**Inspiratory Muscle Training for Post-COVID Syndrome**

**By**

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**Proposal for Dissertation Research**

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# Abstract

More than 10% of adults and 4% of children infected with COVID-19 develop lasting and persistent symptoms that do not resolve with time. While a wide range of symptomatic presentations have been attributed to Post-COVID Syndrome (PCS), the most commonly identified afflictions include chronic and debilitating fatigue, neurocognitive dysfunction, impaired sleep, and disordered breathing. Proposed pathophysiological mechanisms include dysfunction of the vascular, cardiac, respiratory, and autonomic systems. Inspiratory Muscle Training (IMT) is a physical therapy rehabilitation intervention to improve respiratory function and has the potential to enhance vascular, cardiac, and autonomic recovery. A randomized controlled trial (RCT) was conducted to determine the effects of IMT on objective and subjective symptoms of PCS. Between group differences were evaluated using Split Plot ANCOVAs to assess for group x time interactions while controlling for baseline levels of function. Mediation analysis within a hierarchical linear modeling framework was conducted to evaluate if improvements in subjective symptoms are causally mediated by measured physiological adaptations.

# Introduction

## Background

Although the majority of COVID-19 infections do not result in long-term persisting symptoms, approximately 4% of children and at least 10% of adults experience symptoms during COVID-19 infection that do not fully recover with time (Mandel et al., 2025). When symptoms are non-resolving, this condition – commonly referred to as “long COVID”– is indicative of a diagnosis known as Post-Covid Syndrome (PCS). PCS is officially defined by the World Heath Organization (WHO) as a condition “usually 3 months from the onset [of infection], with symptoms that last for at least 2 months and cannot be explained by an alternative diagnosis” (Soriano et al., 2022). In 2022, 144.7 million individuals were diagnosed with PCS (Hou et al., 2025). While over 200 possible symptoms of PCS have been documented throughout every major organ system of human anatomy (Stone et al., 2023)(Davis et al., 2021), the primary complaints affecting PCS patients are chronic and debilitating fatigue, neurocognitive dysfunction, disordered sleep, and dyspnea (Soriano et al., 2022)(Jennings et al., 2021)(Davis et al., 2021)(Vanichkachorn et al., 2021).

## Symptoms of PCS

### Fatigue

Subjects with PCS report chronic and debilitating fatigue. Of importance, PCS patients are particularly vulnerable to a form of fatigue known as “post-exertional malaise” (PEM). PEM is defined by <DEFINITION>.

Over time, fatigue continues to persist without resolution. For instance, a longitudinal study by Spiesshoefer et al. 2024 found that self-reported fatigue remains elevated 14 months following COVID-19 infection, and these levels show no improvement by 31 months post-infection (Spiesshoefer et al., 2024). Over time, this sense of unending fatigue takes its toll on patient’s well-being. The greater the severity of the fatigue, the lower reported social participation, occupational involvement, and quality of life (Twomey et al., 2022)(Walker et al., 2023). Above all else, excessive fatigue is reported to be the strongest determinant among PCS patients of worsened quality of life <CITATION>.

### Neurocognitive Dysfunction

A commonly reported symptom among patients with PCS is a sense of <DEFINITION>. This cluster of subjective symptoms – commonly referred to as “brain fog” by victims – is described to be one of the most debilitating of PCS symptoms (Chasco et al., 2022).

(Asadi-Pooya et al., 2023; Jennings et al., 2022; Vyas et al., 2022) (Davis et al., 2021; Nordvig et al., 2023; Orfei et al., 2022; Sa et al., 2024).

Subjective reports of brain fog are often accompanied objective deficits in cognitive functions including attention, executive functioning, problem-solving, memory, and decision-making (Davis et al., 2021; Jennings et al., 2022).

Given the increased mental and physical burden imposed by these symptoms, it is of no wonder that neurocognitive dysfunction has been associated with reduced physical activity, social isolation, and disability (Nordvig et al., 2023). Brain fog and cognitive dysfunction are often associated with difficulty meeting occupational demands, reduced social participation, and overall lower reported quality of life (Miskowiak et al., 2021; Walker et al., 2023).

### Dyspnea

Dyspnea is common among PCS patients (Harenwall et al., 2022)(Paradowska-Nowakowska et al., 2023)(Jennings et al., 2022) (Bulla et al., 2023) and is commonly reported during physical activity (Frizzelli et al., 2022)(Barbagelata et al., 2022). One report found that 52.7% of PCS patients report symptoms of dyspnea during exercise compared to only 13.7% of healthy controls (Barbagelata et al., 2022).

Concerningly, these respiratory functions do not appear to spontaneously recovery with time. An analysis by Helt et al. found that among subjects with reduced respiratory function following COVID infection, these deficits had not improved 12 months later (Helt et al., 2024). Similarly, a study by Spiesshoefer et al. found significant diaphragm weakness and dyspnea can persist for 31 months following infection with little to no signs of improvement (Spiesshoefer et al., 2024).

### Sleep

A large portion of patients with PCS report significantly impaired sleep (Davis et al., 2021; Sa et al., 2024)(Davis et al., 2021; Jennings et al., 2022; Nordvig et al., 2023). Obstructive sleep apnea (OSA) appears disproportionately common in the PCS population (Davis et al., 2021)(Riou, 2021). Additionally, PCS patients report additional sleep barriers such as nightmares (Davis et al., 2021).

Impaired slight may partly be responsible for a portion of other PCS symptoms. For instance, an analysis by Sunada et al. found that compared to PCS patients without sleep disturbance, PCS patients with impaired sleep were also more likely to experience headache, dysgeusia, dysosmia, anxiety, fever, brain fog, and worsened fatigue (Sunada et al., 2022). Of particular interest, 48.3% of PCS patients with disturbed sleep report brain fog compared to only 19.5% of patients with normal sleep. Of these PCS patients with disturbed sleep, 72% of patients with mid-awakening and 75% of patients with early awakening reported brain fog.

### Quality of Life

Symptoms severely limit PCS patients’ ability to participate in regular activities of daily living. For instance, PCS patients report difficulty with mundane activities within their home such as cleaning or cooking (Chasco et al., 2022). Leaving the home becomes difficult as subjects lose confidence in their ability to drive safely without succumbing to overwhelming brain fog or fatigue. (Chasco et al., 2022).

