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OXYGEN TRANSPORT AND UTILISATION DURING EXERCISE IN CYSTIC FIBROSIS: CONTRIBUTORS TO EXERCISE INTOLERANCE

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NEW FINDINGS (symposium report)

- **What is the topic of this review?**

  This review highlights the central and peripheral mechanisms that alter oxygen transport and utilisation and thereby contribute to exercise limitation in people with cystic fibrosis, considering also viable therapeutic targets for intervention.

- **What advances does it highlight?**

  Although traditionally considered a respiratory condition, pathological intramuscular and cardiovascular changes in people with cystic fibrosis appear to be key determinants of exercise intolerance up until the later stages of respiratory disease. Even young, habitually active patients with normal lung function experience multisystemic abnormalities, which play a role in exercise intolerance.
ABSTRACT

Cystic fibrosis (CF) is a complex condition, commonly associated with exercise limitation. The mechanisms responsible for this in CF are of interest, given that lower aerobic fitness is associated with an increased risk of being hospitalised with pulmonary exacerbation, a poorer quality of life and prognosis. Pathophysiological changes in lung function are considered central to CF, and may contribute to exercise limitation. However, it is now clear that the pathogenesis of exercise limitation in this population is multifactorial, with alterations in cardiovascular, muscle and pulmonary function contributing. Whilst some of these changes are attributable to respiratory disease per se, the CF transmembrane conductance regulator protein is also found in skeletal muscle and the vascular endothelium and can directly alter central and localised oxygen delivery, as well as the ability to effectively extract and utilise oxygen at the myocyte level. Since intense exercise poses considerable challenges to arterial oxygen content and/or blood flow and its supply to the working skeletal muscle, evaluating the exercise physiology of people with CF has helped us understand the mechanisms underlying exercise intolerance. Through several investigations over recent years, we have collectively demonstrated that people with CF exhibit reduced skeletal muscle oxygen extraction and utilisation during exercise, with a lesser contribution from haemodynamic or chronotropic mechanisms. Taken together, our findings highlight the importance of targeting mechanisms of skeletal muscle oxygen utilisation in CF to improve exercise tolerance and we offer potential therapeutic interventional strategies.

(238 / 300 words)
1. INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive disease associated with defective or deficient expression of the CF transmembrane conductance regulator (CFTR) protein, a chloride and bicarbonate ion channel predominantly expressed in epithelial cells. CF pathophysiology is complex, affecting multiple organ systems including the lungs, pancreas, upper airway, liver, intestine, and reproductive organs. Traditionally, CF was characterised by lung congestion and infection and progressive respiratory failure. However, as clinical management and life expectancy improve, the importance of extrapulmonary manifestations is becoming increasingly apparent.

In this review, we highlight several disordered processes inherent to CF pathophysiology that can modulate oxygen (O₂) transport and utilisation. Finally, we consider the current and evolving potential therapeutic strategies that may ameliorate these alterations and convey both physiological and clinical benefits. Understanding and treating the mechanistic basis of impaired aerobic fitness is a clinical priority in people with CF (pwCF), since physical inactivity is associated with a poorer quality of life, increased hospitalisation risk, and higher rates of mortality.

2. TO WHAT EXTENT DOES VENTILATORY LIMITATION CONTRIBUTE TO EXERCISE INTOLERANCE IN CYSTIC FIBROSIS?

Exercise intolerance in pwCF was traditionally considered the result of poor pulmonary function and malnutrition. In the lungs, abnormally thick sticky mucus obstructs the airways and glands, leading to airway damage and alveoli collapse. These pathophysiological changes, typically characterising advanced lung disease, result in increased dead space ventilation and dynamic hyperinflation, exercise-induced ventilation-perfusion abnormalities, arterial desaturation and alveolar hypoventilation during exercise.

These abnormalities may compromise the ability of the lung to meet the ventilatory and gas exchange demands imposed by exercise and thus impair O₂ delivery to the locomotor muscles. However, since the treatment of these pulmonary manifestations has considerably improved, neither ventilatory limitation or arterial hypoxemia are commonly observed in milder CF lung disease (even during maximal exercise). Indeed, recent investigations in physically active, well-nourished children and adolescents with preserved lung function have
provided vital insight into the extrapulmonary factors that contribute to exercise intolerance in pwCF.

