# Using cardiorespiratory fitness assessment to identify pathophysiology in long COVID - Best practice approaches.

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# Using cardiorespiratory fitness assessment to identify pathophysiology in long COVID – Best practice approaches

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ABSTRACT

Cardio-respiratory fitness (CRF) is well-established in the clinical domains as an integrative measure of the body's physiological capability and capacity to transport and utilise oxygen during controlled bouts of physical exertion. Long COVID is associated with >200 different symptoms and is estimated to affect ~150 million people worldwide. The most widely reported impact is reduced quality of life and functional status due to highly sensitive and cyclical symptoms that manifest and are augmented following exposure to physical, emotional, orthostatic, and cognitive stimuli, more commonly known as post-exertional symptom exacerbation (PESE) which prevents millions from engaging in routine daily activities. The use of cardiopulmonary exercise testing (CPET) is commonplace in the assessment of integrated physiology; CPET will undoubtedly play an integral role in furthering the pathophysiology and mechanistic knowledge that will inform bespoke Long COVID treatment and management strategies. An inherent risk of previous attempts to utilise CPET protocols in patients with chronic disease is that these are compounded by PESE and have induced a worsening of symptoms for patients that can last for days or weeks. To do this effectively and to meet the global need, the complex multi-system pathophysiology of Long COVID must be considered to ensure the design and implementation of research that is both safe for participants and capable of advancing mechanistic understanding.

## Introduction

### *Overview of Cardiorespiratory Fitness (CRF) and Methods of Assessment*

The protective benefits of increased CRF have been well-established over decades of research that has demonstrated vast benefits to health and wellbeing and include reducing the risk of noncommunicable diseases[1,2] and mortality.[3] CRF is well-established in the clinical domain as an integrative measure of the body's physiological capability and capacity to transport and utilise oxygen during a controlled bout of physical exertion,[4] which is determined by multiple factors, including genetic potential, health status, and physical activity/exercise training routines.[5] The plasticity of CRF is heavily reliant on regular activities that provide a frequent and appropriate stress/overload stimulus to the body's physiologic systems, which with repetition and time leads to adaptations that reduce the physiologic load associated with completing exercise or functional tasks. Determining and measuring CRF can be achieved via several subjective and objective methodologies, with the gold standard involving online gas analysis during an incremental cardiopulmonary exercise testing (CPET) to exhaustion.[6] However, the requirement for specialist equipment and highly trained staff are often considered a barrier to accessing CPET, and as a result more subjective

*Abbreviation list:* ACE2, angiotensin-converting enzyme 2; a-vO2 diff, arteriovenous oxygen difference; CNS, Central Nervous System; COVID-19, Coronavirus disease 2019; CPET, Cardiopulmonary exercise testing; CTPA, CT pulmonary angiogram; CRF, Cardiorespiratory Fitness; CT, Computerised Tomography; CVD, cardiovascular disease; DECT, Dual-energy computed tomography; ECG, electrocardiogram; eCRF, Estimated Cardiorespiratory Fitness; eCRF, Estimated CRF; FMD, Flow mediated dilation.; HR, Heart rate; IL, Interleukin; ME/CFS, Myalgic encephalomyelitis/chronic fatigue syndrome; MRI, Magnetic Resonance Imaging; O2, Oxygen; OH, orthostatic hypotension.; PA, Physical activity; PESE, Post Exertional Symptom Exacerbation; POTs, postural orthostatic tachycardia syndrome; QoL, quality of life; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TNF-α, tumor necrosis factor alpha; V/Q, Ventilation / Perfusion.

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methods to estimate CRF are adopted.[7] Despite well-documented limitations and a high degree of variability within published data sets that could be considered problematic when making clinical judgments and decisions, estimated CRF (eCRF) approaches are common practice and the suitability and application of eCRF approaches are outside the scope of this article and have been discussed previously.[8] The use of 2-day CPET approaches has also gained popularity in the assessment of chronic disease, this is primarily due to their role in identifying aerobic and physiological deficits and impairments, whereas one-time assessments are better suited to identifying abnormalities with patients often returning a 'normal result'.[9] However, the appropriateness and methods of determining CRF in the context of chronic disease areas remain a pertinent discussion in the context of Long COVID, where a dearth of understanding of the pathophysiology continues to impact the quality of life and functional status of millions of people worldwide and is an urgent threat to global health.[10]

