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

**By**

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**Proposal for Dissertation Research**

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# Introduction

## Background

## Symptoms of PCS

### Fatigue

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). 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).

Of particular interest, PCS patients are particularly vulnerable to a form of fatigue known as “post-exertional malaise” (PEM).

>Whilst symptoms are broad, common symptoms include reduced energy, an increase in muscle soreness, breathing difficulties, and feeling sick/nauseous which occur usually 24-72 h after patients exceed the thresholds of their reduced “energy envelope’.17 [@Faghy2024DevelopingEffectiveStrategies]”

### Neurocognitive Dysfunction

Neurocognitive dysfunction, commonly referred to as “brain fog”, is regularly considered by PCS patients to be one of the most debilitating 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). Given that diaphragm weakness persists for 31 months following infection with no improvement, it is of little surprise that patients do not report spontaneous resolution of dyspnea symptoms during this time frame (Spiesshoefer et al., 2024).

Dyspnea is also more common during physical activity (Frizzelli et al., 2022)(Barbagelata et al., 2022). For instance, 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).

### Sleep

A large portion of patients with PCS report significantly impaired sleep (Davis et al., 2021; Sa et al., 2024). 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 insomnia (Davis et al., 2021; Jennings et al., 2022; Nordvig et al., 2023) and nightmares (Davis et al., 2021).

### 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). Moreover, leaving the home becomes difficult as subjects lose confidence in their ability to drive safely while battling brain fog. (Chasco et al., 2022).

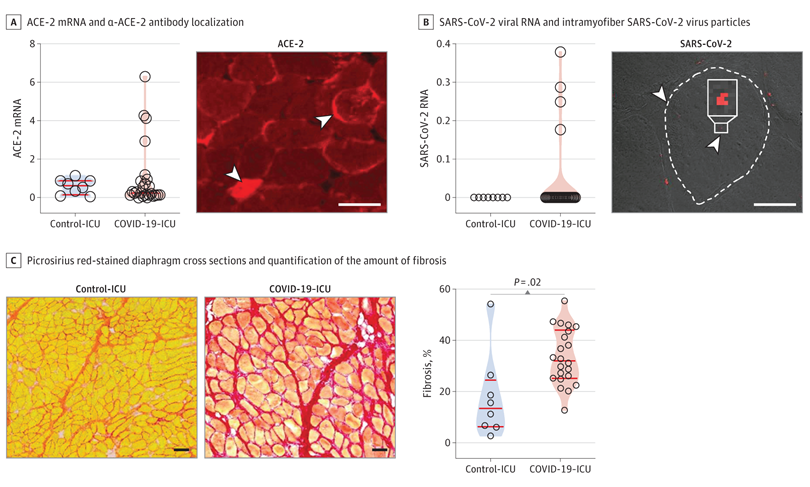
Social relationships are hampered by PCS symptoms dramatically. Many subjects report a sense of stigma for sharing their PCS symptoms, with peers doubting legitimacy of the PCS condition and not understanding the relapsing-remitting nature of the disease (Chasco et al., 2022). Some participants report 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). Parents PCS patients with children report difficulty with being present as a parent, feeling guilty for sometimes shoulder 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. 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), leading to widespread symptoms of myalgia <CITATION>. While all regions of the musculoskeletal system are vulnerable, the diaphragm is particularly vulnerable due to increased proportion of ACE-2 <CITATION>. Consequently, epimysial and perimysial fibrosis of the diaphragm ensues due to direct infiltration by COVID-19 virus RNA (Shi et al., 2021). These structural changes are evident via ultrasound examination in the form of reduced thickness and thickening ratios (Farr et al., 2021). During functional testing, respiratory indices become harmed following COVID-19 infection (Dosbaba et al., 2023; Li et al., 2020).. For instance, an analysis by <EXAMPLE>.



These respiratory deficits persist in PCS. During inhalation, PCS patients demonstrate reduced maximum inspiratory pressure (MIP) (McNarry et al., 2022).

Importantly, the respiratory deficits identified in PCS do not spontaneously resolve over time. For instance, a study using twitch transdiaphragmatic pressure – the gold standard assessment of diaphragm contractility <CITATION> – found insignificant improvement 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, indicating that patients were gradually regaining voluntary control of the diaphragm during their recovery period but were still limited in maximum force production capacity. 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 so 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).

## 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, individuals with dyspnea exhibit worse performance on 6MWT (Cortés-Telles et al., 2021).

### 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 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 inspiratory force required to perform relatively light activities is close to maximal inspiratory capacity. A greater mismatch between neural respiratory drive and mechanical output amplifies this sensation (De Peuter et al., 2004), which likely underlies the observed correlations between dyspnea with inspiratory strength and endurance measures following acute COVID infection (Dosbaba et al., 2023). In the PCS population, longitudinal findings further suggest that improvements in diaphragm strength directly translate to reductions in dyspnea (Spiesshoefer et al., 2024). Inspiratory weakness likely explains debilitating dyspnea among many participants who lack detectible signs of cardiopulmonary pathology (Kaye et al., 2022; von Werder et al., 2025).