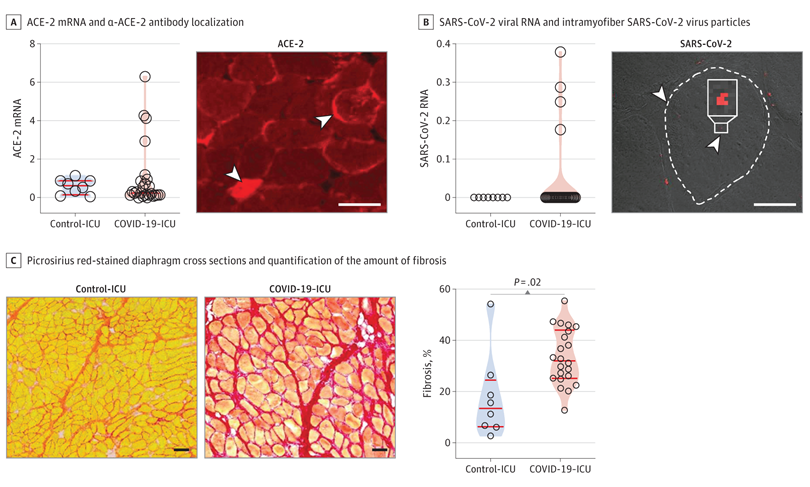
As PCS symptoms manifest, social relationships begin to dwindle. Many subjects report a sense of stigma for sharing their PCS symptoms, with peers doubting legitimacy of the PCS condition and not understanding the relapsing-remitting nature of the disease (Chasco et al., 2022). Some participants report receiving stigma from doctors who did not know how to diagnose, treat, or even acknowledge the existence of their condition (Ladds et al., 2020). Shame and guilt become likely when doctors tell patients their symptoms are not real and they are not taken seriously (Ladds et al., 2020). PCS patients with children report difficulty with being present as a parent, feeling guilty for sometimes shouldering responsibilities to children instead of themselves (Chasco et al., 2022). Romantic relationships become strained, with PCS patients disclosing fear that romantic partners may “grow tired of me” (Chasco et al., 2022).

Above all, many participants report the most challenging component of dealing with PCS to be maintaining occupational responsibilities (Chasco et al., 2022). An analysis by Twomey et al. revealed that 41.8% of PCS patients require a reduced work schedule compared to pre-illness, and an additional 42.3% of PCS patients must stop working entirely due to illness. Only 5.2% of PCS patients reported ability to maintain normal work schedules (Twomey et al., 2022)(Davis et al., 2021). Of those who do return to work, many felt that after communicating the nature of their condition to their supervisors their performance became scrutinized. <CHASCO>. Collectively, reductions in occupational, social, and household activity participation result in high rates of anxiety (Frésard et al., 2022) (Bonner-Jackson et al., 2024; Oh et al., 2024), depression (Jennings et al., 2022) (Bonner-Jackson et al., 2024; Oh et al., 2024), PTSD (Harenwall et al., 2022; Jennings et al., 2022), and reduced quality of life (Jennings et al., 2021)(Oh et al., 2024).

## Pathophysiology of PCS

### Respiratory

During the acute stage of infection, COVID-19 virus can induce myopathic changes by directly binding to ACE-2 receptors localized at the myofiber membrane (Ferrandi et al., 2020; Shi et al., 2021) and initiating a cytokine storm (dos Santos et al., 2022; Mittal et al., 2021). Skeletal muscle throughout the entire body is susceptible to attack, which could explain upper and lower extremity myalgia symptoms commonly reported during acute COVID19 infection (dos Santos et al., 2022; Kucuk et al., 2020). Unlike limb muscles, however, the diaphragm musculature appears uniquely vulnerable to myopathic atrophy. For instance, an analysis by Bhattarai et al. found that after COVID-19 infection the quadriceps displayed no detectible loss of size while the diaphragm myofiber cross-sectional area declined by 48%, despite greater viral infiltration found in the quadriceps (Bhattarai et al., 2025). The most likely explanation for this stark disparity is that compared to other skeletal muscles, the myofiber tissue of the diaphragm reacts with increased sensitivity to cytokine-mediated inflammation and proteolytic signaling (Bhattarai et al., 2025; Mittal et al., 2021). Consequently, epimysial and perimysial fibrosis of the diaphragm ensues (Image 1) accompanied by histological evidence of myofiber remodeling (Shi, Bogaards, et al., 2021; Shi, de Vries, et al., 2021). During ultrasound examination, these changes manifest among COVID-19 infected patients as reduced thickness and altered thickening ratios (Farr et al., 2021; Hadda et al., 2023).



**Image 1**

As the diaphragm becomes compromised, indices of respiratory strength such as maximum inspiratory pressure (MIP), sustained MIP (SMIP), and fatigue index test (FIT) worsen (Dosbaba et al., 2023; Li et al., 2020) and persist into PCS (Helt et al., 2024; Nagel et al., 2022). Importantly, these deficits identified do not spontaneously resolve over time; a study using twitch transdiaphragmatic pressure found insignificant recovery of diaphragm function between 14 months and 31 months post-infection (Spiesshoefer et al., 2024). Interestingly, improvement was observed in Diaphragm Voluntary Activity Index (DVAI) and Sniff Pes-Pdi, suggesting that patients gradually regain neural control of the diaphragm but remain limited in maximum force production capacity. Taken together, these findings are consistent with an underlying pathology of viral-induced diaphragm myopathy.

### Vascular

Along with the respiratory musculature, endothelial cells lining the vascular walls are rich in ACE-2 inhibitors and vulnerable to attack from COVID-19 virus <CITATION>.

These vascular deficits persist in PCS. Compared to healthy controls, PCS patients have been demonstrated to exhibit reduced markers of macrovascular health including flow-mediated dilation (FMD) (Ambrosino, 2021; Ergül et al., 2022; Riou, 2021) endothelial quotient index (Charfeddine et al., 2021), and increased circulating endothelial cell (CEC) counts (Chioh et al., 2021). For instance, an analysis by <EXAMPLE>.

Ultimately, these endothelial deficits have been associated with a wide range of negative outcomes, During exercise, patients with low FMD are more likely to exhibit poor VO2max, reduced end-tidal CO2, and higher VE/VCO2 slope (Ambrosino et al., 2022). When examining patient subjective symptoms, reduced FMD is associated with increased likelihood of fatigue or neurocognitive dysfunction (Charfeddine et al., 2021).

Along with macrovascular deficits, long COVID has also been associated with damage to the capillaries. PCS patients demonstrated 41% less capillary recruitment compared to healthy controls, and capillary density was particularly reduced in subjects reporting symptoms of neurocognitive fatigue (Osiaevi et al., 2023).

