.8 (134.3; 217.0)	**0.01**	177.5 (140.5; 213.3)	162.2 (128.4; 249.4)	200.0 (127.0; 254.4)	0.74
Neural control							
DVAI, %	37.3 (27.1; 73.3)	81.1 (58.4; 92.0)	**<0.001**	68.3 (45.5; 83.2)	86.9 (53.3; 93.6)	90.9 (72.2; 94.3)	**0.03**
Diaphragm ultrasound							
Thickness at TLC, mm	3.8 (3.2; 4.8)	3.1 (2.3; 4.1)	0.51	3.8 (3.1; 4.5)	3.2 (2.6; 4.6)	3.1 (3.1; 4.1)	0.72
Thickness at FRC, mm	1.9 (1.6; 2.2)	2.5 (1.7; 3.1)	**0.005**	2.0 (1.7; 3.0)	1.9 (1.6; 2.9)	2.2 (2.0; 2.4)	0.89
DTR	2.0 (1.7; 2.3)	1.6 (1.4; 1.8)	**0.002**	1.6 (1.3; 2.0)	1.6 (1.4; 1.9)	1.5 (1.4; 1.8)	0.82
Parasternal intercostal ultrasound							
Thickness at TLC right, mm	-	3.9 (3.2; 5.0)	-	4.3 (3.6; 5.4)	3.2 (3.0; 4.7)	3.4 (3.1; 5.0)	0.20

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Thickness at FRC right, mm	-	3.3 (2.7; 4.7)	-	3.7 (3.0; 4.9)	3.3 (2.2; 4.0)	3.1 (2.6; 4.7)	0.32
ITR right	-	1.2 (1.1; 1.4)	-	1.2 (1.1; 1.2)	1.5 (0.9; 1.6)	1.1 (1.0; 1.3)	0.47
Thickness at TLC left, mm	-	3.8 (3.4; 4.9)	-	4.6 (3.7; 5.4)	3.9 (3.5; 5.5)	3.5 (3.2; 3.8)	0.11
Thickness at FRC left, mm	-	3.0 (2.6; 4.3)	-	3.4 (2.8; 4.4)	2.7 (2.0; 4.3)	2.7 (2.4; 3.2)	0.18
ITR left	-	1.3 (1.2; 1.5)	-	1.3 (1.1; 1.4)	1.4 (1.2; 1.5)	1.3 (1.1; 1.5)	0.50
Endurance testing	-		-				
Time to task failure, sec	-	324.0 (241.0; 524.0)	-	283.5 (223.3; 354.8)	321.0 (249.5; 642.5)	412.0 (249.5; 1162.5)	0.15
Resistance set, cmH_2O	-	70.0 (50.0; 82.0)	-	58.0 (38.5; 71.5)	63.0 (46.3; 77.5)	80.0 (72.0; 98.0)	**0.04**§
6MWD, m	480.0 (440.0; 545.0)	480.0 (410.0; 552.5)	0.11	395.0 (290.0; 500.0)	465.0 (423.8; 547.5)	509.0 (480.0; 575.0)	**0.02**§
6MWD, % predicted	77.9 (55.9; 98.5)	87.3 (76.5; 100.3)	0.41	80.0 (56.5; 93.7)	84.3 (80.0; 103.3)	95.0 (81.7; 101.9)	0.13

Values are median (interquartile range).

6MWD, 6-minute walking distance; CMS, cervical magnetic stimulation (of the phrenic nerve roots); CRQ, Chronic Respiratory Disease Questionnaire; DTR, diaphragm thickening ratio; DVAI, diaphragm voluntary activation index; FRC, functional residual capacity; FVC, forced vital capacity; ITR, parasternal intercostal thickening ratio; MCR, maximum contraction rate; MFIS, Modified Fatigue Impact Scale; mMRC; Modified Medical Research Council; MRR, maximum relaxation rate; Pdi, transdiaphragmatic pressure; Pes, esophageal pressure; Pgas, gastric pressure; RMS, respiratory muscle strength; RV, residual volume; TH10, tenth thoracic vertebrae; tw, twitch; TLC, total lung capacity.

The first *P*-value relates to the differences between first follow-up (median 14 months after discharge) and second follow-up (median 31 months after discharge). *P*-ANOVA values relate to differences in dyspnea subgroups at second follow-up.

†Significant differences ($P<0.05$) within paired t tests between moderate and severe dyspnea groups, if ANOVA is significantly different ($P<0.05$).

‡Significant differences ($P<0.05$) within paired t tests between mild and moderate dyspnea groups, if ANOVA is significantly different ($P<0.05$).

§Significant differences ($P<0.05$) within paired t tests between mild and severe dyspnea groups, if ANOVA is significantly different ($P<0.05$).

