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Exercise Intolerance in Cystic Fibrosis: Importance of Skeletal Muscle

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Abstract

Purpose: Exercise intolerance, evaluated by O_2 consumption, predicts mortality in Cystic Fibrosis (CF). People with CF exhibit skeletal muscle dysfunctions that may contribute to an imbalance between O_2 delivery and utilization. Sildenafil, a phosphodiesterase type 5 (PDE5) inhibitor, increases blood flow and improves O_2 consumption, although the exact mechanisms in CF have yet to be elucidated. Thus, we hypothesized that exercise intolerance in CF is limited primarily by an impaired skeletal muscle O_2 utilization, and sildenafil improves exercise tolerance in CF by addressing this mismatch between O_2 demand and extraction.

Methods: Fifteen individuals with mild-to-moderate CF and eighteen healthy controls completed an incremental exercise test and measurements of gaseous exchange, chronotropic response, hemodynamics, and O_2 extraction and utilization. People with CF also completed a four-week-treatment with sildenafil with a subsequent follow-up evaluation after treatment.

Results: Skeletal muscle O_2 extraction and utilization during exercise were reduced in people with CF when compared to controls. Exercise capacity in our CF population was minimally limited by hemodynamic or chronotopic responses, while peripheral O_2 extraction was more closely associated with exercise capacity. The study also demonstrated that four weeks of sildenafil improved skeletal muscle O_2 utilization during exercise to similar values observed in healthy individuals.

Conclusions: Individuals with mild to moderate CF exhibit exercise intolerance secondary to a reduction in O_2 utilization by the exercising skeletal muscle. The present study demonstrated that

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four weeks of sildenafil treatment improves the capacity of the skeletal muscle to utilize O_2 more efficiently during exercise. Findings from the present study highlight the importance of targeting skeletal muscle O_2 utilization to improve exercise tolerance in CF.

Keywords

Exercise capacity; cystic fibrosis; skeletal muscle; O_2 utilization; sildenafil

INTRODUCTION

Cystic Fibrosis (CF) is an autosomal recessive genetic disorder caused by a mutation in the CF transmembrane conductance regulator (CFTR) protein. Exercise intolerance is a common phenotype and an independent predictor of mortality in people with CF (1). Previous reports have described a 20% decline in exercise capacity during adolescence in individuals with CF, that appears to be associated with lung disease severity and CF-related diabetes, yet independent of pulmonary function or body mass (2). Thus, a regular assessment of exercise capacity to evaluate oxygen consumption (VO_2) as well as ventilatory efficiency and peak work rate are considered an important and recommended prognostic approach in the evaluation of disease progression in CF (1).

According to the Fick principle, O_2 consumption depends on both central (O_2 delivery) and peripheral (O_2 utilization) physiological mechanisms. Four major factors may limit VO_2 at maximal exertion, including lower pulmonary diffusing capacity, reductions in O_2 delivery, decrease in O_2 carrying capacity, and limitations in skeletal muscle O_2 utilization (3). Previous reports in CF have described the existence of dysfunctions in some of these physiological mechanisms evaluated independently, including an impaired use of O_2, mitochondrial deficiencies and/or abnormal skeletal muscle metabolism (4–7). These observations support the evaluation of potential therapies to mitigate these dysfunctions.

Sildenafil is a selective phosphodiesterase type 5 (PDE-5) inhibitor approved by the Food and Drug Administration for the use in pulmonary arterial hypertension and erectile dysfunction. In addition, a novel role of sildenafil has been described with direct action on rescuing CFTR trafficking and correcting deficient CFTR transport activity (8, 9), opening new possibilities for patients with CF. As a result, treatment with sildenafil has resulted in improvements in vascular function (10), inflammation (11), and exercise capacity (12) in CF. Despite these early compelling observations, there is no information about the effect of sildenafil on the mechanisms involved in skeletal muscle O_2 consumption.