## Pathophysiology of long COVID

Defined as a persistent and episodic symptom profile that presents after a suspected or confirmed infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2[11];), Long COVID is associated with >200 different symptoms[12] and is currently estimated to affect ~150 million people worldwide.[13] The most widely reported impact is reduced quality of life (QoL) and functional status[14] due to highly sensitive and cyclical symptoms that manifest and are augmented following exposure to physical, emotional, orthostatic, and cognitive stimuli, preventing millions from engaging in routine daily activities, including employment, social activities and even family roles.[15] Research to test developed hypothesis and mechanistic understanding is ongoing, the emergent theories point to persistent issues with the transport, delivery, and function of vital systems within the body that broadly impacts functional status and QoL.[16] These will be discussed briefly here in the context of Long COVID pathophysiology (Fig. 1) to inform the importance of diagnostic assessment using CPET and readers wishing to seek extensive insight are recommended to review the work by Davis *et al*[17] and Altman et al.[18] Whilst a plethora of research exists and demonstrates a need for integrative assessments using CPET, careful consideration is needed to prevent the use of excessive exertion that could result in perpetuating patient symptoms and can in some cases carry a significant risk to participants, which will be discussed later in the article. Here we provide an overview of the most important pathophysiological considerations that may impair exercise performance and provide a summary of the current understanding that could be furthered by detailed investigations and assessments of CRF.

### *Pulmonary abnormalities and perfusion*

Long COVID patients often present with unexplained persistent chest pain, breathlessness, exercise limitation, and fatigue, in the weeks after hospitalisation or the onset of symptoms. Whilst the cause of these symptoms has yet to be understood in its entirety there is evidence that various interrelating factors might be responsible. From a pulmonary perspective, these include impaired lung function and gas exchange, which could also be caused by haemodynamic derangements in the pulmonary and circulatory vascular beds. Imaging studies have demonstrated significant lung abnormalities which include pulmonary thrombosis, fibrosis, thromboembolism, and small airways diseases following infection with COVID-19[19] and ventilation defects and regional reductions in pulmonary perfusion have been demonstrated utilizing hyperpolarised xenon magnetic resonance imaging (MRI).[20] Paired expiratory axial computerised tomography (CT) images also demonstrate widespread lobular and regional low attenuation[21] and are indicative of air trapping in people with Long COVID, which is irrespective of acute infection severity[22] and persists for longer than 12 months.[23] Whilst common in other chronic respiratory conditions such as Chronic Obstructive Pulmonary Disease and viral pneumonia, the cause in Long COVID patients has yet to be established but it has been suggested to occur because of chronic airway inflammation, pulmonary endotheliitis, and/or fibrosis.[24] Reduced lung volumes indicate likely