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Table S3. Twitch transdiaphragmatic pressure, diaphragm voluntary activation index and electromyography findings of specified respiratory muscles at the beginning and end of an endurance test in patient subgroups based on the severity of dyspnea on exertion at median 31 months of follow-up.

	Endurance test (n=30)				%Change (end vs. beginning) in dyspnea severity subgroups			
	Beginning	**End**	**% Change**	***P*-value (end vs. beginning)**	**Severe dyspnea**	**Moderate dyspnea**	**Mild dyspnea**	***P*-ANOVA**
twPdi, cmH_2O	14.8 (9.10; 21.4)	5.5 (3.5; 8.2)	-63.8 (-77.1; -29.7)	**<0.001**	-58.0 (-76.9; -24.9)	-64.1 (-75.2; -35.4)	-76.6 (-79.9; -40.8)	0.52
DVAI	80.3 (51.6; 91.1)	80.7 (57.4; 94.2)	7.2 (-20.2; 32.6)	0.96	32.6 (6.5; 113.2)	-0.1 (-35.5; 21.3)	-2.7 (-63.6; 17.0)	0.07
Right diaphragm amplitude, µV	26.9 (14.8; 39.3)	30.3 (15.0; 42.4)	-0.9 (-34.5; 29.9)	0.75	10.1 (-22.4; 54.8)	-26.1 (-54.4; 31.0)	14.5 (-12.2; 200.2)	0.30
Right diaphragm AUC, µV·s	47.3 (27.7; 64.7)	47.9 (27.7; 65.5)	-3.3 (-28.2; 28.4)	0.18	3.4 (-36.7; 32.9)	-26.2 (-40.1; -2.0)	15.8 (-12.8; 311.7)	**0.05**
Left diaphragm amplitude, µV	21.5 (13.3; 38.0)	26.7 (18.4; 39.9)	3.2 (-14.8; 41.3)	0.23	-4.0 (-41.6; 44.4)	3.7 (-14.5; 58.4)	22.97±52.64	0.45
Left diaphragm AUC, µV·s	41.4 (26.6; 53.8)	38.3 (27.0; 59.0)	-5.5 (-20.2; 26.0)	0.28	-14.6 (-23.4; 1.6)	-26.2 (-40.1; -2.0)	15.8 (-12.8; 311.7)	0.37
Parasternal intercostal muscle amplitude, µV	53.0 (33.5; 78.6)	58.7 (38.0; 96.7)	1.6 (-10.2; 39.0)	0.22	1.1 (-36.9; 8.5)	-4.5 (-24.4; 23.9)	13.9 (-11.4; 61.9)	0.98
Parasternal intercostal muscle AUC, µV·s	88.7 (73.6; 144.1)	83.5 (57.3; 153.3)	-10.5 (-24.7; 6.6)	0.71	-9.5 (-18.1; 0.4)	5.2 (-5.1; 40.1)	15.1 (-12.6; 63.9)	0.57
Sternocleidomas-id muscle amplitude, µV	139.1 (62.3; 224.3)	117.4 (80.2; 227.2)	-9.7 (-29.7; 14.4)	0.98	-9.8 (-40.9; 7.0)	-11.4 (-44.7; 18.0)	-3.0 (-27.5; 60.4)	0.43
Sternocleidomas-id muscle AUC, µV·s	210.5 (108.8; 335.8)	192.3 (93.4; 337.3)	-15.2 (-31.2; 7.0)	0.69	-16.4 (-30.5; -6.1)	-10.4 (-20.0; 28.7)	1.4 (-21.1; 29.4)	**0.05**

Values are median (interquartile range).

AUC, area under the curve; DVAI, diaphragm voluntary activation index; twPdi, twitch transdiaphragmatic pressure.

The first *P*-value relates to the differences in absolute values end versus beginning of the endurance tests. *P*-ANOVA values relate to relative differences in values in dyspnea subgroups at the beginning and at the end of the endurance tests.

†Significant differences ($P<0.05$) within paired t tests between moderate and severe dyspnea groups, if ANOVA is significantly different ($P<0.05$).

‡Significant differences ($P<0.05$) within paired t tests between mild and moderate dyspnea groups, if ANOVA is significantly different ($P<0.05$).

§Significant differences ($P<0.05$) within paired t tests between mild and severe dyspnea groups, if ANOVA is significantly different ($P<0.05$).

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Table S4. Inspiratory muscle training (IMT) device data throughout the 6 weeks of IMT in the treatment and sham arms.