3. OTHER PATHOPHYSIOLOGICAL ALTERATIONS THAT MAY IMPAIR O₂ DELIVERY AND/OR O₂ UTILISATION IN PEOPLE WITH CYSTIC FIBROSIS DURING EXERCISE

Evidence is growing to support the existence of myocardial impairment in CF (Labombarda, 2016), even in youth (Giacchi et al., 2015; Eising et al., 2018), indicated by an increased pulmonary blood pressure (Giacchi et al., 2015) and diminished right and left ventricular function (Eising et al., 2018). CF-related chronic hypoxaemia, inflammation, infection and/or diabetes have been implicated, but CFTR dysfunction may also directly affect cardiac function since it is expressed in cardiac muscle (Hart et al., 1996; Chen et al., 2004). Moreover, CFTR can regulate cardiomyocyte contraction rate in neonatal mice and loss of CFTR in mice without lung pathology also leads to vascular remodelling and increased aortic stiffness (Sellers et al., 2013).

Increased arterial stiffness is also evident in adults and children with CF, even at a very young age (Eising et al., 2018). Recent data from our team also demonstrates peripheral micro- and macrovascular endothelial perturbations in young pwCF (Poore et al., 2013; Rodriguez-Miguelez et al., 2016). Questions remain regarding the mechanisms underlying vascular changes in CF, nevertheless CFTR has been linked, since it is expressed in human umbilical vein endothelial cells (Tousson et al., 1998), human pulmonary artery endothelial cells (Tousson et al., 1998), and human lung microvascular endothelial cells (HLMVECs). Furthermore, we recently demonstrated that CFTR is involved in the regulation of oxidative stress, inflammation and endothelial cell signalling in HLMVECs (Khalaf, 2020). Unpublished data from one of our laboratories, using the orbital shaker method, demonstrated that CFTR appears to regulate endothelial actin cytoskeleton dynamics and cellular alignment in response to flow, which is important for maintaining cellular integrity and promoting alignment with flow.

Skeletal muscle is also altered (Gruet et al., 2017). Reduced muscle mass and strength, particularly in the lower limbs, is common and not solely attributable to physical inactivity and malnutrition. CF is consistent with a scenario in which two major factors act synergistically to favour the development of skeletal muscle dysfunction: 1) more pulmonary/systemic inflammation leading to a greater exposure of the muscles to pro-
inflammatory mediators, and 2) greater sensitivity of the pro-inflammatory mediator response to muscles (due to dysfunctional CFTR), which leads to exaggerated upregulation of atrophy pathways as well as impairment of contractile mechanisms (Gruet et al., 2017). Furthermore, reduced antioxidant capacity, due to increased systemic inflammation and/or oxidative stress, which we have recently phenotyped even in those with stable CF (Causer et al., 2020), may further impair mitochondrial efficiency and contribute to peripheral (and respiratory) muscle dysfunction. Similar to the cardiovascular system, CFTR is expressed in skeletal muscle (Lamhonwah et al., 2010) and clearly contributes to the pathophysiology; indicated by abnormal intracellular calcium and contractile responses to depolarisation in muscle with dysfunctional CFTR (Divangahi et al., 2009; Lamhonwah et al., 2010). CFTR has also been shown to mediate glutathione flux in epithelial cells (Linsdell & Hanrahan, 1998) and thus plays a role in controlling the redox state, which is an important modifier of calcium release. Lack of CFTR has also been shown to predispose muscle atrophy and diaphragm muscle pump failure using a murine model (Divangahi et al., 2009).

4. O₂ DELIVERY AND UTILISATION DURING EXERCISE IN PEOPLE WITH CYSTIC FIBROSIS: RECENT ADVANCES IN OUR UNDERSTANDING

The technological advancement of non-invasive monitoring systems, particularly those suitable for paediatric participants, recently permitted the study of the dynamic balance of O₂ delivery-to-extraction in pwCF. Near-infrared spectroscopy (NIRS) and thoracic impedance cardiography have been used by our team and others to evaluate muscle oxygenation and oxidative capacity, an index of mitochondrial function, and cardiac function, respectively. In a series of experiments, we have concurrently measured the dynamics of pulmonary oxygen uptake (VO₂) and oxygenation within the microcirculation of exercising muscle, with additional measures of systemic O₂ delivery, for the first time. This has allowed investigation into the dynamic balance between O₂ delivery to and localised extraction and utilisation at the peripheral locomotor muscle of pwCF during exhaustive exercise, constant work rate exercise of different intensities, and in response to experimental manipulations of systemic O₂ delivery.