### Exercise Capacity

#### VO2

PCS has been associated with a wide range of impairments to exercise capacity. During tests of physical function, such as the 6 Minute Walk Test (6MWT), PCS patients score lower compared to healthy control participants (Paradowska-Nowakowska et al., 2023). The lower the 6MWT scores, the more likely these subjects were to report symptoms of fatigue and dyspnea (Paradowska-Nowakowska et al., 2023). Cardiopulmonary exercise testing (CPET), which is considered to be the gold standard form of assessment of exercise capacity (Ross et al., 2016), stark differences between PCS and healthy control subjects have been revealed. Particularly, vo2max – a marker of overall aerobic function that is associated with a wide range of health metrics (Ross et al., 2016) -- is disturbingly low in patients with PCS (Barbagelata et al., 2022) (Baratto et al., 2021) (Contreras et al., 2023) (Durstenfeld et al., 2022) (Ambrosino et al., 2022) (Frizzelli et al., 2022). In some estimates, 61% of PCS patients exhibit impaired cardiorespiratory fitness (<70% of predicted) compared to 17% of healthy controls (Baratto et al., 2021). According to a meta-analysis of 9 studies, PCS is associated with a mean reduction of 4.9 mL/min/kg in peak VO2. Furthermore, during testing, overall workload and VO2 / work slope is reduced (Baratto et al., 2021)(Frizzelli et al., 2022).

#### Exertional Tolerance

Subjectively, PCS patients report a greater degree of physical exertion during exercise testing as measured by the Berg <CITATION>. Consequently, a notably large portion of PCS patients are unable to tolerate activity to the point of reaching anaerobic threshold (AT). In a study by Barbagelata, only 50.9% of PCS patients reached AT compared to 72.7% of healthy controls (Barbagelata et al., 2022). Similarly, an analysis by Cherneva et al. found only 48.2% of moderate-to-severe cases of PCS patients reached AT compared to 75% of mild cases and 87.% of healthy controls (Cherneva et al., 2025)

#### Cardiac Weakness

PCS patients exhibit reduced O2 pulse, indicating reduced force production of the cardiac muscle during systole (Frizzelli et al., 2022) (Kersten et al., 2022) (de Boer et al., n.d.). Interestingly, patients with a history of PEM appear to demonstrate significantly reduced O2 pulse in the 24 hours following physical activity, suggesting a possible connection (Thomas et al., 2025).

#### Respiratory/Ventilatory Influence

Respiratory dysfunction has the potential to serve as a major limiting factor of exercise capacity in PCS patients. Following acute COVID infection, 95% of patients demonstrate breathing dysregulation during CPET (van Voorthuizen et al., 2022). In PCS, an analysis by Frésard et al. concluded that 84.3% were limited by dysfunctional breathing or respiratory limitation, compared to only 11.8% who were limited by oxygen delivery and utilization (Frésard et al., 2022). These findings are supported by additional studies that have observed abnormal ventilatory patterns in PCS, including lower tidal volume (Baratto et al., 2021)(Frizzelli et al., 2022), higher respiratory rates (Baratto et al., 2021), lower peak minute ventilation (Baratto et al., 2021; Contreras et al., 2023)(Frizzelli et al., 2022), and elevated VE/VCO2 ratio (Ambrosino et al., 2022; Baratto et al., 2021)(Frizzelli et al., 2022). Moreoever, TI/TOT is significantly lower both at rest and during activity, indicating that PCS patients experience greater diaphragm load and fatigue susceptibility at a given ventilation (Frizzelli et al., 2022). Collectively, these data suggest that ventilatory dysfunction may play a central role in limiting exercise tolerance among patients with PCS.

### Autonomic

PCS patients have higher resting heart rate (Frizzelli et al., 2022). At rest, PCS patients demonstrate worsened metrics of heart rate variability (HRV) including SDNN, RMSSD, RR Tri, TINN, LF, LF/HF, SD1, ApEN (Santos-de-Araújo, Bassi-Dibai, et al., 2024).

Heart rate recovery is worse in PCS patients (Frizzelli et al., 2022). In the first minute following exercise, healthy controls dropped an average of 25 BPM whereas PCS patients dropped by 20 (Frizzelli et al., 2022).

During exercise, chronotropic incompetence has been identified among PCS patients (Contreras et al., 2023) (Baratto et al., 2021). For instance, an analysis by Contreras et al. indicated that 44% of PCS patients exhibited chronotropic incompetence during CPET compared to only 34% of healthy control subjects (Contreras et al., 2023).

Compared to healthy controls, PCS patients reporting fatigue as their primary symptoms demonstrated elevated resting heart rate (Baker et al., 2023), reduced HRV (Baker et al., 2023), impaired galvic skin response (Baker et al., 2023), elevated core body temperature (Baker et al., 2023),

Compared to PCS patients without dysautonomia, patients with dysautonomia present with lower VO2 peak (Ladlow et al., 2022).

Autonomic dysfunction may contribute to symptoms of PCS. Compared to PCS patients without autonomic dysfunction, autonomic dysfunction patients are more likely to report, headache (Ladlow et al., 2022), low mood (Ladlow et al., 2022), and some forms of neurocognitive dysfunction such as poor attention (Ladlow et al., 2022). Markers of HPA dysfunction are linked with higher likelihood of sleep disturbance (Sunada et al., 2022).

## Respiratory Influence on PCS Condition

### Respiratory Effects on Vascular Dysfunction

#### Retrograde Shear

In healthy populations, aerobic exercise enhances via two distinct mechanisms: anterograde shear and retrograde shear. Increased anterograde shear occurs due to increased pulses of blood emitting from the heart during systole, stimulating endothelial remodeling. In contrast, retrograde shear is not directly affected by cardiac output and instead occurs due to increased respiration. By reducing intrathoracic pressure, the process of inspiration facilitates retrograde shear by drawing blood through the vessels back towards the heart (Tavoian et al., 2023). Unlike anterograde shear – which appears to affect endothelial remodeling through nitric oxide – the effects of oscillatory retrograde shear appears to involve distinct signaling cascades including redox balance, mechanosensitive pathways (kLF2, Nrf2), and endothelial glycocalyx remodeling <CITATION>. Therefore, subjects with intact respiratory systems will reap the dual and concurrent benefits of anterograde and retrograde shear during exercise. In contrast, individuals with compromised ability to reduce intrathoracic pressure via the respiratory musculature may become limited in their potential for vascular adaptations.

#### Metaboreflex

Another mechanism by which IMT can influence blood flow is through the metaboreflex. During physical activity, lactate and H+ ions accumulate as a result of muscle glycogen breakdown, and these metabolic byproducts stimulate group III/IV phrenic afferent input. Along with lactate and H+, other metabolites such as inorganic phosphate, phosphocreatine depletion, adenosine, and nitric oxide could also contribute to group III/IV phrenic afferent stimulation. This input is sent to the medulla oblongata and pons, which stimulates the sympathetic nervous system (SNS) to create vasoconstriction in the peripheries. As a result, blood supply becomes restricted to the locomotor muscles in order to increase blood supply to the respiratory musculature.