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	*P*-value for Week 1 vs. Week 6
Treatment arm							
Sniff nasal pressure, cmH_2O	70.0 (54.6; 77.7)	70.0 (58.0; 76.4)	74.0 (63.0; 84.0)	74.2 (51.1; 83.3)	80.5 (57.4; 86.8)	88.2 (60.2; 95.2)	**0.02**
Resistance, cmH_2O	50.0 (45.0; 50.0)	50.0 (45.0; 55.0)	55.0 (50.0; 60.0)	65.0 (60.0; 70.0)	65.0 (55.0; 72.5)	70.0 (60.0; 72.5)	**<0.0001**
Power, W	5.1 (2.8; 8.0)	5.8 (3.1; 8.7)	5.6 (3.7; 10.9)	7.3 (4.5; 12.2)	6.5 (3.7; 13.4)	9.5 (5.3; 14.7)	**0.01**
Energy, J	170.0 (86.7; 264.4)	196.6 (141.7; 273.6)	197.7 (136.5; 303.8)	235.0 (157.9; 358.2)	177.3 (144.3; 380.7)	178.0 (123.1; 332.9)	**0.002**
Volume, L	1.9 (1.2; 3.1)	1.8 (1.4; 3.3)	1.8 (1.3; 2.9)	1.7 (1.3; 3.1)	1.8 (1.2; 3.0)	1.7 (1.3; 3.0)	0.54
Sham arm							
Sniff nasal pressure, cmH_2O	71.0 (62.3; 77.7)	65.4 (55.5; 77.8)	69.0 (67.2; 77.0)	74.0 (58.6; 84.2)	77.7 (54.6; 84.6)	74.9 (63.7; 81.2)	0.17
Resistance, cmH_2O	10.0 (10.0; 11.0)	10.0 (8.5; 11.0)	10.0 (10.0; 11.0)	10.0 (10.0; 11.0)	10.0 (10.0; 11.0)	10.0 (10.0; 11.0)	0.35
Power, W	1.1 (0.9; 1.4)	1.2 (0.9; 1.5)	1.3 (1.0; 1.4)	1.2 (1.1; 1.5)	1.1 (1.0; 1.5)	1.2 (1.1; 1.7)	0.18
Energy, J	39.4 (24.0; 48.7)	33.0 (26.1; 45.7)	35.1 (29.3; 49.9)	31.4 (28.4; 50.7)	30.9 (28.9; 41.0)	32.2 (27.4; 50.7)	0.68
Volume, L	1.8 (1.5; 2.0)	1.8 (1.4; 1.9)	1.7 (1.4; 1.9)	1.5 (1.4; 1.8)	1.5 (1.4; 1.8)	1.6 (1.4; 1.8)	**0.03**

Values are median (interquartile range).

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Table S5. Persistence of effects of inspiratory muscle training at the end of the 6-week training period and at 6 weeks after the end of the 6-week training period.