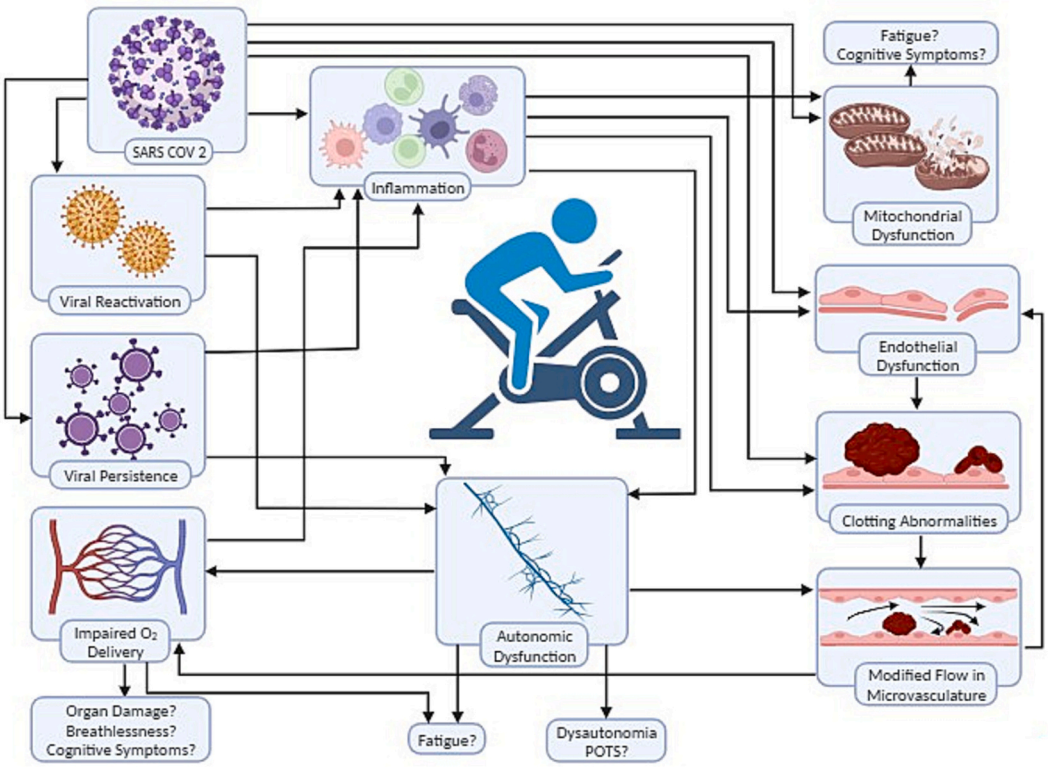


**Fig. 1.** A schematic to represent the complex/dynamic and interrelated pathophysiology of Long COVID.

restrictive parenchymal physiology[25] and air trapping has been demonstrated by reduced end-expiratory volume and hyperinflation and a presentation of breathing difficulties, chest tightness, and dyspnoea.[24] Air trapping prompts a ventilation/perfusion (V/Q) mismatch contributing to reduced physical performance and hypoxaemia downstream as oxygen transfer is impeded. Dual energy computed tomography (DECT) and V/Q have been shown to be more sensitive at detecting microperfusion defects in the lungs of Long COVID patients when compared with CT pulmonary angiogram (CTPA).[26] Dhawan et al[27] used lung V/Q scintigraphy, with single-photon emission computed tomography, to assess for residual clots and small pulmonary vessel disease for patients who have recovered from COVID-19 but continue to demonstrate persistent respiratory symptoms. Imaging to determine V/Q mismatch is important as it could play a leading role in the evaluation of pulmonary small vessel disease, which is not optimally demonstrated via CT pulmonary angiography. Whilst there is still a need for more detailed and investigative approaches, initial data shows patterns of small vessel disease and lung parenchymal disease, and therefore V/Q scanning could play a crucial role in elucidating the evolution of vascular disease and long-term pulmonary vascular sequela of COVID-19 and Long COVID.[27]

*Endothelial dysfunction*

Cardiovascular complications following COVID-19 are relatively common and include arrhythmia, pericarditis, myocarditis, microvascular angina, myocardial infarction, stroke and heart failure. SARS-CoV-2 has been shown to cause endothelial dysfunction. Endothelial dysfunction is the precursor to the development of atherosclerosis.[28,29] Atherosclerosis is the disease process that causes angina, myocardial infarction, stroke, transient ischemic attack, stroke, vascular dementia and peripheral vascular disease. Endothelial dysfunction has also been implicated in platelet activation, hypercoagulopathy and thrombotic disease. Evidence shows that SARS-CoV-2 can damage the vascular endothelium which could be a trigger for vascular disease and impact physiological function.[30] The vascular endothelium is the innermost layer of blood vessels and provides a dynamic interface between circulating blood and tissues/organs of the body. The endothelium is widely recognised as playing an integral part in regulating tissue homeostasis via the production of vasoactive molecules that tightly control vasodilatory and vasoconstrictory, pro-proliferative and anti-proliferative, pro-thrombotic and anti-thrombotic, pro-oxidant and antioxidant, fibrinolytic and anti-fibrinolytic, and pro-inflammatory and anti-inflammatory responses.[31] Subsequently, the endothelium plays an important physiological role in maintaining barrier integrity, regulating vascular tone, maintaining anti-inflammatory, anti-oxidant, and anti-thrombotic interface, anti-proliferative properties, and the regulation of cellular metabolism of ATP, glucose, and amino acids.[32] Due to their superficial nature, endothelial cells are a preferential target for SARS-CoV-2 which attacks these cells once it binds to the angiotensin-converting enzyme 2 (ACE2) receptor[33] and transmembrane serine protease 2 facilitates the disassociation of the viral spike protein.[34] The subsequent occurrence of endocytosis of the complex of ACE2 receptor in the presence of the virus reduces the number of ACE2 receptors that are available on the cell surface, leading to a dysregulation of ACE2 receptor expression and causing endothelial dysfunction and activation of prothrombotic state commonly seen in COVID-19.[35] Flow mediated dilation (FMD) techniques have been regularly used to determine endothelial dysfunction and Nandadeva et al.[36] demonstrated reduced FMD values in COVID-19 patients that were symptomatic and recovered when compared to asymptomatic controls. Importantly dyspnoea, cough, and chest pain, which are among the most common symptoms were demonstrated to be associated with impaired endothelial function. To date, there remains a need to determine whether endothelial function plays a causative role in the presentation of these symptoms or if indeed they co-exist together. Arterial stiffness has also been demonstrated in acute COVID-19 and Long COVID cases which is significantly elevated when compared to healthy controls.[30] Importantly here is the association between arterial stiffness and endothelial damage where the integrity of vascular endothelium is compromised by increased systemic hyperinflammation and cytokine release.[37] Insight derived from CPET by assessing changes in the blood pressure response and electrocardiogram (ECG) in responses to controlled stimuli can play an integral role in determining the integrative cause and response to cardiovascular responses which when paired with knowledge up and downstream can lead to increased awareness about the extent to which endothelial dysfunction impairs physiologic function.