Based on the complicated pathophysiology of CF and the recognized role of muscle O_2 extraction on exercise capacity (13), we hypothesized that when evaluated simultaneously, people with mild to moderate CF would exhibit exercise intolerance that was limited in part by an inability of the skeletal muscle to utilize O_2 efficiently and sildenafil would promote a better balance between O_2 supply and O_2 extraction. Thus, the purpose of this study was 1) to determine the contributions of each component of O_2 consumption to exercise capacity in CF and 2) to assess the efficacy of a sub-acute treatment with sildenafil on exercise intolerance-mediated mechanisms.

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MATERIAL AND METHODS

Experimental Design

Both participants with CF (n=18) and healthy control (n=15) participants reported to the Laboratory of Integrative Vascular and Exercise Physiology (LIVEP) at 8:00 AM for baseline testing following an overnight fast of at least 8 hours and with *ad libitum* water. Exercise capacity, chronotropic and hemodynamic responses, measurements of expiratory gas exchange, and O_2 utilization were evaluated at baseline in all participants. Individuals with CF returned for follow-up testing after four weeks of treatment with sildenafil (20 mg, three times a day). For each visit, participants were instructed to adhere to the timing of their daily treatments and come to the laboratory following their morning airway clearance techniques and inhaled medicines. Patients were taking standard CF therapy (i.e., bronchodilators, daily multivitamin supplements, and pancreatic enzyme supplements) during their participation and did not change medications during the investigation. Two of the participants with CF were on CFTR modulators for more than 6 months prior enrolling in this study. Patients were only tested if they had been clinically stable (no exacerbations or need for antibiotic treatment) for at least 2 weeks prior to testing.

Participants

An initial power calculation was performed based on the anticipated effect size estimated for the primary outcome variable (exercise capacity) previous studies(14, 15). Power analysis and sample size were calculated before initiating the study considering that under most circumstances, an α=0.05 and a statistical power ≥0.85 is well accepted. The analysis has previously described in more detail (12).

Initially, a total of 19 participants with CF were recruited from the Augusta University Cystic Fibrosis Center. All were eligible to participate with the exception of 1. Of the 18 participants with CF recruited, 3 withdrew from the study [change of residence (1), missed final visit (1), and personal reason (1)], resulting in a total of 15 participants with CF (8 males and 7 females; 23 ± 11 years), that completed the study. The majority of patients with CF were homozygous ΔF508del (n=13); however, one patient was ΔF508/621+1G->T, and another was ΔF508/G551D. One of the participants had a diagnosis of CF related diabetes (CFRD). In addition, eighteen demographically-matched controls (9 males and 9 females; 27 ± 8 years) from the Central Savannah River Area were also included in the study to determine pre-treatment differences between groups. Exclusion criteria for all participants consisted of the following: (1) a forced expiratory volume in one second (FEV_1) <50% predicted following American Thoracic Society recommendations for mild or moderate lung disease (16), (2) a resting O_2 saturation <90%, (3) a clinical diagnosis of pulmonary hypertension or cardiovascular disease, (4) use of vasoactive medications, (5) diagnosed with sleep apnea or sleep disorders, or (6) pregnant or self-reported smoker. Individuals with CF completed four weeks of sildenafil (20 mg, three times a day). No serious adverse events were reported and the side effects were usually mild and consistent with those previously reported and indicated on the label.

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All participants and parents of minors were informed of the objectives, and possible risks of the investigation before written consent/assent for participation was obtained. The study (clinicaltrials.gov #NCT02057458) followed the principals of the Declaration of Helsinki and was approved by the Institutional Review Board at Augusta University (No. 10-07-019).

Demographic Characteristics and Clinical Laboratory Values

Baseline anthropometric measurements of height, weight, calculated body mass index (BMI), and body composition using dual-energy x-ray absorptiometry (DXA; QDR-4500W, Hologic, Inc., Marlborough, MA) were obtained. Resting systolic and diastolic blood pressures were evaluated in triplicate using Omron HEM705 (Omron, Lake Forest, IL), and the average of 3 assessments was used to calculate mean arterial pressure.