Multiple adaptations to IMT can decrease the magnitude of this metaboreflex, including decreased oscillatory ventilation, Group III/IV phrenic afferent desensitization, and reduced metabolite accumulation due to improved oxidative capacity of the respiratory musculature (Sadek 2022). As a result of decreased SNS-mediated vasoconstriction, this results in reduced vascular resistance which could potentially improve cardiac output by increasing venous return and reducing the amount of work needed to be performed by the heart during systolic contraction to overcome the vascular resistance (ventricle-arterial coupling). Even in the absence of improvements in VO2max, attenuation of the metaboreflex could still induce benefits in physical capacity by enhancing oxygenation status and blood volume of the locomotor muscles (Borghi-Silva 2008).

### Respiratory Effects on Exercise Capacity

#### AVO2 Difference

##### Tidal Volume

*One mechanism by which inspiratory muscle training (IMT) may increase VO2 is by increasing tidal volume during ventilation due to an improved ability for the diaphragm to decrease intrathoracic pressure by creating a greater strength of contraction. In the short-term, this can occur due to improved activation and excursion of the diaphragm muscle. In the long-term, diaphragm thickness could increase and force production may improve. Along with diaphragm strength, IMT also stimulates improvements in endurance of the diaphragm musculature so that it may become more resistant to fatigue during activity. The diaphragm is a highly oxidative muscle that requires sufficient blood flow and becomes fatigued at exercise intensities greater than 80% of VO2max* (Sheel, 2002)*.* For individuals with restrictive deficits that interfere with the ability to move air into the lungs, these factors could ultimately increase the amount of oxygen that is available to diffuse from the alveoli into the bloodstream, thereby improving VO2 by raising AV-difference. To support this possibility, an analysis by Frizzelli et al. identified that PCS patients with a TI/TOT below 0.38 had significantly worsened VO2max and workload during activity, suggesting inspiratory load may be a rate-limiting factor (Frizzelli et al., 2022).

#### Stroke Volume

##### Respiratory Pump

Another mechanism by which IMT could enhance VO2 is by increasing stroke volume via the “respiratory pump” (Salah 2022). When respiratory muscle strength increases, this leads to a decrease in intrathoracic pressure and increase in intraabdominal pressure. This leads to an increased atrial transmural pressure gradient, which reduces atrial pressure and allows for more atrial filling. An increase in ventricle filling at lower pressures with higher volumes leads to an improvement in ventricular compliance. Ultimately, this pressure gradient enhances venous return and myocardial stretch (preload), leading to increased stroke volume on the right. Therefore, the increased negative intrathoracic pressures created by the diaphragm and accessory muscles have the potential to increase stroke volume by increasing venous return (Uva 2015).

##### Vascular Compliance

*End-systolic volume (ESV) also plays a significant role in determining cardiac output. ESV represents the amount of blood remaining after a systolic heart contraction. If ESV increases, this will lead to a decrease in VO2 due to a reduced stroke volume and cardiac output. One factor that goes into determining ESV is ventricle-arterial coupling, which can be defined as end-systolic elastance divided by arterial elastance. In a perfect system, the ratio between these values should be as near to 1 as possible, indicating that the heart does not need to spend excessive energy to overcome the arterial resistance. In cases of increased vascular resistance, however, the heart must work harder to overcome the resistance which can constrain stroke volume and reduce VO2. Additionally, chronic adaptations to aerobic exercise can reduce vascular resistance. This may occur via chemical mechanisms such as improved nitric oxide bioavailability via endothelial function enhancement or improved antioxidant status. Ultimately, by reducing vascular resistance, this enhances venous return and thus increases stroke volume by increasing EDV. Also, reduced vascular resistance resulting from chronic endurance training could also improve ventriculoarterial coupling, reducing ESV because the heart does not need to produce as much energy to overcome the vascular resistance. Furthermore, contractility, could be higher due to increased venous return causing an increase in the Frank-Startling mechanism.*

In PCS, respiratory dysfunction is associated with worse performance on 6MWT and greater reports of dyspnea (Cortés-Telles et al., 2021) (Hennigs et al., 2022).

### Respiratory Effects on Autonomic Nervous System

During physical activity, lactate and H+ ions accumulate as a result of muscle glycogen breakdown, and these metabolic byproducts stimulate group III/IV phrenic afferent input. Additionally, this phrenic afferent input may also be increased by other metabolite such as phosphocreatine depletion, nitric oxide, or adenosine. These afferents from the lungs travel to regions of the limbic system such as the amygdala, thalamus, and insula, which regulates activity of the hypothalamic pituitary adrenal (HPA) axis. Therefore, breathing is intricately interlinked with activity of the Autonomic Nervous System (ANS) and HPA. As breathing increases, SNS activity subsequently increases. One of the key adaptations to IMT is reduced respiratory rate and reduced fatigue of the diaphragm which leads to reduced afferent input stimulating SNS activity. Along with reducing respiratory rate and respiratory muscle fatigue, IMT could also reduce SNS activity by improving baroreflex sensitivity and inducing the lung inflation reflex through improved tidal volume. For these reasons, IMT has been demonstrated to improve markers of ANS function such as heart rate variability (HRV) and chronotropic index (CI). This may be particularly important for long COVID patients, which is a population characterized by high prevalence of ANS dysfunction. In fact, a secondary analysis of IMT for long COVID patients determined that the long COVID patients that were most likely to exhibit improvements in VO2 peak from IMT were those who had signs of autonomic dysfunction at baseline (Palau 2024).

Compared to healthy controls, PCS patients reporting fatigue as their primary symptoms demonstrated elevated resting heart rate (Baker et al., 2023), reduced HRV (Baker et al., 2023), impaired galvic skin response (Baker et al., 2023), elevated core body temperature (Baker et al., 2023),

Dysautonomia is more common among PCS patients with signs of respiratory dysfunction including higher respiratory rate at rest (Ladlow et al., 2022) or elevated VE/VCO2 (Ladlow et al., 2022).

### Respiratory Effects on Dyspnea

The subjective sensation of dyspnea appears directly related to the proportion of inspiratory force produced at any given time relative to an individual’s maximum inspiratory force generating capacity (O’Donnell et al., 1997). Individuals with low respiratory strength are therefore more vulnerable to sensations of breathlessness when the force requirements for relatively light activity approximates near maximal inspiratory capacity.