	IMT				Sham			
	Before training	**After training**	**6 weeks after the end of training**	***P*-value**	**Before training**	**After training**	**6 weeks after the end of training**	***P*-value**
Dyspnea								
mMRC dyspnea scale score	3.0 (2.0; 3.0)	2.0 (1.0; 3.0)	2.0 (1.5; 2.0)	0.96	2.0 (2.0; 3.0)	2.0 (2.0; 2.0)	2.0 (2.0; 2.0)	0.35
Borg dyspnea scale score	7.0 (5.5; 8.0)	6.0 (4.0; 7.0)	5.0 (3.5; 6.0)	0.24	5.0 (5.0; 6.5)	5.0 (4.0; 6.0)	5.0 (5.0; 6.0)	0.62
MFIS score	43.0 (20.0; 57.0)	27.0 (23.0; 52.0)	42.0 (30.0; 52.0)	**0.03**	35.0 (27.0; 43.0)	29.0 (15.5; 46.0)	35.0 (15.0; 42.5)	0.52
CRQ dyspnea domain score	2.4 (2.1; 2.8)	3.2 (2.3; 3.7)	3.0 (2.2; 3.8)	0.53	2.8 (2.0; 3.5)	3.0 (2.4; 3.5)	2.5 (2.1; 3.9)	0.85
CRQ fatigue domain score	4.3 (2.9; 5.1)	4.3 (3.5; 5.0)	4.3 (3.3; 5.1)	0.90	3.8 (2.9; 4.5)	3.5 (2.6; 4.6)	3.8 (3.1; 4.3)	0.78
CRQ emotional function score	4.1 (3.1; 5.6)	4.7 (3.7; 5.3)	4.3 (4.1; 6.4)	0.21	4.4 (3.6; 5.9)	5.3 (3.9; 5.9)	4.7 (4.1; 5.8)	0.90
CRQ mastery score	4.5 (4.1; 5.8)	4.8 (4.3; 6.0)	5.3 (4.5; 6.3)	0.27	5.3 (4.6; 6.1)	5.3 (4.8; 6.3)	5.2 (4.6; 6.1)	0.39
Non-volitional invasive RMS								
CMS twPdi, cmH_2O	10.7 (6.7; 16.4)	12.0 (8.9; 21.2)	17.0 (9.7; 22.8)	0.35	15.0 (5.6; 21.8)	21.5 (8.6; 24.1)	18.4 (12.5; 23.8)	0.18
CMS twPes, cmH_2O	-8.9 (-13.6; -6.3)	-7.8 (-14.6; -6.2)	-8.7 (-13.3; -5.3)	0.15	-10.9 (-12.9; -5.0)	-8.1 (-12.6; -4.0)	-8.0 (-10.2; -4.7)	0.22
CMS twPgas, cmH_2O	1.1 (0.2; 7.6)	3.5 (2.4; 5.0)	8.7 (5.1; 9.5)	**0.02**	2.4 (0.7; 7.6)	8.0 (3.1; 12.7)	9.9 (5.5; 15.1)	0.88
CMS MRR normalized, cmH_2O/msec	-16.5 (-20.4; -11.5)	-12.4 (-17.4; -8.6)	-13.3 (-19.1; -8.1)	0.36	-14.8 (-29.5; -11.1)	-10.3 (-21.8; -9.0)	-13.1 (-19.8; -10.7)	0.67
CMS MCR normalized, cmH_2O/msec	45.6 (18.3; 115.9)	50.0 (33.2; 110.9)	70.8 (39.8; 121.6)	0.13	25.6 (23.2; 47.1)	38.4 (32.8; 70.2)	32.1 (24.7; 69.4)	0.23
TH10 twPgas, cmH_2O	3.7 (2.1; 12.5)	10.8 (10.0; 18.1)	12.6 (10.9; 19.2)	0.44	4.1 (3.2; 13.9)	16.4 (10.2; 25.0)	17.2 (11.6; 27.0)	0.63
Sniff nasal pressure, cmH_2O	70.0 (54.6; 77.7)	88.2 (60.2; 95.2)	86.8 (54.6; 96.6)	0.17	71.4 (62.3; 77.7)	74.9 (63.7; 81.2)	77.0 (66.5; 80.0)	0.38
Volitional invasive RMS								
Sniff Pdi, cmH_2O	83.0 (75.1; 90.8)	100.1 (81.4; 113.2)	110.0 (97.9; 126.7)	0.27	96.3 (73.2; 127.0)	102.0 (76.6; 132.4)	116.8 (80.6; 141.5)	0.59
Sniff Pes, cmH_2O	-57.0 (-72.3; -49.0)	-79.5 (-105.5; -60.0)	-110.0 (-114.9; -97.2)	0.06	-68.5 (-82.6; -42.5)	–-67.7 (-90.4; -50.6)	-76.0 (-111.0; -59.9)	0.56
Mueller Pdi, cmH_2O	85.7 (57.4; 98.2)	96.0 (69.5; 123.2)	117.0 (38.3; 137.8)	0.42	64.0 (23.2; 100.7)	104.4 (44.4; 114.2)	24.9 (0.0; 100.7)	0.31
Mueller Pes, cmH_2O	-58.0 (-73.6; -46.2)	-80.2 (-102.3; -65.6)	-109.7 (-126.8; -92.0)	0.10	-52.0 (-78.6; -38.0)	-95.7 (-107.1; -48.9)	-55.0 (-97.2; -38.3)	0.61
Valsalva Pgas, cmH_2O	187.0 (100.5; 260.3)	230.0 (157.5; 309.6)	287.2 (170.9; 305.0)	0.16	101.2 (72.9; 155.2)	116.0 (108.0; 226.4)	126.1 (110.8; 241.5)	0.86
Cough Pgas, cmH_2O	183.6 (145.0; 217.9)	248.9 (180.2; 325.5)	259.4 (188.0; 314.0)	0.88	172.7 (135.6; 249.4)	184.0 (135.7; 274.0)	198.3 (139.8; 277.7)	0.73
Neural control								
DVAI, %	78.6 (62.9; 91.5)	89.1 (74.8; 93.5)	88.9 (71.9; 91.7)	0.97	60.7 (30.4; 91.5)	71.4 (53.1; 82.7)	78.9 (66.2; 85.5)	0.75
Diaphragm ultrasound								
Thickness at TLC, mm	4.0 (2.8; 4.5)	4.1 (3.0; 5.1)	3.3 (3.1; 4.9)	0.22	3.1 (2.8; 3.4)	3.2 (2.9; 3.9)	3.3 (3.1; 3.9)	0.09