Our investigation of the dynamic adjustments in localised muscle (vastus lateralis) fractional O₂ extraction during exhaustive ramp incremental exercise in youth with mild-to-moderate CF lung disease (Saynor et al., 2014b), relative to pulmonary gas exchange and ventilation
and systemic O₂ delivery, was the first of its kind. The NIRS-derived muscle oxygenation signal provides insight into the local ratio of muscle O₂ delivery-to-utilisation, with the deoxyhaemoglobin ([HHb]) signal considered a proxy for O₂ extraction. This methodology permits inferences regarding blood flow within the microcirculation of exercising muscle. More rapid [HHb] dynamics typically occur in the face of reduced systemic or localised O₂ delivery and vice versa. As expected, aerobic fitness was reduced in youth with mild-to-moderate CF. However, despite impaired central O₂ delivery (reduced SpO₂ at exhaustion and O₂ pulse, which were associated with VO₂peak), they did not compensate by accelerating muscle O₂ extraction (Figure 1). This raised questions regarding the capacity of CF skeletal muscle and/or its microcirculation to extract and use O₂.

We took this further to, for the first time, study O₂ transport and utilisation during physical activity that more closely reflects activities of daily living, i.e. bouts of constant work rate exercise (Saynor et al., 2016). Our finding that the phase II and overall VO₂ on-kinetic response, a proxy for muscle O₂ consumption, was slowed during very heavy- but not moderate-intensity exercise supported the notion that impaired muscle oxidative metabolism in children and adolescents with mild-to-moderate CF lung disease is exercise intensity-dependent. Furthermore, we observed a significant reduction in the arterio-venous O₂ content difference (C(a-v)O₂), a measure of the amount of O₂ taken up by the tissues – with a greater difference reflecting greater O₂ extraction- , during the time interval corresponding to the phase II VO₂ response. ΔC(a-v)O₂ was also associated with the VO₂ kinetic response during very heavy-intensity exercise in CF but not healthy controls, suggesting a mechanistic link and further supporting the notion that pwCF are principally limited by intramuscular factors, which limit O₂ extraction and/or utilisation during exercise. Physiological compensation for this does however only appear viable at lower exercise intensities in milder disease, a finding that likely reflects the greater physiological challenge more intense exercise poses to mitochondrial aerobic metabolism.

One of our laboratories was also the first to use NIRS during a non-invasive recovery kinetics test evaluating skeletal muscle oxidative capacity in pwCF. Measuring the recovery rate of oxygen consumption of the m. vastus lateralis following 15 s of electrical stimulation (4 Hz) and subsequent repeated transient arterial occlusions (Erickson et al., 2015), we identified impaired skeletal muscle oxidative capacity in pwCF. A significant inverse relationship between skeletal muscle oxidative capacity and age was also observed in pwCF, but not...
healthy individuals, indicating that an age-related reduction in skeletal muscle oxidative capacity may be evident in CF.

Manipulating O₂ delivery and extraction can also provide insight into the locus of exercise limitation. Various strategies exist, such as reducing the work of breathing during exercise via inspiration of hyperoxic gas. This should increase systemic O₂ delivery, however early evidence in pwCF suggested that whilst O₂ supplementation could normalise O₂ saturation, this does not improve ŔVO₂peak (Nixon et al., 1990) or ŔVO₂ kinetics (Kusenbach et al., 1999). Another established experimental manipulation to reveal the role of central limiting factors is ‘priming’ exercise. Priming exercise has been shown to speed ŔVO₂ kinetics in individuals, through enhanced muscle O₂ availability, microvascular oxygenation and activation of the mitochondrial pyruvate dehydrogenase complex, in addition to improved muscle perfusion secondary to the vasodilatory effects of metabolic acidaemia. If priming exercise increased systemic O₂ delivery via an increase in cardiac output in pwCF and improved the matching of O₂ delivery-to-utilisation (i.e. reduced Δ[HBB]/ampled ŔVO₂), this would provide insight into the O₂ delivery-dependence of aerobic fitness. However, currently unpublished data from our laboratory has revealed that priming exercise cannot increase cardiac output in youth with CF and did not accelerate ŔVO₂ kinetics. These observations further support the mechanistic role of localised intramuscular consequences of CF to limit exercise tolerance rather than central factors.