*Clotting abnormalities*

Fibrin amyloid microclots have been demonstrated in Long COVID[38] and articulately described outside of this article.[38,39] Importantly in the context of this paper is the role that fibrin amyloid microclots may play in increasing levels of tissue hypoxia and impairing oxygen exchange. These clots are resistant to fibrinolysis and are large enough to temporarily block capillaries, which if restored rapidly may lead to tissue damage known as ischemic reperfusion injury[40] both of which can significantly impact the ability of the body's system to match the external demands with an 'appropriate' physiological response thus impairing functional status and QoL. Furthermore, ischemic reperfusion injury results in the production of reactive oxygen species, which in turn increases oxidative stress and is known to increase inflammatory cytokines and tissue hypoxia which may persist and present in a multitude and complex symptom profiles.[38] SARS-CoV-2 has also been demonstrated to interact with platelets and fibrinogen to induce changes in hypercoagulability which are suggestive of a direct pathological effect upon cellular function without being directly taken up by cells.[39] Whilst not directly assessed by CPET, indirect assessments of ECG and blood pressure alongside gas transfer variables will indicate how well the cardio-pulmonary interface is working at baseline and in response to an exercise stimulus. Biochemical insight from blood gas analysis and blood lactate assessments conducted before and after 2-day CPET approaches can be used to indicate changes in aerobic/anaerobic metabolism that is known to be a response to impaired gas transfer which has been shown in Long COVID.

*Chronic inflammation and immune dysregulation*

Acute COVID-19 is associated with an immune system response that stimulates polyclonal T-cell activation which releases an array of inflammatory molecules, such as cytokines, interleukins, and chemokines. Whilst a 'typical' response to a virus is indeed helpful in fighting infection, overproduction of these inflammatory molecules is often referred to as a 'cytokine storm' which has a very distinct immunopathological feature that is associated with COVID-19. The term cytokine storm refers to a broad systemic inflammatory response, which involves elevated circulating cytokine profiles that occur following systemic infection. As the production and release of cytokines increase in magnitude, inflammatory molecules, such as serum amyloid A, von Willebrand factor, interleukin (IL)-6, IL-8, IL-10, and tumor necrosis factor-alpha (TNF-α) increase drastically.[38] It is established that coronaviruses are neurotropic and may invade the blood-brain barrier and access the central nervous system (CNS) through periphery or olfactory neurons and the hippocampus is particularly vulnerable to infection, which could contribute to post-infection reductions in cognitive function[41] and whole body activities. Ortelli et al.[42] demonstrated a hyper-inflammatory response in Long COVID patients with a presentation of fatigue and cognitive deficit. The authors found evidence for central abnormal neuromuscular fatigue, impaired cognitive control, reduced global cognition, apathy, and executive dysfunction in the post-COVID period which was confirmed against healthy controls. The authors concluded that altered neuronal function in the context of the profound