This weakness is likely the key driver of dyspneic sensations in PCS. Following acute COVID infection, inspiratory strength measures appear relatively low and directly correlated with dyspnea (Dosbaba et al., 2023). These dyspneic changes typically occur in the absence of identifiable cardiopulmonary pathology (Kaye et al., 2022; von Werder et al., 2025). The explanation behind these counterintuitive findings was discovered in an analysis by Nagel et al. which found that FEV1, FVC, DLCO, and radiological findings in patients with PCS were unable to explain dyspnea symptoms. Instead, the only metric that associated strongly with dyspnea was MIP (Nagel et al., 2022). Further corroborating these findings is a longitudinal analysis by Spiesshoefer et al. indicating that changes in diaphragm strength over time are strongly and directly correlated with corresponding changes in dyspneic symptomology (Spiesshoefer et al., 2024).

### Respiratory Effects on Fatigue

Chronic fatigue prevalence is disproportionately represented among patients with respiratory conditions (Gruet, 2018), and the magnitude of fatigue is proportional to the severity of dyspnea (Kapella et al., 2006). Following acute COVID infection, fatigue problems are more common in patients with dyspnea compared to patients with normal breathing (Cortés-Telles et al., 2021). In PCS, a significant correlation persists between breathing difficulty and self-reported fatigue (Twomey et al., 2022).

### Respiratory Effects on Neurocognitive Dysfunction

Despite the severe and debilitating neurocognitive symptoms reported by patients with PCS, MRI of the cerebrum has identified abnormalities in only a small percentage of subjects reporting cognitive dysfunction (Davis et al., 2021), Similarly, no cerebral pathology has been identified that has associated with chronic and debilitating fatigue.

Rather than cerebral damage, one potential pathophysiological explanation may be weakness to the diaphragm.

In a study of 1680 PCS patients, the number one predictor of neurocognitive dysfunction was respiratory dysfunction. Subjects with respiratory dysfunction were 1.95 times more likely to develop brain fog compared to subjects without respiratory dysfunction, whereas no other medical pathologies demonstrated any relationship (Asadi‐Pooya et al., 2022). Similarly, a study of 29 PCS patients concluded that worsened respiratory symptoms were associated with worsened global cognitive impairment and executive function (Miskowiak et al., 2021).

Symptoms of dyspnea have been identified to be associated with increased likelihood of experiencing brain fog. For instance, an analysis by Bulla et al. found that 37.5% of PCS patients with neurocognitive dysfunction reported major signs of dyspnea, compared to only 6.3% of patients without neurocognitive dysfunction (Bulla et al., 2023). Similarly, Nordvig et al. found that 48% of patients with neurocognitive dysfunction reported dyspnea compared to 18% without (Nordvig et al., 2023).

### Respiratory Effects on Sleep

During sleep, upper airway narrowing and increased airflow resistance coupled with reduced contribution from the intercostals instill increased force production demands of the diaphragm (Severin, 2022; Yokoba et al., 2016). In healthy individuals, the diaphragm can meet these demands with relatively low neural drive, permitting stable ventilation without allowing cortical arousal to disrupt deep sleep. When diaphragm force-generating capacity is compromised, however, greater neural respiratory drive is required to sustain tidal volume. Evidence from Lueo et al. demonstrates that elevated neural drive to the diaphragm to cope with apnea — rather than apnea itself — is a primary generator of excessive cortical arousal (Luo et al., 2008). Consequently, in individuals with weakened inspiratory muscles, repeated drive-induced cortical arousal throughout the night may fragment REM and non-REM sleep to the extent to which restorative functions become compromised.

Indicators of respiratory endurance such as SMIP slope have been associated with sleep quality (Severin, 2022).

### Respiratory Effects on Quality of Life

Indicators of respiratory endurance such as ID have been associated with self-reported physical activity (Severin, 2022).

Greater degree of respiratory dysfunction is associated with worsened ability to participate in occupational activities (Hennigs et al., 2022).

## Inspiratory Muscle Training for PCS

### IMT on Respiratory Function and Dyspnea

# IMT has been shown to improve mechanical properties of the diaphragm including contraction velocity (Benli et al., 2024), excursion (Benli et al., 2024), and thickness (Tanriverdi et al., 2023). These structural adaptations translate into improvements in clinical metrics such as MIP (Gosselink et al., 2011). Most importantly, IMT improves PDIsniff and PESsniff (Langer et al., 2018) (Spiesshoefer et al., 2024), which is the gold standard of diaphragm strength testing <CITATION>.

# By improving diaphragm function, IMT has a direct beneficial effect on symptoms of dyspnea (Gosselink et al., 2011)(Spiesshoefer et al., 2024), and the degree of dyspnea reduction appears directly correlated with the magnitude of enhanced diaphragm functioning (Spiesshoefer et al., 2024). These benefits carryover to reduced dyspnea during physical activity (Campos et al., 2018; Langer et al., 2018; Spiesshoefer et al., 2024).

### IMT on Vascular Function

IMT acutely alters shear stress patterns, which is believed to be the proximal stimulus for improvements in FMD. For 1-2 heart beats per load-resisted breath, retrograde shear rate effectively doubles during inspiration and subsequently returns to normal during expiration (Tavoian et al., 2023). Over time, this pulsatile shear stimulus can positively stimulate adaptations for vascular remodeling. Chemical changes indicating improved endothelial function include reduced syndecan-1, angiopoietin-2, and endothelin-1 (Campos et al., 2018).

IMT attenuates the respiratory metaboreflex, thereby permitting increased blood flow distribution to the locomotor muscles. For instance, an analysis by Yanez-Sepulveda identified tissue saturation index (TSI) of the quadricep muscles changed significantly in response to IMT, indicating enhanced extraction secondary to improved perfusion (Yáñez-Sepúlveda et al., 2022). This hemodynamic redistribution may partly explain some studies finding improved lower extremity strength in response to IMT (Tanriverdi et al., 2023) (Katayıfçı et al., 2022).

Consequently, prolonged IMT has been demonstrated to have long-term effects on FMD (Craighead et al., 2022; Freeberg et al., 2023). For instance, <EXAMPLE>. In some instances this can reduce systolic blood pressure (Craighead et al., 2022; Freeberg et al., 2023).

### IMT on Exercise Capacity

IMT can improve functional exercise capacity in tests such as 6MWT (Abodonya et al., 2021; Ammous et al., 2023; Chen et al., 2023; Katayıfçı et al., 2022; Tanriverdi et al., 2023) (Gosselink et al., 2011).

During CPET testing, IMT has been shown to improve VO2 peak (Yáñez-Sepúlveda et al., 2022) (Jimeno-Almazán et al., 2023) (Chen et al., 2023)

The effect of IMT on VO2 appears influenced by degree of respiratory weakness. For instance, an analysis by Trevizan et al. found that IMT improved VO2 peak by 20% in patients with baseline inspiratory muscle weakness compared to only 8% with normal inspiratory strength (Trevizan et al., 2021).

### IMT on Autonomic Function

IMT reduces resting heart rate (Campos et al., 2018).