A venous blood sample was also collected in all the volunteers following an overnight fast for assessment of standard clinical laboratory values. Fasting concentrations of standard biochemical values for lipids (total cholesterol (TC), high-density lipoproteins (HDL), low-density lipoproteins (LDL), and triglycerides) and glucose concentrations were obtained using the Cholestech LDX point of care analyzer (Alere, Providence, RI). Hemoglobin and hematocrit values were obtained using the HemoPoint H2 analyzer (Stanbio Laboratory, Boerne, TX). Concentrations of high-sensitivity C-reactive protein (hsCRP) and hemoglobin A_{1c} (HbA_{1c}) were obtained from standard core laboratory techniques (Laboratory Corporation of America Holdings, Burlington, NC).

Pulmonary Function Testing

A pulmonary function test was completed by all participants using the EasyOne Pro Lab (NDD, MA, USA) to evaluate FEV_1, forced vital capacity (FVC), FVC/FEV_1 ratio, and forced expiratory flow (FEF_{25-75}). Pulmonary function testing was conducted following the standards of the American Thoracic Society. The European Respiratory Society Global Lung Function Initiative spirometric reference standards (17) were used to determine the percent predicted data set.

Cardiopulmonary Exercise Testing

A maximal exercise test using the Godfrey protocol on a cycle ergometer was conducted in all participants following the American College of Sport Medicine guidelines (18) and the recently published recommendations for cardiopulmonary exercise testing in chronic lung diseases (19). A detailed description of the methodology used has previously been reported (12).Briefly, expired gases were analyzed in a mixing chamber by a TruOne® 2400 metabolic cart (ParvoMedics, Sandy, UT) and analyzed as 30 s averages to obtain VO_2 peak and pulmonary ventilation (VE). VO_2 peak relative to fat-free mass (FFM) was evaluating using ratio-standard scaling. The ventilatory threshold was determined using the v-slope method (20), and additional ventilatory parameters, including VE/VO_2 peak, VE/VCO_2 peak, end-tidal CO_2, and the VE/VCO_2 slope were determined according to previously published methodology (21).

VO_2 response time (VO_2 RT) and functional VO_2 gain, as indices of gas exchange, were determined using the breath by breath metabolic data that were averaged into 5-second

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intervals as previously described (22). Briefly, VO_2 RT was evaluated at the time interval between the onset of loaded ramp cycling and the intersection between resting VO_2 and the linear VO_2/time slope. Functional VO_2 gain was calculated by the slope of the ratio between the change in VO_2 with the change in work rate (22).

Hemodynamic and Chronotropic Responses

Hemodynamic responses were monitored non-invasively both at rest and throughout maximal exercise testing by the patented technology of signal morphology-based impedance cardiography (PhysioFlow®, Ebersviller, France). Disposable electrodes were placed on the neck, the chest, and the back according to manufacturer's instructions to record electrical and impedance changes in the thorax which allow for the calculation of cardiac output (CO), cardiac index (CI), stroke volume (SV), SV index (SVI), early diastolic filling ratio (EDFR) and systemic vascular resistance (SVR). Sensitivity and reproducibility of PhysioFlow® has been validated against the invasive thermodilution Swan-Ganz catheter technique (23) and also validated in people with CF (24).

Chronotropic response index (CRI), an indicator of the ability of the heart to increase its rate proportional to the increase in demand, was also evaluated. CRI was calculated using resting heart (HR_{rest}), maximal HR achieved during the exercise test (HR_{peak}), and the age-predicted maximal HR (HR_{APM}), using the following equation:

$$CRI = \frac{(HR_{peak} - HR_{rest})}{(HR_{APM} - HR_{rest})}$$

Failure to achieve a CRI of 0.8 was considered to be chronotropic incompetence (25).