The influence of respiratory capacity on dyspnea becomes particularly evident during physical activity when ventilatory demand may exceed available capacity. Given that elevations in arterial CO2 can independently provoke feelings of breathlessness in mechanically ventilated or paralyzed subjects, dyspnea may partly be attributed to physiological drive to correct blood gas disturbances (De Peuter et al., 2004). In the case of PCS, impaired ventilation during activity (Baratto et al., 2021; Contreras et al., 2023) likely compounds these mechanisms, further intensifying symptoms.

### 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 easily generate these forces with relatively low neural drive, allowing stable ventilation without cortical arousal disrupting deep sleep. However, when diaphragm force-generating capacity is compromised, greater neural respiratory drive is required to sustain tidal volume. Evidence from Lueo et al. demonstrates that elevated neural drive to the diaphragm — rather than the apnea event 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 during sleep 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).

## 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

# 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

## Population

## 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.

## 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

Because prior studies have demonstrated respiratory measures may decline substantially following physical activity in patients vulnerable to inspiratory weakness, all measures were taken immediately before CPET and again immediately afterwards to examine post-activity changes.

#### Measures

A PrO2 manometer was used to record MIP, FIT, SMIP, SMIP Slope, and Inspiratory Duration. A PowerBreathe KH2 was used to record PIF, S-Index, and Volume.

### Vascular Function

#### Methods

Seated resting blood pressure was measured in the arm 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, participants were instructed to sit with feet flat, legs uncrossed, back supported, and not to talk during in line with American Heart Association guidelines (Muntner et al., 2019).

High resolution Duplex ultrasound imaging was conducted using a 5-13MHz linear probe (Prosound Alpha 7, Hitcahi-Aloka, Japan). The brachial artery was imaged using a dual screen with simultaneous determination of artery diameter (B-mode) and flow velocity (Doppler mode). Subjects were instructed to rest for 10 minutes supine before recording was initiated and were instructed not to speak or move during recording. After one minute of baseline recording, a blood pressure cuff placed on the forearm was inflated to double of resting systolic pressure and maintained for 5 minutes. Following rapid release of cuff pressure, changes in Doppler flow and arterial diameter were recorded and evaluated using Quipu FMD Studio edge detection software. Values for analysis were selected from the time period 1-2 minutes following cuff deflation.

#### Outcomes

Blood flow and intra-arterial shear rate will be determined by following formulas: Blood Flow L. min−1 , where πr2 equals the cross-sectional area of ( ) = πr2 ( )× Velocity cm. s−1 () the artery. Shear Rate (1. s−1) = 4× Velocity cm.s−1 () diameter (cm) Arterial Vascular conductance (CVC) will be determined by accounting for changes in mean arterial pressure (MAP) assessed through finger photoplethysmography (Finapres Nano, Finapres, ADInstruments, Colorado, USA). Blood flow and vascular conductance will be normalized to limb lean mass to account for differences or changes in lean mass.

### Cardiopulmonary Exercise Testing (CPET)

#### Methods

Conventional incremental treadmill protocol (Bruce) will be conducted involving graded increases of intensity and incline at period intervals. Oxygen uptake was measured using a ParvoMedics TrueOne metabolic system. Peak effort will be determined using <ELABORATE>. The highest 30 second average oxygen uptake reached during the test will be used as the peak value. Heart rate will be monitored continuously using <HEART RATE>.

#### Outcomes

HRR was measured during CPET. Following termination of peak activity, participants walked for 2 minutes at 1.5 MPH at an incline of 2.5% inline with clinical guidelines <CITATION>.

### Autonomic Function

#### Methods

To obtain HRV measurements, subjects were instructed to lie supine for 10 minutes. Following 10 minutes of rest, RR-intervals were captured using a <HEART RATE MONITOR> which has established concurrent validity with EKG (r=????) <CITATION>. Patients were instructed not to speak or move during measurement. RR-Intervals were imported into Kubos Software an analyzed using parameters previously validated in the PCS population (Santos-de-Araújo et al., 2024). <KUBOS PROCESS> This method for calculating HRV has established high reliability (ICC >= 0.95; CV < 10%) (Santos-de-Araújo, Oliveira, et al., 2024).

#### Outcomes

HRV: SDNN, RMSSD, LF/HF

CPET: HRR1, HRR2, CI

### Dyspnea

### Functional Capacity

### Sleep

### Fatigue

### Neurocognitive Dysfunction

## Statistical Analysis

### Statistical Software

Statistical analyses were performed using lme4 and mediation packages in R 3.12.4.

### 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 and 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 Type I error (Valente & MacKinnon, 2017; Vickers, 2001; Vickers & Altman, 2001). Breusch-Pagan tests were used along with visual inspection of the residuals plot to assess for homoscedasticity, 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.

### Mediation

Mediation analyses will be conducted using a hierarchical linear modeling framework using the following 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) will be coded categorically and serve as the independent variable. Objective parameters of long COVID (respiratory muscle strength, FMD, VO2max) will be specified as mediators, with patient-reported outcomes (e.g., fatigue, neurocognitive dysfunction) as the dependent variables. Models will include baseline values and time 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.