IMT reduced muscle sympathetic nerve activity (MSNA) by 26% in patients with inspiratory muscle weakness compared to 10% in patients with normal inspiratory strength (Trevizan et al., 2021).

IMT improves multiple metrics of HRV including RMSSD (Edgell et al., 2025; Tanriverdi et al., 2023), LF/HF (Edgell et al., 2025), pRR50 (Edgell et al., 2025). During exercise, chronotropic exercise improves significantly following an intervention of IMT (Palau et al., 2022).

### IMT on Dyspnea

IMT reduces dyspnea (Saglam et al., 2015).

### IMT on Sleep

IMT improves objective markers of sleep quality such as apnea hypopnea index (AHI) (Azeredo et al., 2022), leading to improvements in subjective reports of sleep quality (Edgell et al., 2025) (Azeredo et al., 2022)

Another benefit of repeated IMT is improved tone of the upper airway musculature. In particular, strengthening of the pharyngeal dilator muscles such as stylopharyngeus and palatopharyngeus may be beneficial for maintaining airway tone during sleep. Previous research literature has indicated that IMT can be effective for reducing severity of sleep apnea or sleep-disordered breathing. This is particularly important for long COVID patients due to high rates of sleep disorders in this population.

### IMT on Fatigue

IMT reduces fatigue for patients with CHF (Tanriverdi et al., 2023) (Katayıfçı et al., 2022), OSA (Azeredo et al., 2022), PHA (Saglam et al., 2015).

### IMT on Neurocognitive Dysfunction

Cranial blood vessels respond to repeated bouts of shear stress to stimulate dilation (Smith 2017). During IMT, large retrograde shear forces induced by loaded inspiration repeatedly expose the cerebrum to transiently elevated arterial CO2. Over time, the blood vessels adapt by increasing their capacity for dilation in response to hypercapnic stimuli. Supporting this possibility, an analysis by Freeberg et al. reported a 120% improvement in cerebrovascular reactivity to hypercapnia following 6 weeks of IMT, consistent with enhanced endothelial function in the cerebral circulation (Freeberg et al., 2023).

These cerebral changes have the potential to translate into enhanced cognitive function. For instance, following a course of IMT, Freeberg et al. found considerable improvements in episodic memory (Freeberg et al., 2023).

### IMT on Quality of LIfe

IMT improves self-reported functional status (Palau et al., 2022; Tanriverdi et al., 2023) and physical activity (Katayıfçı et al., 2022) (Jimeno-Almazán et al., 2023). Ultimately, these gains translate to self-reported improvements in quality of life (Gosselink et al., 2011).

## Research Questions and Hypotheses

### Gaps in the Literature

### Aims

In order to address these distinct gaps in the literature, a randomized controlled trial is warranted to address the following research questions:

**Aim 1)** Can IMT improve objective physiological parameters in PCS?

**Aim 2)** Can IMT improve subjects self-reported symptoms of PCS?

**Aim 3)** Are changes in self-reported symptoms following IMT mediated by changes in objective physiological parameters?

By addressing these aims, this analysis will help to gain insight into the pathophysiological mechanisms underpinning PCS symptoms and guide clinical practice on physical therapy rehabilitation treatment.

# Methods

## Ethics

### Informed Consent

Prior to enrollment in the study, participants were provided with written informed consent procedures describing the protocols of the study, the intended risks, and the expected benefits of participation. After reviewing the written information in the informed consent and discussing verbally with research personnel, individuals were offered the option to decline to participate.

## Study Design

## Participants

### Sample Size

A meta-analysis by <AUTHOR> examining IMT on long COVID identified a <EFFECT SIZE> for parameter 1 and a <EFFECT SIZE> for parameter 2 <CITATION>. A power analysis was performed using GPower <VERSION> using the following settings: ANOVA Repeated Measures, within-between interaction F-Test, 5% alpha. With these parameters, it was estimated that <SUBJECTS> would provide 80% power to detect a difference between groups. <SUBJECTS> were recruited to account for potential dropout.

### Inclusion Criteria

????

### Exclusion Criteria

????

## Intervention

### Inspiratory Muscle Training

#### Intervention Group

IMT was performed using techniques validated in previous clinical studies. Participants in the intervention group performed IMT using a PowerBreathe KH2 (HaB International, UK) (Langer et al., 2018) (Spiesshoefer et al., 2024). This device features a one-way electronical-loaded valve at one end and a mouthpiece on the other end through which subjects will be required to breathe in hard. The electronical-loaded valve (tapered flow-resistive loading) which will gradually introduce resistance or load to breathing during the treatment session until reaching the target loading.

Intensity was initially set to 60% of baseline MIP and resistance was updated weekly to maintain 60% of MIP while accommodating continuous weekly strength gains (Bhatnagar et al., 2021; Katayıfçı et al., 2022; Krause-Sorio et al., 2021)(Figueiredo et al., 2018)(Archiza et al., 2018).

Participants were instructed to perform 30 breaths twice per day (Ahmadnezhad et al., 2020; Benli et al., 2024; Chung et al., 2021; Schaeffer et al., 2023; Spiesshoefer et al., 2024) on 7 days per week (Ahmadnezhad et al., 2020; Azeredo et al., 2022; Jimeno-Almazán et al., 2023; Langer et al., 2018; Winkelmann et al., 2009). Participants performed IMT remotely and communicated with research staff a minimum of once per week (Alwohayeb et al., 2018).

#### Control Group

Subjects assigned to the control group will receive a <DEVICE NAME>. The resistance will be set to its minimal resistance, which is 10 cmH2O. Subjects were instructed to maintain the load at 10 cmH20 throughout the duration of the study.

## Measures

### Respiratory

#### Methods

A handheld digital manometer (PrO2TM, PRO2Fit Health Inc., Smithfield, RI, USA) was used to assess MIP, SMIP, Slope of SMIP, FIT, and inspiratory duration (ID). A flow-based device (PowerBreathe KH2, POWERbreathe International Ltd., Southam, UK) was used to obtain PIF, S-Index, and inspiratory volume (IV). PrO2 maneuvers followed the Test of Incremental Respiratory Endurance protocol, consisting of a maximal sustained inhalation from residual volume (RV) to total lung capacity (TLC) held for as long as possible. PowerBreathe maneuvers consisted of sharp maximal inhalations from RV to TLC. All procedures were performed in accordance with American Thoracic Society/European Respiratory Society guidelines (Laveneziana et al., 2019) and have previously demonstrated excellent reliability (Formiga et al., 2018). A minimum of three trials were performed and additional trials were added if MIP values differed by more than 10% from one another. The highest reproducible MIP, SMIP, PIF, S-Index, and volume were recorded. FIT, Slope of SMIP, and ID values were obtained from the trial producing the highest SMIP (Formiga et al., 2018).