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Thickness at FRC, mm	2.3 (1.7; 3.1)	2.1 (1.8; 2.6)	1.7 (1.4; 2.4)	0.81	1.7 (1.7; 2.3)	2.0 (1.6; 2.5)	1.7 (1.3; 1.8)	0.99
DTR	1.6 (1.4; 2.1)	1.8 (1.7; 2.1)	2.12 ± 0.97	0.22	1.6 (1.4; 1.9)	1.7 (1.4; 1.9)	2.0 (1.8; 2.8)	**0.04**
Parasternal intercostal ultrasound								
Thickness at FRC right, mm	3.1 (2.6; 4.7)	3.8 (3.2; 5.3)	4.5 (2.5; 4.6)	0.49	3.7 (3.3; 5.1)	4.2 (3.1; 5.3)	4.5 (3.5; 5.1)	0.37
Thickness at TLC right, mm	4.3 (3.4; 5.4)	5.7 (4.6; 6.8)	5.5 (4.0; 6.2)	0.54	4.0 (3.4; 5.2)	4.8 (3.9; 6.4)	4.9 (4.5; 8.2)	0.07
ITR right	1.2 (1.2; 1.4)	1.4 (1.3; 1.5)	1.4 (1.3; 1.5)	0.67	1.2 (0.8; 1.5)	1.4 (1.3; 1.6)	1.4 (1.3; 1.6)	0.98
Thickness at FRC left, mm	3.8 (2.3; 4.4)	3.7 (3.0; 4.8)	3.8 (2.5; 4.4)	0.22	3.0 (2.5; 4.3)	4.0 (2.7; 4.4)	4.6 (3.2; 5.2)	0.15
Thickness at TLC left, mm	4.8 (3.7; 5.3)	5.7 (5.1; 6.9)	5.3 (4.1; 6.4)	0.39	4.0 (3.5; 5.8)	5.5 (4.3; 6.3)	7.1 (4.5; 7.3)	**0.03**
ITR left	1.3 (1.1; 1.6)	1.5 (1.3; 1.6)	1.5 (1.2; 1.6)	0.57	1.4 (1.2; 1.5)	1.4 (1.3; 1.7)	1.5 (1.4; 1.5)	0.76
Pulmonary function parameters								
TLC, % predicted	102.0 (87.5; 110.0)	101.5 (95.0; 102.8)	99.0 (94.0; 118.0)	0.55	103.0 (93.5; 114.0)	112.0 (90.5; 116.0)	104.5 (94.0; 119.3)	0.71
Vital capacity, % predicted	95.0 (78.0; 108.5)	98.5 (92.5; 103.8)	101.0 (97.0; 103.0)	0.33	98.0 (81.5; 111.5)	101.0 (81.5; 117.5)	99.0 (73.3; 114.5)	0.61
RV/TLC, % predicted	118.0 (100.0; 124.5)	105.0 (97.3; 108.0)	100.0 (78.0; 125.0)	0.70	108.0 (101.0; 120.5)	108.0 (98.0; 119.0)	115.0 (99.3; 147.3)	0.84
FEV_1, % predicted	82.0 (66.5; 93.0)	89.0 (82.3; 98.8)	95.0 (79.0; 96.0)	0.65	91.0 (71.5; 103.0)	91.0 (70.5; 102.0)	87.5 (49.8; 105.8)	0.80
FEV_1/FVC, %	102.0 (93.0; 108.5)	99.5 (97.3; 107.0)	100.0 (90.0; 108.0)	0.25	101.0 (96.0; 106.0)	97.0 (87.5; 105.0)	99.0 (83.5; 103.8)	0.10
Endurance testing								
Time to task failure, sec	365.0 (284.0; 700.5)	983.0 (551.0; 1494.0)	939.0 (648.0; 1221.0)	0.32	285.6 (279.8; 289.2.8)	573.5 (474.0; 610.8.0)	646.3 (521.9; 700.6)	0.99
6MWD, m	420.0 (385.0; 570.0)	465.0 (427.5; 585.0)	510.0 (420.0; 570.0)	0.99	410.0 (290.0; 472.5)	367.5 (292.5; 570.0)	370.0 (225.0; 600.0)	0.20
6MWD, % predicted	89.2 (78.0; 103.2)	88.0 (68.3; 11.4)	86.8 (31.5; 109.2)	0.34	77.9 (54.6; 93.8)	74.2 (63.1; 97.5)	56.2 (0.0; 91.4)	0.03

Values are median (interquartile range).

6MWD, 6-minute walking distance; CMS, cervical magnetic stimulation (of the phrenic nerve roots); CRQ, Chronic Respiratory Disease Questionnaire; DTR, diaphragm thickening ratio; DVAI, diaphragm voluntary activation index; FEV_1, forced expiratory volume in 1 second; FRC, functional residual capacity; FVC, forced vital capacity; ITR, intercostal thickening ratio; MCR, maximum contraction rate; mMRC; Modified Medical Research Council; MRR, maximum relaxation rate; Pdi, transdiaphragmatic pressure; Pes, esophageal pressure; Pgas, gastric pressure; RMS, respiratory muscle strength; RV, residual volume; TH10, tenth thoracic vertebrae; tw, twitch; TLC, total lung capacity.

The *P*-values relate to comparisons between values immediately after training and 6 weeks after completion of training.

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Table S6. Twitch transdiaphragmatic pressure, diaphragm voluntary activation index and electromyography findings of specified respiratory muscles at the beginning, mid-way point and end of an endurance test before and after intervention in the treatment and sham arms.