increase of circulating cytokines (particularly IL-6), appears to contribute to CNS complications and may have broad implications downstream in the regulation and delivery of physical activity and exercise. Plasma IL-4 is also involved in brain function, such as memory, and has been demonstrated to be increased in COVID-19 patients which can be linked to ongoing neuroinflammation following a COVID-19 infection.[43] Koumpa et al studied protein markers of neuronal dysfunction including amyloid-beta, neurofilament light chain, neurogranin, total tau, and pT181-tau which they demonstrated to be increased in the neuronal-enriched extra-cellular protein of those recovering from COVID when compared to pro-COVID-19 historical controls. Hyperactivation and/or dysregulation of immune cell activation can occur and cause clinically significant and irreversible multi-organ damage, failure, and even death.[37] Severe COVID-19 infections have also been demonstrated to induce B cell and T cell lymphocyte deficiencies which in turn can cause hyperinflammation as lymphocytes are actively involved in the resolution of inflammation after infection. Depleted T cell and B cell numbers are strongly associated with persistent SARS-CoV-2 shedding and may contribute to chronic immune activation in Long COVID[44]. Mast cell activation has also been described as part of the body's hyperinflammatory responses in both acute COVID-19 infection and Long COVID. Mast cell activation is responsible for repeated and severe allergic symptoms that broadly affect bodily systems and consequently, a multitude of symptoms which include gastrointestinal upset and shortness of breath[45] which is linked to impaired exercise performance.

*Autonomic dysfunction*

Whilst widely reported as a symptom that affects the quality of life and functional status, symptoms of autonomic dysfunction that are consistent with dysautonomia are common, but the index of a formal diagnosis is varied. Common presentations of dysautonomia include orthostatic intolerance, fatigue, palpitations, cognitive impairment, nausea, and temperature dysregulation which has also been observed with reports demonstrating that Long COVID patients have lower heart rate variability when compared with matched controls.[46] Whilst the mechanisms of these symptoms have yet to be understood in their entirety, multiple suggestions have been discussed. These include relative hypovolemia caused by failed peripheral vasoconstriction which is consistent with both postural orthostatic tachycardia syndrome (POTS) and orthostatic hypotension (OH).[47,48] The impact here is an increase in cardiac stress via a reduction in both stroke volume and cardiac output which impacts tissue oxygenation, and metabolic function and can result in tachycardia and compensatory overdrive in sympathetic activity. Assessing arteriovenous oxygen difference (a-vO2) during CPET might shed light on potential autonomic dysfunction as a-v$O_2$ difference continues to increase in line with increased physical exertion, producing a linear or curvilinear rise in the $O_2$ pulse. If there is a plateau in $O_2$ pulse with increasing work this may be indicative of low stroke volume and an indicator of autonomic dysfunction.[49] This data can be coupled alongside measures of cardiovascular parasympathetic function by analysing heart rate variability and blood pressure response to exercise. Whilst more interrogative methods such as transcranial Doppler and neurological assessment are required for clinical diagnosis, CPET methods offer an opportunity to assess the during and post-exercise response of multiple systems in response to the same stimuli, which might provide a more useful interpretation of understanding of impairment.