5. TREATMENT OPTIONS FOR EXERCISE IN TOLERANCE IN PEOPLE WITH CYSTIC FIBROSIS

In the cases where an O₂ utilisation limitation to ŔVO₂ kinetics is responsible for the reduced tolerance to aerobic exercise in pwCF, for example those with preserved lung function, exercise training to improve mitochondrial function, such as high-intensity interval training (HIIT), might be particularly beneficial. Reuveny and colleagues (Reuveny et al., 2020) recently provided the first evidence that 8 weeks HIIT can accelerate ŔVO₂ kinetics and improve exercise tolerance in pwCF. Although central and peripheral function were not independently assessed; considering that HIIT is particularly suited to inducing peripheral adaptations, their findings provide further evidence that a peripherally-based factor is principally rate limiting in pwCF.
Aside from exercise, Sildenafil, a phosphodiesterase type 5 inhibitor, has shown promising results. Sildenafil can improve CFTR chloride channel function (Lubamba et al., 2011), inflammation (Taylor-Cousar et al., 2015), and blood flow to and within muscles (Sperandio et al., 2012). Data from our team recently reported improvements in vascular function (Rodriguez-Miguelez et al., 2018) and exercise capacity after a single dose, which was maintained throughout 4 weeks of treatment (Rodriguez-Miguelez et al., 2019). A more in-depth analysis supports that a sub-acute treatment with sildenafil was able to improve skeletal muscle O₂ extraction and utilisation in pwCF during maximal exertion (Figure 1). Not only does this data provide further evidence regarding the vascular and intramuscular involvement in exercise limitation characterising pwCF, but importantly offers a therapeutic strategy to, at least in part, treat this.

A pharmacological ‘helping hand’ could also be administered. We have previously demonstrated in a small case series that Ivacaftor, a CFTR modulator therapy, improved aerobic fitness in pwCF with the G551D mutation (Saynor et al., 2014a), due to improvements in both O₂ delivery and extraction in the absence of training. Currently unpublished data from one of our laboratories has more recently shown that Elexacaftor-Tezacaftor-Ivacaftor, can improve exercise capacity in adolescents with CF, with early indicators suggesting improvements in O₂ transport/utilisation in the exercising muscle independent of changes in ventilatory function.

6. SUMMARY AND CONSIDERATIONS

As highlighted in this review, exercise intolerance in pwCF is multifactorial with a mechanistic basis that shifts with age and disease severity. To date ventilatory, cardiovascular and intramuscular abnormalities have been reported and we suggest that mechanisms of exercise intolerance is likely distributed differently and dependent on disease severity. At lower intensities of exercise and in mild CF lung disease, physiological compensation is possible. However, with more severe dysfunction and more intense exercise, a pharmacological ‘helping hand’ may be needed to improve exercise capacity.
REFERENCES


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COMPETING INTERESTS

None to declare

AUTHOR CONTRIBUTIONS

All authors contributed conceptually to the concepts detailed in this manuscript and the drafting of the article. All authors read and approved the final version of the article and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Figure 1. Skeletal muscle $O_2$ utilisation during peak exercise. Change in oxygenated haemoglobin ($\Delta O_2Hb$; panel A) and deoxygenated haemoglobin ($\Delta HHb$; panel B) in healthy controls (grey circles), people with cystic fibrosis (CF) before (black circles) and following four weeks of sildenafil (SIL, white circles) from rest to peak $O_2$ consumption. All measures are expressed as individual data and overall group means. All statistical analyses were performed using SPSS Version 25 (SPSS Inc., Chicago, IL, USA), and significance was set at $p < 0.05$. Independent group $t$-tests were used to compare controls and people with CF (pwCF). Paired $t$-tests were performed to identify differences between baseline and treatment with sildenafil within the pwCF. *Significant difference between pwCF and controls. #Significant difference ($p < 0.05$) between baseline and four weeks of treatment with sildenafil in pwCF. Figure represents more detailed analysis of data derived from Rodriguez Miguelez et al. (Rodriguez-Miguelez et al., In Press).