	Before Training				After training			
	Beginning	**End**	**Relative Change**	***P*-value**	**Beginning**	**End**	**Relative Change**	***P*–value**
Treatment arm								
twPdi, cmH_2O	10.7 (6.7; 16.4)	5.4 (3.3; 8.5)	52.5 (22.9; 76.7)	**0.008**	12.0 (8.9; 21.2)	4.4 (3.5; 10.6)	55.4 (22.4; 71.7)	**0.01**
DVAI, %	78.6 (62.9; 91.5)	82.1 (69.5; 93.8)	11.6 (-4.1; 28.0)	0.99	89.1 (74.8; 93.5)	84.1 (69.1; 92.4)	-9.4 (-22.8; 27.9)	0.77
Right diaphragm amplitude, µV	30.61 (14.39; 63.76)	35.20 (14.92; 45.28)	-12.22 (-30.91; 48.13)	0.48	19.80 (15.54; 21.96)	15.71 (10.94; 25.67)	-11.32 (-31.90; 0.48)	0.19
Right diaphragm AUC, µV·s	48.30 (29.39; 65.60)	32.22 (22.79; 59.45)	3.38 (-37.58; 20.92)	0.92	23.25 (19.60; 35.87)	22.99 (16.59; 32.24)	-5.39 (-20.24; 6.04	0.42
Left diaphragm amplitude, µV	20.26 (12.90; 42.27)	19.29 (12.71; 39.95)	-6.04 (-27.73; 23.59)	0.91	32.0 (15.3; 49.8)	32.3 (15.2; 39.3)	-13.2 (-20.3; 31.4)	0.63
Left diaphragm AUC, µV·s	49.9 (25.2; 62.9)	26.7 (22.0; 49.7)	-18.3 (-21.9; -2.5)	0.86	40.7 (16.5; 78.5)	34.8 (17.0; 62.7)	-18.2 (-35.1; 10.4)	0.20
Parasternal intercostal muscle amplitude, µV	47.6 (29.6; 114.9)	48.1 (30.8; 78.1)	1.1 (-42.9; 6.8)	0.27	31.8 (26.7; 46.7)	41.5 (28.3; 76.5)	8.9 (-2.8; 66.2)	0.71
Parasternal intercostal muscle AUC µV·s	74.83 (49.10; 172.16)	64.50 (43.25; 98.58)	-11.39 (-21.10; -0.51)	0.13	41.61 (33.77; 73.39)	43.95 (39.86; 76.05)	10.8 (-27.7; 33.3)	0.44
Sternocleidomastoid muscle amplitude, µV	173.1 (87.9; 310.1)	156.2 (83.0; 269.7)	-10.4 (-42.2; 9.1)	0.16	109.4 (53.0; 252.1)	177.8 (66.8; 244.5)	18.5 (-17.4; 36.4)	0.57
Sternocleidomastoid muscle AUC, µV·s	210.5 (148.6; 416.0)	191.7 (72.9; 322.6)	-22.5 (-40.6; -5.7)	**0.02**	115.4 (73.4; 312.1)	139.2 (85.6; 281.4)	4.0 (-24.2; 44.3)	0.85
Sham arm								
twPdi cmH_2O	14.99 (5.64; 21.78)	6.86 (2.99; 14.17)	48.21 (26.97; 61.01)	**0.005**	21.5 (8.6; 24.1)	13.8 (8.0; 18.3)	21.3 (5.8; 47.1)	**0.03**
DVAI, %	60.7 (30.4; 91.5)	66.3 (38.0; 92.8)	8.1 (-10.7; 11.6)	0.80	71.4 (53.1; 82.7)	92.8 (90.7; 96.3)	30.0 (2.8; 63.4)	0.07
Right diaphragm amplitude, µV	23.1 (20.1; 38.7)	22.6 (14.1; 28.6)	-33.1 (-63.8; 24.6)	0.39	21.1 (18.0; 46.5)	37.7 (16.2; 42.3)	-7.9 (-34.7; 31.8)	0.79
Right diaphragm AUC, µV·s	53.5 (25.2; 66.5)	48.0 ± 32.0	-21.9 (-40.6; -0.4)	0.43	49.8 (36.9; 67.8)	43.6 (28.1; 70.8)	-10.9 (-32.2; 10.3)	0.87
Left diaphragm amplitude, µV	20.3 (12.1; 30.7)	25.8 (21.8; 29.6)	4.2 (-26.1; 117.5)	0.46	29.2 (20.4; 33.5)	32.6 (17.5; 52.2)	6.5 (-35.9; 54.5)	0.27
Left diaphragm AUC, µV·s	49.0 (24.2; 53.3)	38.6 (25.8; 84.3)	-7.7 (-31.1; 79.0)	0.79	48.7 (34.5; 55.9)	50.9 (27.4; 81.2)	-2.2 (-29.7; 60.0)	0.22
Parasternal intercostal muscle amplitude, µV	53.2 (21.1; 90.2)	63.0 (46.1; 141.2)	22.5 (-0.1; 114.4)	0.14	41.8 (25.5; 63.1)	45.7 (31.2; 66.4)	3.6 (-10.9; 24.5)	0.15
Parasternal intercostal muscle AUC, µV·s	88.0 (62.4; 141.8)	118.8 (72.8; 217.9)	-2.3 (-8.4; 29.4)	0.88	75.5 (53.9; 144.8)	84.6 (57.9; 115.4)	1.6 (-23.5; 6.3)	0.99
Sternocleidomastoid muscle amplitude, µV	71.6 (37.7; 190.2)	84.0 (54.9; 179.4)	5.4 (-14.7; 34.9)	0.44	90.9 (59.0; 148.6)	81.7 (52.8; 157.7)	-18.2 (-46.0; 10.0)	0.47
Sternocleidomastoid muscle AUC, µV·s	115.9 (89.6; 264.8)	129.4 (78.3; 203.2)	-15.2 (-16.2; 18.0)	0.63	166.2 (73.7; 205.5)	116.2 (63.5; 208.3)	-13.1 (-49.9; 11.9)	0.49