*Mitochondrial dysfunction*

Recent developments have demonstrated mitochondrial dysfunction and as a consequence impaired exercise tolerance in Long COVID patients.[50] As the body's powerhouse for aerobic metabolism, impaired mitochondrial activity has a widespread impact on bodily systems and functions which has been demonstrated in major organs such as the lungs, heart, liver, kidneys, and brain.[51] Mitochondrial dysfunction following viral infection is not a new phenomenon with previous data highlighting increased tissue damage during infection and recovery phases which is attenuated with time.[52] Nasopharyngeal samples from SARs-COV-2 patients demonstrate impaired transcription of nuclear DNA, and mitochondrial OXPHOS genes and triggered an antiviral immune response that impairs mitochondrial function[51] and remains impaired despite viral resolution and could contribute to severe Long COVID pathology. In essence, these mechanisms will affect the uptake and utilisation of oxygen within skeletal muscles, thus affecting aerobic and anaerobic contributions to the provision of energy at a cellular level. It has been postulated that anaerobic thresholds are reduced in athletic populations with Long COVID which the authors attribute to virally mediated mitochondrial dysfunction that extends beyond expected post-viral infection deconditioning and could be associated with impaired tissue oxygenation and substrate oxidation.[53] In context, structural and mechanistic impairments result in reduced total aerobic contribution and increase the reliance on anaerobic provisions which is time limited. It is plausible that patients with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and Long COVID could breach aerobic thresholds and work in an anaerobic state at markedly lower intensities compared to healthy controls and as a result mitochondrial dysfunction could play an integral role in the clinical presentation of post-exertional symptom exacerbation (PESE), which is triggered by physical, cognitive, orthostatic and emotional stimuli and remains one the biggest challenges to Long COVID. Physical and biochemical responses to physical exercise tasks can be determined with 2-day CPET designs that include pre and post-test assessments via venepuncture/blood gas analysis to quantify aerobic/anaerobic thresholds which can be used to support the development of bespoke, person-centred rehabilitative and management pathways.[54] The addition and monitoring of near-infrared spectroscopy (NIRS) techniques that quantifies tissue oxygenation and tissues saturation could also provide novel insights into an abnormal exercise response and between day impaired physiologic function and dysfunction[55] but have yet to be tested in the context of PESE and Long COVID.

## Role of 2-day CPETs to examine long covid pathophysiology

CPET remains highly relevant and indicated to evaluate individuals living with chronic conditions such as ME/CFS and Long COVID. Whilst caution is advised to prevent PESE, measurements obtained from cardiorespiratory responses to physiological stress could provide insight regarding the integrity of the pulmonary-vascular interface and characterisation of any impairment or abnormal cardio-respiratory function.[56] To date, there is clear evidence that CRF is compromised following acute infection with COVID-19[57] and in patients diagnosed with Long COVID[58]. This is not surprising as the virus and the resulting impact upon the body's pathophysiology (outlined above) adversely affects the cardiac, pulmonary, and skeletal muscular systems, which are well documented to influence a person's CRF.[59] A recent meta-analysis by Lim *et* al[60] that reviewed the use of CPETs in PESE identified that overall mean values of all parameters are reduced on day two when compared with healthy controls, especially when analysed that the ventilatory threshold (overall mean: −10.8 at Test 1 vs. −33.0 at Test 2, $p < 0.05$). The authors conclude that two-day CPET serves as an objective assessment of PESE in ME/CFS patients but there is great consideration required for future clinical trials to determine the feasibility of working with patients from fatigue-inducing disorders and to prevent excessive and unnecessary exertion that could result in a relapse in symptoms. In the context of chronic disease, Long COVID, the assessment of CRF via CPET provides an opportunity to utilise expertise from clinical exercise domains to: 1) provide mechanistic knowledge of Long Covid; 2) quantify functional status and inform the development, implementation, and monitoring of safe interventions to support rehabilitation; and 3) monitor the aerobic/anaerobic response to drug therapies.[61,62] In the context of COVID-19, CPET provides an ideal

approach to assess the intersection between pathophysiologic and clinical manifestations, allowing for a refined account of the impact the viral infection has both cross-sectionally and longitudinally, most importantly during a bout, or repeated bouts of physical exertion in a highly controlled environment. However, the use and implementation of CPET in Long COVID must consider the use of novel/adapted protocols to mitigate against a potential and excessive exacerbation of symptoms that may result following the completion of maximal protocols.

## Consideration for Using 2-Day CPET in Long COVID

Without question, CPET will play an integral role in furthering the pathophysiology and it remains the gold standard assessment, providing for the most non-invasive, comprehensive, and accurate assessment of CRF. Typical CPET approaches are conducted to exhaustion and require maximal effort, as governed by established test termination criteria.[63] However, its application in Long COVID has been criticised by patient groups for not considering and even prioritising patient safety. The ability of Long COVID patients to produce maximal efforts will likely induce PESE over a course of days and even weeks which will drastically reduce QoL. Therefore, care must be taken to ensure protocols are suitably adapted and cognisant of the challenges associated with the nuances of chronic disease settings. In a recent meta-analysis, Lim et al demonstrated significant reductions in all parameters from a review of studies using 2-day CPET approaches,[60] however, they determined that all variables were reduced at the ventilatory threshold and this could serve as an objective endpoint rather than completing protocols to volitional tolerance. This is pertinent in the context of ME/CFS and Long COVID where PESE and a worsening of symptoms can be exacerbated with minimal physical, emotional, cognitive, and/or orthostatic stimuli. This should include giving thought to the wrap-around services that mitigate risks to patients during and following testing that might impact patients. We, therefore, encourage engraining all research design processes with the lived experience to inform the investigation of informative research questions that adopt innovative protocol designs and produce accessible and meaningful insight that can be used to advance clinical practice and inform effective management and restorative pathways that are underpinned with interdisciplinary[64] and whole system thinking[65]. To assist, we outline some important steps and considerations that we have developed with a national patient and public involvement and engagement representatives that can be used to address previously documented challenges in this area to help establish research in this area to progress.