Peripheral Oxygen Content

Peripheral O_2 saturation (SpO_2), an adequate reflection of arterial blood oxygenation (SaO_2), was evaluated using a fingertip pulse oximeter (PureSat, Nonin Medical, MI, USA) both at rest and throughout the maximal exercise test. The pulse-by-pulse system used in the present study shows an accuracy in SpO_2 ≥90% of −0.5±1 related with SaO_2, making it possible to assume that SpO_2 is highly representative of SaO_2. Mixed venous O_2 saturation ($S\bar{v}O_2$) represents a direct evaluation of tissue O_2 delivery and was calculated using a derivation of the Fick equation, considering cardiac output (CO) and hemoglobin (Hb) values (26):

$$S\bar{v}O_2 = SpO_2 - \left(\frac{VO_2}{13.9 \times CO \times Hb}\right)$$

Peripheral O_2 extraction ratio (O_2ER), was estimated based on the difference between SpO_2 and $S\bar{v}O_2$. Exercise factor (EF) ratio, an index of the relative contribution of O_2 extraction to exercise capacity, was calculated through the evaluation of the CO/VO_2 slope (27).

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Skeletal Muscle Oxygen Utilization

Muscle oxygenation was evaluated using a near-infrared spectroscopy (NIRS) device (Portamon, Artinis Medical Systems, Elst, The Netherlands) and reliable data was obtained in a subset of participants (healthy controls, n=14; patients with CF before sildenafil, n=14; patients with CF following four weeks of sildenafil, n=11). NIRS provides a noninvasive surrogate of muscle O_2 delivery and extraction using two continuous wavelengths of near-infrared light (780 and 850 nm) to measure tissue oxygenated hemoglobin/myoglobin (O_2Hb) and deoxygenated hemoglobin/myoglobin (HHb) concentrations (μM). In addition, the arithmetic sum of O_2Hb and HHb is defined as total hemoglobin (tHb) and used as an index of change in regional blood volume (28).

The placement of the NIRS device was carefully chosen based on the high activity that the *vastus lateralis* has during cycling, making it suitable for examining exercise-induced changes in active muscle oxygenation. The device was placed on the belly of the right *vastus lateralis* muscle, midway between the lateral femoral epicondyle and greater trochanter of the femur. Distance from the patella to the lowest point of the device was quantified for reproducibility purposes. To ensure that NIRS penetration depth was enough to reach the skeletal muscle, we evaluated adipose tissue thickness (ATT) in all the participants at the site of the NIRS placement using B-mode ultrasound imaging (LOGIQ 7; GE HealthCare, USA). The averaged values of skin and subcutaneous tissue thickness were 50.5 ± 10.0 and 60.7 ± 30.9 mm in the CF and control group, respectively. Considering the separation of the three light transmitters (3, 3.5 and 4 cm) from the receiving optode of the NIRS device, the light penetration was appropriate to interrogate the O_2 delivery and extraction of skeletal muscle in all our participants.

The intensity of incident and transmitted light of the NIRS device was continuously recorded at 10 Hz during the entire cardiopulmonary exercise test. With the leg placed in a stationary position, we normalized the results using a 2-min baseline period (arbitrarily defined as 0 μM) immediately before beginning the exercise protocol. Data was interpolated to 1 s intervals and expressed as a change from baseline. Data were then aligned to exercise intensity based on individual's VO2 peak response.

Statistical Analysis

All measurements are expressed as mean ± standard deviation (SD) unless otherwise noted. All statistical analyses were performed using SPSS version 25 (SPSS Inc., Chicago, IL, USA) and significance was set at $p<0.05$. Significance for a set of variables is expressed as greater than the lower value ("≥") or lower than the higher value ("≤"). Significance for a unique variable is expressed as equal to ("="). The Shapiro-Wilk test was used to assess the normality of the distribution of the data. Baseline characteristics between controls and patients with CF were compared using independent group *t*- tests. Paired *t*- tests were performed to identify differences between baseline and treatment within the patients with CF. Box and whisker plots were used to evaluated reserve capacity. The line in the shaded box represents the median value and the whiskers represent the minimum and maximum value. Effect size (Cohen's *d*) determined the magnitude of the difference between the main

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variables of interest. Cohen's *d* values of 0.2, 0.5 and 0.8 represented small, medium, and large effect sizes, respectively.