Because prior studies have demonstrated inspiratory weakness can result in declining respiratory measures following physical activity (Coast et al., 1990; Severin, 2022), all measures were taken immediately before CPET and again immediately after maximal exercise testing to capture potential post-activity changes.

#### Outcomes

MIP (Maximum Inspiratory Pressure) – greatest negative pressure sustained for at least one second

SMIP (Sustained Maximal Inspiratory Pressure) – calculated as the area under the pressure-time curve

Slope of SMIP – rate of pressure development measured by slope of the SMIP plot

FIT (Fatigue Index Time) – combined ratio measure of MIP, SMIP, and Slope of SMIP (SMIP x ID / MIP / Slope)

ID (Inspiratory Duration) – maximal length of sustained inhalation

PIF (Peak Inspiratory Flow) – maximum liters of air flow per minute

S-Index –highest point of the pressure x time graph representing peak inspiratory pressure under dynamic flow

IV (Inspiratory Volume) – liters of volume of air inhaled in a single breath

### Vascular Function

#### Methods

Seated resting blood pressure (SBP, DBP) was measured on subjects’ arms using an automated microprocessor controlled ambulatory blood pressure monitor (Mobil-O-Graph 24 PWA, I.E.M, Stolberg, Germany). To ensure reliability of obtained values, American Heart Association guidelines were followed including instructing participants to sit with feet flat, legs uncrossed, back supported, and to avoid speaking while measurements were collected (Muntner et al., 2019).

High-resolution Duplex ultrasound (Prosound Alpha 7, Hitcahi-Aloka, Japan) was used to image the brachial artery using a 5-13MHz linear probe. Simultaneous determination of artery diameter (B-mode) and flow velocity (Doppler mode) were implemented. Subjects rested supine for 10 minutes beforehand and were instructed not to speak or move during imaging. A pneumatic cuff was placed on the forearm approximately 3 cm distal to the antecubital fossa (Mućka et al., 2022). The cuff was inflated to double of patients’ resting SBP and maintained for 5 minutes to induce ischemia. Following rapid release of cuff pressure, changes in Doppler flow and arterial diameter were recorded and evaluated using Quipu FMD Studio edge-detection software (Cardiovascular Suite, Quipu, Pisa, Italy). Baseline arterial diameter was taken from 1-minute pre-occlusion. Maximum diameter was taken 1-2 minutes after cuff release.

#### Outcomes

SBP (Systolic Blood Pressure) – peak arterial pressure during ventricular contraction

DBP (Diastolic Blood Pressure) – minimum arterial pressure during ventricular relaxation

FMD (Flow Mediated Dilation) – percent change in brachial artery diameter relative to baseline (maximum diameter – baseline diameter) / (baseline diameter)

Velocity – peak blood flow velocity recorded by Doppler averaged over several cardiac cycles

SR (Shear Rate) – estimate of frictional force on the endothelium due to blood flow (4 × Velocity / diameter)

Flow – liters of blood flow passing through brachial artery per minute (velocity \* π \* radius2 \* 60)

AUC (Area Under Curve) – total shear stimulus over time after cuff release

### Cardiopulmonary Exercise Testing (CPET)

#### Methods

Conventional incremental treadmill protocol (Bruce) was conducted involving graded increases of intensity and incline every 3 minutes. Participants were fitted with a mouthpiece to obtain ventilatory rate volume and expired gases which were analyzed with raw data recorded breath-by-breath through a metabolic cart and compiled into 30-second rolling averages for analysis (Parvo TrueOne 2400, Parvo Medics, Sandy. UT) using techniques consistent with guidelines from the American Heart Association (AHA) (Balady et al., 2010) and American Thoracic Society/American College of Chest Physicians (ATS/ACCP) (“ATS/ACCP Statement on Cardiopulmonary Exercise Testing,” 2003). Heart rate was monitored continuously using telemetric cardiac monitor (Polar® T31, Polar Electro oy, Kempele, Finland). Consistent with previous literature on PCS, few participants reached traditional maximal effort termination criteria and thus all tests were classified as symptom limited (Thomas et al., 2025). The highest 30 second average oxygen uptake reached was used as the peak value. Following termination of peak activity, participants walked for 2 minutes at 1.5 MPH at an incline of 2.5% to capture HRR <CITATION>.

Anaerobic Threshold (AT) was estimated using the V-Slope method which identifies the inflection point where carbon dioxide (VCO2) increases disproportionately relative to oxygen (VO2). V-Slope method has previously been used in PCS literature (Cherneva et al., 2025) and has demonstrated to be of acceptable validity for estimating true lactate threshold relative to other methods (Gaskill et al., 2001). Breath-by-breath VO₂ and VCO₂ data were first smoothed using a centered rolling average to reduce noise. The smoothed dataset was then iteratively partitioned across candidate breakpoints, and linear regressions were fitted to the segments before and after each breakpoint. A breakpoint was accepted as the AT if (1) the slope of VCO₂ vs. VO₂ increased by a minimum threshold between the two segments, and (2) both regressions demonstrated acceptable linearity (R² > 0.95). The candidate breakpoint with the best overall fit was selected as the AT. In cases where no breakpoint met these strict criteria, a secondary (“fallback”) V-slope procedure was applied. This method used a lighter smoothing filter and more permissive thresholds for slope change and model fit, to allow detection of subtler inflection points. If an inflection point was still not detected, the AT was recorded as not reached.

#### Outcomes

VO2 (absolute) – liters of oxygen per minute

VO2 (relative) – milliliters of oxygen per kilogram body mass per minute

AT (absolute) – VO2 at anaerobic threshold

AT (relative) – (VO2 at anaerobic threshold) / (VO2 at peak)

VCO2 – liters of carbon dioxide per minute

RR (respiratory rate) – breaths per minute

Vt (tidal volume) – liters of air inhaled per breath

VE (minute ventilation) – RR x Vt

VE/VO2 – ventilatory equivalent for oxygen

VE/VCO2 – ventilatory equivalent for carbon dioxide

VE/VCO2 slope – slope of the linear regression for VE versus VCO2

O2 Pulse – mL of oxygen consumed / heart beat (VO2 relative / BPM)

PetCo2 – partial pressure of end-tidal carbon dioxide

RER – VCO2 / VO2

OUES (Oxygen uptake efficiency slope) – slope of the regression of VO₂ on log-transformed VE