Values are median (interquartile range).

AUC, area under the curve; DVAI, diaphragm voluntary activation index; twPdi, twitch transdiaphragmatic pressure.

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The *P*-values relate to the comparisons between absolute values at the beginning and at the end of the endurance tests.

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Table S7. Respiratory ultrasound values and pulmonary function values in both groups before and after IMT intervention.

	Treatment arm			Sham arm			Mean between-group difference (95% CI)	*P*-value for between-group difference
	Before training	After training	*P*-value	Before training	After training	*P*-value		
Diaphragm ultrasound								
Thickness at TLC, mm	4.0 (2.8; 4.5)	4.1 (3.0; 5.1)	0.84	3.1 (2.8; 3.4)	3.2 (2.9; 3.9)	0.34	-0.08 (-1.30, 1.14)	0.43
Thickness at FRC, mm	2.3 (1.7; 3.1)	2.1 (1.8; 2.6)	0.98	1.7 (1.7; 2.3)	2.0 (1.6; 2.5)	0.34	-0.15 (-0.79, 0.49)	0.28
DTR	1.6 (1.4; 2.1)	1.8 (1.7; 2.1)	0.89	1.6 (1.4; 1.9)	1.7 (1.4; 1.9)	0.93	0.02 (-0.45, 0.48,)	0.62
Parasternal intercostal ultrasound								
Thickness at TLC right, mm	4.3 (3.4; 5.4)	5.7 (4.6; 6.8)	0.08	4.0 (3.4; 5.2)	4.8 (3.9; 6.4)	0.18	0.99 (-0.97, 2.97)	0.86
Thickness at FRC right, mm	3.1 (2.6; 4.7)	3.8 (3.2; 5.3)	0.19	3.7 (3.3; 5.1)	4.2 (3.1; 5.3)	0.33	-0.61 (-0.75, 0.45)	0.60
ITR right	1.2 (1.2; 1.4)	1.4 (1.3; 1.5)	0.70	1.2 (0.8; 1.5)	1.4 (1.3; 1.6)	0.13	-0.20 (-0.55, 2.15)	0.14
Thickness at TLC left, mm	4.8 (3.7; 5.3)	5.7 (5.1; 6.9)	**0.04**	4.0 (3.5; 5.8)	5.5 (4.3; 6.3)	0.17	0.80 (-0.93, 2.53)	0.98
Thickness at FRC left, mm	3.8 (2.3; 4.4)	3.7 (3.0; 4.8)	0.29	3.0 (2.5; 4.3)	4.0 (2.7; 4.4)	0.11	0.37 (-1.02, 1.78)	0.79
ITR left	1.3 (1.1; 1.6)	1.5 (1.3; 1.6)	0.46	1.4 (1.2; 1.5)	1.4 (1.3; 1.7)	0.38	0.01 (-0.34, 0.35)	0.52
Pulmonary function parameters								
TLC, % predicted	102.0 (87.5; 110.0)	101.5 (95.0; 102.8)	0.39	103.0 (93.5; 114.0)	112.0 (90.5; 116.0)	0.31	-7.21 (-18.80, 4.38)	0.13
Vital capacity, % predicted	95.0 (78.0; 108.5)	98.5 (92.5; 103.8)	0.91	98.0 (81.5; 111.5)	101.0 (81.5; 117.5)	0.94	-1.94 (-11.19, 7.30)	0.28
RV/TLC, % predicted	118.0 (100.0; 124.5)	105.0 (97.3; 108.0)	0.10	108.0 (101.0; 120.5)	108.0 (98.0; 119.0)	0.81	-9.98 (-23.82, 3.85,)	0.13
FEV_1, % predicted	82.0 (66.5; 93.0)	89.0 (82.3; 98.8)	0.31	91.0 (71.5; 103.0)	91.0 (70.5; 102.0)	0.38	7.44 (-3,41, 18.29)	0.94
FEV_1/FVC, %	102.0 (93.0; 108.5)	99.5 (97.3; 107.0)	0.71	101.0 (96.0; 106.0)	97.0 (87.5; 105.0)	0.51	3.88 (-7.80, 15.55)	0.57