RESULTS

Subjects Characteristics

Demographic characteristics and laboratory values for all individuals with CF and control participants are presented in Table 1. No differences in age, sex, weight, height, or body mass index were observed between both groups. Participants with CF, however, exhibited significantly ($p \leq 0.02$) lower concentrations of lipids (TC, HDL, and LDL) when compared to controls, as others have previously described in this population (29). Additionally, as expected, pulmonary function was significantly ($p \leq 0.008$) reduced in individuals with CF compared to apparently healthy controls and no changes in lung function were observed after four weeks of sildenafil.

Exercise Capacity

Exercise testing values for people with CF and demographically-matched controls are summarized in Table 1. Given that only 32 of 33 participants achieved a true maximal test following the criteria defined by the American College of Sports Medicine (18), all values are expressed as peak instead of max. Functional capacity, indicated by VO_2 peak, VO_2 peak/body weight, VO_2 peak/FFM, and VO_2 peak (% predicted), were all significantly ($p \leq 0.02$) lower in individuals with CF compared to the control group. Additionally, as previously reported in greater detail (12), participants with CF showed a significant ($p \leq 0.03$) improvement in O_2 consumption (VO_2 peak, VO_2 peak/body weight, VO_2 peak/FFM, and VO_2 peak (% predicted)) following four weeks of treatment with sildenafil. Although individuals with CF achieved improvements on average of 4.7% increase in exercise capacity, results were still lower than healthy individuals.

Indices of Gaseous Exchange—During the exercise test, people with CF exhibited a significantly ($p=0.006$; Cohen's $d=1.12$) slower VO_2 RT than the control group (CF: 50.7 ± 18.6 s vs. Controls: 32.8 ± 16.4 s). Notably, a faster ($p=0.02$; Cohen's $d=1.03$) response time was identified in the CF group following sildenafil treatment (37.9 ± 14.3 s), which resulted in a similar ($p=0.35$) post-treatment value compared to controls. On the other hand, no differences ($p=0.21$; Cohen's $d=0.75$) were observed in functional VO_2 gain between CF and controls at baseline (8.76 ± 1.01 *vs.* 9.26 ± 1.25 ml/watts) or following four weeks of sildenafil treatment in patients with CF (8.45 ± 1.11 ml/watts; $p=0.99$).

Hemodynamic and Chronotropic Responses

Hemodynamic variables are presented in Table 2. Both resting and at peak exercise values for CO, CI, SV, and SVI were similar ($p \geq 0.10$; Cohen's $d \geq 1.04$) between patients with CF and control participants. In addition, EDFR and SVR indices were both similar ($p \geq 0.081$; Cohen's $d \geq 0.84$) between groups at rest and during maximal exercise. Following treatment with sildenafil, no differences ($p \geq 0.17$; Cohen's $d \geq 0.91$) were observed in any of the hemodynamic variables analyzed, with the exception of EDFR, which was significantly ($p=0.022$; Cohen's $d=0.61$) reduced following 4 weeks of PDE-5 inhibition.

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Blood pressure at rest was similar ($p \geq 0.089$; Cohen's $d \geq 0.69$) between patients and controls; however, patients had a significantly ($p=0.036$; Cohen's $d=0.74$) lower mean arterial blood pressure in response to maximal exercise than the demographically matched controls. After four weeks of sildenafil, no changes ($p \geq 0.48$; Cohen's $d \geq 0.88$) were observed in blood pressure either at rest or during maximal exertion in the CF group.