### Autonomic Function

#### Methods

To obtain HRV measurements, subjects were instructed to lie supine for 10 minutes prior to recording and were instructed not to move or speak once recording was initiated. RR-intervals were captured during spontaneous breathing for a minimum of 10 minutes using a heart rate monitor chest strap with a sampling rate of 1000 Hz which has previously established strong concurrent validity with HRV measurements using ECG (r >= 0.95) (Gilgen-Ammann et al., 2019; Schaffarczyk et al., 2022). RR-Intervals were imported into Kubos Software 4.2.0 (Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Kuopio, Finland) and analyzed using parameters previously validated in the PCS population (Santos-de-Araújo et al., 2024). Specifically, the R-R interval series underwent detrending using the smoothing method with lamda of 500 and cubic interpolation of 4 Hz. Artifacts were corrected using the low filter settings. A 5-minute segment with the greatest signal stationarity was selected for analysis with the following criteria: 1) no large R-R outliers 2) equidistant RR-intervals 3) normal distribution of R-R intervals visually observed in graphs. For the linear analysis in the frequency domain, spectral analysis was performed using Fast Fourier Transform (FFT). This method for calculating HRV has established high reliability (ICC >= 0.95; CV < 10%) (Santos-de-Araújo, Oliveira, et al., 2024).

#### Outcomes

RHR – resting heart rate beats per minute in supine

SDNN – standard deviation of all normal N-N intervals

RMSDD – square root of successive mean squared differences of RR

RR Tri – integral of the density of the RR interval histogram divided by its height

TINN – baseline width of a histogram displaying NN intervals

LF –low-frequency domains (0.04 – 0.15 Hz)

HF – high-frequency domains (0.15 – 0.4 Hz)

SD1 – standard deviation perpendicular to the line of identity

SD2 – plot the standard deviation along the line of identity

ApEn – approximate entropy

SampEn – sample entropy (embedding dimension: 2; tolerance: 0.2 x SD)

DFa1 – short-term (4-12 beats) fluctuations (1 = chaotic, 1.5 = regular, 0.5 = randomness)

DFa2 – long-term (13 – 64 beats) fluctuations (1 = chaotic, 1.5 = regular, 0.5 = randomness)

HRR1 – (peak HR) – (HR at 1 minute post-activity following CPET)

HRR2 – (peak HR) – (HR at 2 minutes post-activity following CPET)

CI (Chronotropic Index) – (peak HR during CPET – RHR supine) / (220 – age – RHR supine)

### Dyspnea

#### Methods

Dyspnea was measured using Modified Medical Research Council (MMRC) and Transitional Dyspnea Index (TDI) scales.

#### Outcomes

MMRC – grade between 0 to 4

TDI – grade between -3 to -9

### Physical Functioning

#### Methods

To assess patients’ ability to participate in physical activities, the Physical Function subdomain was used from the Short Form-36 (SF-36).

#### Outcomes

SF-PA – score between 0 to 100

### Sleep

#### Methods

Sleep was assessed using the Pittsburgh Sleep Quality Index (PSQI).

#### Outcomes

PSQI Disturbances – subcomponent of PSQI

PSQI Total – cumulative total of 7 PSQI subcomponents

### Fatigue

#### Methods

Fatigue was measured using the Fatigue Severity Scale (FSS) and Visual Analogue Scale (VAS). To assess the PEM component of fatigue, the Depaul Symptom Questionnaire (DSQ) was used.

#### Outcomes

FSS – cumulative sum of FSS score

VAS – scale of 0-10

DSQ – cumulative sum of Depaul Symptom questionnaire

### Neurocognitive Dysfunction

#### Methods

To assess subjective reports of brain fog, the Woods Mental Fatigue Inventory (Woods) was used.

#### Outcomes

Woods – cumulative sum of items

## Statistical Analysis

### Statistical Software

Statistical analyses were performed using lme4 and mediation packages in R V4.3.1.

### Descriptive Statistics

Descriptive statistics were presented as means ± standard deviations for continuous variables and as frequencies (percentages) for categorical variables. Independent samples t-tests were used to compare baseline continuous variables between the intervention and control groups. Chi-square tests were used to compare categorical variables. Normality was assessed using visual inspection of Q-Q plots, and Mann-Whitney U tests were used in place of independent samples t-tests for non-normally distributed data.

### Group Comparison

Within-group changes were assessed using paired samples t-tests. Normality was assessed using visual inspection of QQ-plots, and Wilcoxon signed-rank tests were used in place of Paired T-tests for non-normally distributed data.

Between group changes were assessed using Split Plot ANCOVAS to evaluate for group x time interactions. ANCOVA models have previously been used in IMT clinical trials (Dos Santos et al., 2019; Tanriverdi et al., 2023) and are considered to be optimal for group comparisons due controlling for baseline differences with reduced risk of Type I error (Valente & MacKinnon, 2017; Vickers, 2001; Vickers & Altman, 2001). Normality of residuals were assessed by visually inspecting Q-Q plots. Homoscedasticity was assessed through visual inspection of the residuals plot, and robust standard errors (HC3 sandwich estimator) were used when heteroscedasticity was detected. To confirm homogeneity of regression slopes in ANCOVA models, an interaction term between baseline outcome and group (baseline outcome × group) was included; a non-significant interaction indicated that the assumption of homogeneity of slopes was met. In such instances, a general linear model (GLM) was created with the independent variable, baseline covariate, and independent variable x baseline covariate interaction term to evaluate differences in responsiveness to intervention depending on baseline status.

### Mediation

Mediation analyses were be conducted using the following hierarchical linear mixed regression model:

MIPij = B0 + B1 \* Timeij + uoi + Eij

FMDij = B0 + B1 \* Timeij + uoi + Eij

Vo2maxij = B0 + B1 \* Timeij + uoi + Eij

Outcomeij = yo + y1 \* Timeij + y2 \* MIPij + y3 \* FMDij + y4 \* Vo2maxij + u0i + Eij

Where:

i = participant, j = time

uoi = random intercept for participant i

The indirect effect is represented as Path A x Path B:

A = effect of time on mediator (from mediator models)

B = effect of mediator on outcome (from outcome models)

Time (pre-intervention, post-intervention) was coded categorically and serve as an independent variable. Objective parameters of long COVID (respiratory muscle strength, FMD, VO2max) was specified as mediators, with patient-reported outcomes (e.g., fatigue, neurocognitive dysfunction) as dependent variables. Models included time and mediators as fixed effects, with subject ID included as a random intercept to account for repeated measures.

For each mediator, three pathways will be estimated: (i) the effect of time on the mediator (Path A), (ii) the association between the mediator and the outcome while controlling for time (Path B), and (iii) the total (Path C), direct (Path C′), and indirect (Path AB) effects of time on the outcome. Indirect effects will be tested using nonparametric bootstrapping with 1,000 simulations, and mediation will be considered present when the 95% confidence interval (CI) for the indirect effect does not include zero.