### *Adapting approaches*

Conventional CPET to volitional exhaustion are supported by decades of research demonstrating immense clinical value, however, the confounders of PESE provide a challenge to the design and implementation of research in COVID-19/Long COVID. Therefore, it is appropriate to establish protocols that acknowledge the previously reported impairment and explore the physical determinants in a way that captures the relevant and important data (i.e., ventilatory and anaerobic thresholds) without exercising to maximal levels that might induce PESE that will affect patients in the aftermath of testing for days or even weeks. Suitable adaptations to the protocols are well established in other clinical areas and consideration should be given to the starting intensity, within test increments, and the test termination criteria monitored continuously to prevent excessive workloads and risk to some patients. Consideration should also be given to the use and choice of terminology that is used during tests. Phrases like 'just keep going as long as you can' or 'let us know if you want to stop' should be avoided and there needs to be a recognition of the following: 1) that some patients will not have knowledge of PESE and/or its consequences and will therefore have no concept that they should be limiting their exertion to prevent a worsening of symptoms; and 2) Researchers/health practitioners are in a position of power, and trust and retain a duty of care for participants and whilst participants will want to help and will comply with instructions, this needs to be considered from a risk/benefit perspective that prioritises patient safety. Therefore, phrases like 'I want you to stay comfortably within your capacity, I don't want you to push yourself, it is very important that you don't overdo it' should be used to replace more traditional phrases. Ensuring that participants have their accessibility requirements considered and are given plenty of time to complete all study protocols and not rushed is important and the provision of a place to rest following participation is also key to participant safety. This might include completing additional surveys/questionnaires that are important to the study design in advance of CPET which could also be made available digitally, to reduce the physical and cognitive load imposed during testing.

### *Inclusion/exclusion criteria*

The risk of causing inadvertent harm can also be mitigated by implementing strict inclusion/exclusion criteria which include identifying and screening individuals who are at risk of a severe PESE reaction. Pre and post-test screening measures should also include objective determination of symptoms and details of any relapses that might also pose additional risks to patients. These methods should also consider the use of digital apps that can profile symptoms systematically. *COVID-19 mitigations:* COVID-19 is being allowed to circulate worldwide with variants of concern contributing to sustained levels of transmission with persistent symptoms reported in every one in ten infections.[17] For those with Long COVID, the threat of further infection and therefore a worsening of symptoms is a very real consideration, and all laboratories should still implement strict COVID-19 mitigation strategies which include regular testing, use of masks, ensuring adequate ventilation, thorough and regular cleaning processes to reduce the risk to all involved.

### *Communication*

The art of communication between clinical professionals and patients has been the center of much discussion and even debate previously. Qualitative research in Long COVID highlights that patients do not feel heard and are even gaslit by some healthcare practitioners,[15,66] creating a disconnect between two key stakeholders. Demonstrating knowledge, awareness, understanding, and being proactive for participants is crucial not only to make participants feel comfortable and at ease with the study, which for some will represent a big decision.

## Conclusion

Without question, CPET will play an integral role in furthering the knowledge of the pathophysiology of Long COVID and it remains the gold-standard method for most non-invasive, comprehensive, and accurate assessments of CRF. However, the nuances and multi-system pathophysiology of Long COVID creates additional considerations that must be considered to ensure the design and implementation of research that is both safe for participants and capable of advancing mechanistic understanding.

## Disclosures

None.

## CRediT authorship contribution statement

**Mark A. Faghy:** Supervision. **Caroline Dalton:** Supervision. **Rae Duncan:** Supervision. **Ross Arena:** Supervision. **Ruth E.M. Ashton:** Supervision.

## Declaration of competing interest

None.

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