The chronotropic response at rest and peak exercise for all the participants are presented in Table 2. HR at rest was similar ($p \geq 0.62$; Cohen's $d \geq 0.69$) between groups; however, maximal HR during exercise was significantly ($p=0.04$; Cohen's $d=0.84$) lower in patients with CF when compared to controls. In addition, a significantly ($p \leq 0.001$) greater number of patients showed chronotropic incompetence (n=5) when compared to the control group (n=0). Treatment with sildenafil did not impact ($p=0.14$) chronotropic response to maximal exercise in patients with CF.

Oxygen Content and Utilization

Peripheral Capillary O_2 Saturation—Data for O_2 content and utilization are presented in Table 2. Peripheral capillary O_2 saturation was similar ($p \geq 0.14$; Cohen's $d=1.12$) between patients and controls both at rest and during maximal exercise. Treatment with sildenafil did not affect ($p \geq 0.67$; Cohen's $d=1.09$) SpO_2 in CF either at rest or during exertion. No changes were observed in pulmonary function after four weeks of sildenafil.

Mixed Venous O_2 Content—Mixed venous O_2 content results are shown in Table 2. Although $S\bar{v}O_2$ was similar ($p=0.54$; Cohen's $d=0.23$) between CF and controls at rest, individuals with CF exhibited a significantly ($p=0.008$; Cohen's $d=1.08$) higher $S\bar{v}O_2$ during maximal exercise when compared to their healthy counterparts. In addition, after the four weeks of sildenafil treatment, a significant ($p<0.001$; Cohen's $d=2.37$) reduction in $S\bar{v}O_2$ at maximal exertion was observed, restoring it to a similar value ($p=0.70$) observed in the controls.

O_2 Extraction Ratio—O_2 extraction ratio results are described in Table 2. At rest, the extraction of O_2 was similar ($p=0.73$; Cohen's $d=0.13$) between groups. However, during maximal exertion, O_2ER was significantly ($p=0.003$; Cohen's $d=1.21$) lower in people with CF when compared to controls. Treatment with sildenafil resulted in 10% greater O_2 extraction during exertion ($p=0.001$; Cohen's $d=2.34$), restoring the values to a similar response ($p=0.46$) observed in demographically matched controls.

Skeletal Muscle Oxygen Utilization—Muscle O_2 utilization results between CF and controls are illustrated in Figure 1. At rest, no differences ($p=0.92$; Cohen's $d=0.84$) in muscle blood volume were identified between CF and controls. However, ΔtHb was significantly ($p \leq 0.021$; Cohen's $d=0.91$) lower in the CF group compared with the control when exercise intensity was 50% VO_2peak or greater. During maximal effort, the CF group exhibited a significantly ($p=0.006$) lower muscle blood volume than their healthy counterparts.

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ΔO_2Hb was similar between both groups at low intensities of the exercise test. However, at high intensities (80% VO_2 peak or greater), ΔO_2Hb was significantly ($p \leq 0.04$; Cohen's $d=0.98$) higher in the CF group than in the control group, with a marked differenced ($p=0.006$) at peak exercise. Similarly, ΔHHb increased gradually in both groups along with exercise intensity, and at high intensities (70% VO_2 peak or greater), the increase in ΔHHb in the CF group was significantly ($p \leq 0.004$; Cohen's $d=1.07$) lower than the response observed in the control group, with an overall ($p=0.004$) lower O_2 extraction at peak exercise.

Muscle O_2 utilization in the CF group before and after four weeks of sildenafil is also illustrated in Figure 1. No meaningful differences were observed at rest or low intensities. Conversely, muscle blood volume was significantly ($p=0.04$; Cohen's $d=1.11$) greater after the sub-acute treatment with sildenafil and similar ($p=0.22$) to the control group. In the same way, statistically significant changes were observed in muscle O_2 utilization post-treatment (ΔO_2Hb; $p \leq 0.04$; Cohen's $d=0.90$: ΔHHb; $p \leq 0.02$; Cohen's $d=0.98$) at high intensities of exercise capacity (80% VO_2 peak or greater). Notably, individuals with CF after four weeks of sildenafil exhibited similar muscle O_2 utilization than the control group (ΔO_2Hb; $p \geq 0.16$; ΔHHb; $p \geq 0.15$).