Values are median (interquartile range). Before IMT, none of the variables shown differed significantly between individuals randomized to the IMT or sham control group (all $P>0.05$).

DTR, diaphragm thickening ratio; FEV_1, forced expiratory volume in 1 second; FRC, functional residual capacity; FVC forced vital capacity; ITR, intercostal thickening ratio; RV, residual volume; TLC, total lung capacity.

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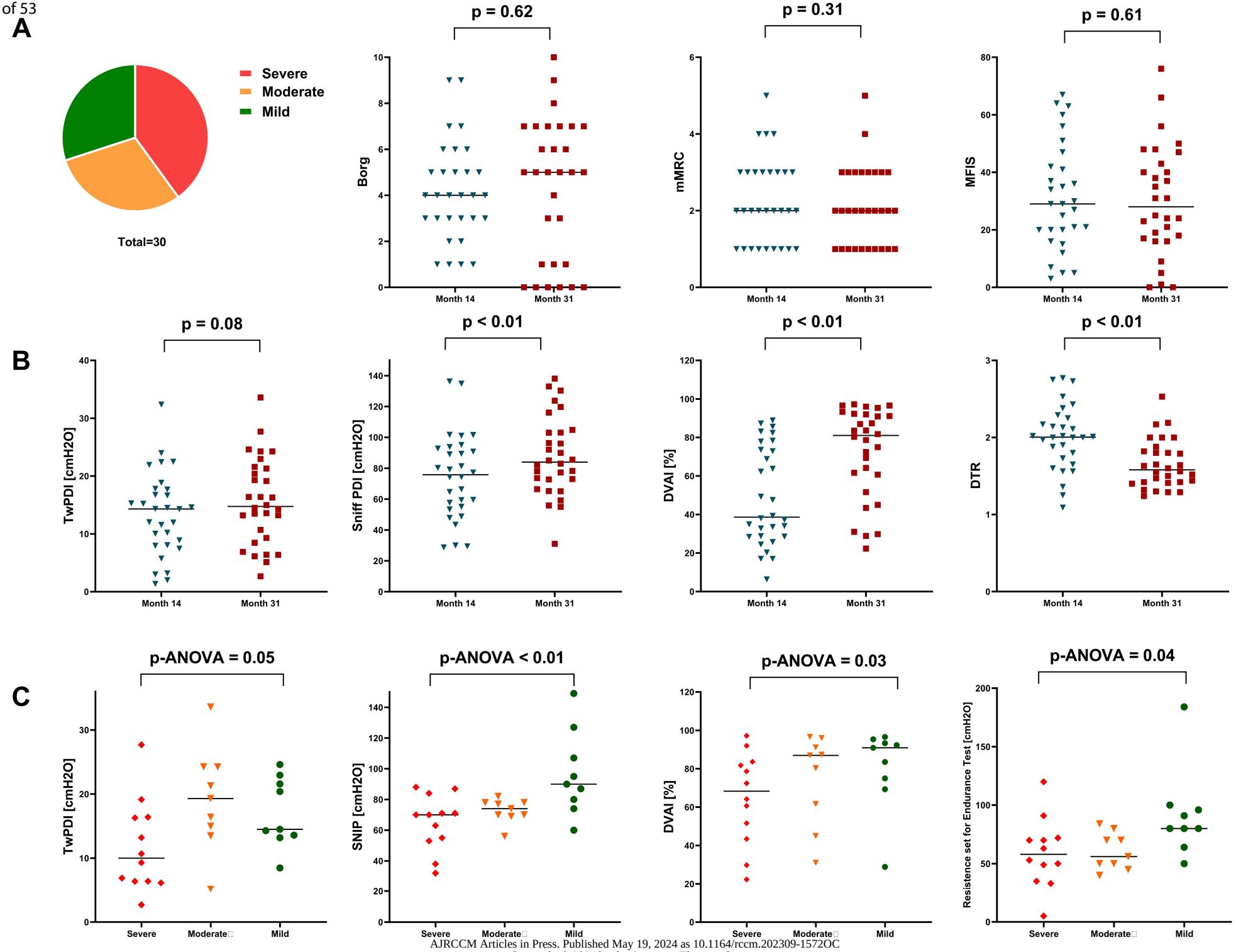

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