Exercise Factor Ratio—EF ratio was significantly ($p=0.03$; Cohen's $d=0.85$) higher in the participants with CF (7.9±3.1 a.u.) when compared with controls (5.7±2.1 a.u.). After four weeks of sildenafil, the CF group exhibited a significantly ($p=0.03$; Cohen's $d=1.29$) lower EF ratio (6.2±1.9 a.u.) than prior to the treatment. Notably, post-sildenafil EF was similar to the efficiency observed in demographically-matched controls ($p=0.51$).

DISCUSSION

Findings of the present study have demonstrated that individuals with mild-to-moderate CF lung disease exhibit a reduction in skeletal muscle O_2 extraction and utilization during exercise when compared to demographically-matched controls. Our findings also reveal that four weeks of treatment with sildenafil can improve skeletal muscle O_2 utilization during exercise, restoring values to thoseobserved in healthy individuals. In addition, we have identified that mechanisms for exercise intolerabce in our CF population were minimally limited by hemodynamic or chronotopic responses, while stronger contributions were linked to peripheral O_2 extraction. Taken together, our findings highlight the importance of targeting mechanisms of skeletal muscle O_2 utilization in CF to augment exercise tolerance and the therapeutic potential of sildenafil.

Factors Limiting Exercise Intolerance in Cystic Fibrosis

Exercise intolerance, evaluated through O_2 consumption, represents a critical phenotype in CF disease severity since it predicts survival in this population (1). According to the Fick equation, O_2 consumption is determined by the balance between central factors such as O_2 content, transport and delivery, and peripheral factors such as O_2 extraction and utilization (3).

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Oxygen Content—In a healthy individual, the capacity of the lungs to transfer O_2 to the arterial blood is commonly preserved. However, when the lungs have a deteriorated function, compensatory mechanisms are triggered to increase the affinity of O_2 to hemoglobin and secure O_2 loading within pulmonary capillaries. In this situation, most individuals hyperventilate and increase the concentration of hemoglobin to facilitate the defense against arterial hypoxia and hypercapnia. However, that is not the situation for individuals with CF that present with similar hemoglobin concentrations compared with healthy controls. Although pulmonary limitations are critical in the prognosis of CF, the observed exercise intolerance in CF is independent of lung function (30). Indeed, individuals with mild to moderate disease, similar to the present CF cohort, are able to maintain adequate arterial O_2 saturation and appropriate ventilation during exertion, supporting the idea that pulmonary impairment is not a limiting factor of exercise intolerance.

Oxygen Transport and Delivery—Central mechanisms, including hemodynamics and chronotropic response, are critical for the adequate supply of O_2 during periods of increased demand. In healthy individuals, CO typically increases 3- to 5-fold during exercise to support the metabolic requirements and facilitate the delivery of O_2 to cardiac, respiratory, and skeletal muscles. Previous reports have described the involvement of the CFTR in the regulation of cardiomyocyte contraction (31) and a potential association with early signs of cardiac dysfunction in younger individuals with CF (32). In this line, CFTR modulators may have the ability to improve cardiac function, as prevailing reports have demonstrated (33). However, similar to our results with sildenafil, contrasting information has also been reported with no observed changes in the cardiac response to exercise (34, 35). It is important to keep in mind the heterogeneity of the CF population and the broad range of symptoms and progression that these individuals present with. Indeed, a recent report identified that certain CF genotypes develop more severe cardiac dysfunctions than others (36) warranting future investigations into this hypothesis.

Heart rate constitutes a major determinant of CO and the chronotropic response, especially following the plateau in stroke volume. Consistent with a previous report (37), five participants with CF were unable to achieve 85% of their HR_{APM} during maximal exercise, whereas all of the healthy controls were able to meet the same criteria. In addition, six of the fifteen individuals from the CF cohort (40%) exhibited chronotropic incompetence during the maximal exercise test at baseline, with a CRI as low as 0.68. It is important to note that these CRI values were achieved with maximal ventilation and even with RER values equal or greater than 1.17, ruling out the possibility that chronotropic incompetence contributed to a poor or non-maximal effort during the exercise test.

Adequate O_2 delivery can also be evaluated through changes in tissue blood volume (28). As expected, findings of the present study indicate that both groups exhibit a progressive increase in muscle blood volume proportional to exercise intensity (38). However, the overall increase in muscle blood volume was significantly lower in CF compared to controls, indicating the possibility of reduced muscle perfusion. In addition, the CF group exhibited delayed muscle perfusion at the onset of exercise (<60% VO_2 peak). These results are consistent with previous observations in people with CF that have not only described

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impairments in both micro- (39) and macro- (40) vascular function, but reduced blood flow during maximal exercise as well (41).

Oxygen Extraction and Utilization—After convective delivery stay of O_2 to the skeletal muscle, the diffusion and utilization of O_2 depend on the exchange between microcirculation and skeletal muscle units and can be evaluated indirectly using venous O_2 saturation. At rest, adequate tissue oxygenation is reflected by $S\bar{v}O_2$ levels from 65 to 77%. During exercise, an efficient extraction is reflected by a fall in $S\bar{v}O_2$, while the working muscles are extracting O_2 to maintain aerobic metabolism. Higher values or a lack of change from rest directly reflect impairments in tissue perfusion (26). In the present study, individuals with CF and healthy controls exhibited a similar $S\bar{v}O_2$ at rest. However, during maximal exertion, tissue oxygenation in the CF group was significantly lower when compared to the control group, indicating impairment in muscle perfusion during exertion.

Muscle fractional O_2 extraction, indirectly measured through HHb, also reflects the local balance between O_2 delivery and utilization within the muscle. Findings from the present investigation provide evidence that during exertion, people with CF exhibit a severe impairment in O_2 extraction, especially during high intensities (>70% VO_2peak). This is consistent with earlier observations that also described similar deficits in an intensity-dependent pattern in young individuals with CF (35). Several factors may explain this deficit in O_2 utilization. For example, our data support that individuals with CF exhibited reduced recruitment of oxidative fibers (sigmoidal phase HHb) at the start of exercise that resulted in less efficient extraction of O_2 (plateau phase HHb) compared with their healthy counterparts, as described in young individuals (42). Another possibility that may explain the observed reduced capacity of the muscle to utilize O_2 is mitochondrial dysfunction. Prevailing reports have described impairments in skeletal muscle oxidative capacity in CF (5, 6, 43, 44) in an intensity-dependent manner (35), which conceivably may prevent efficient extraction of O_2 from the working muscles (45). In addition, these dysfunctions have been previously associated with the dysregulation of calcium mobilization in muscle cells (46, 47). Consistent with this hypothesis, the plateau phase of HHb curve has previously been considered as a functional evaluation of skeletal muscle oxidative metabolism (48). It is also noteworthy that EF ratio, an indirect index of oxidative metabolism (49), and the observed delay in VO_2 response time observed in CF (7, 22, 50) also support the existence of reductions in oxidative metabolism (51). Another factor contributing to the observed impairment of O_2 extraction in CF may be in part from a reduced dissociation of O_2 from Hb (28) since individuals with CF commonly present with CF-related diabetes, and glycated hemoglobin can impact O_2Hb dissociation. However, the average HbA_{1c} in the investigated CF cohort was 6.1%, and the aforementioned impairment is typically observed with HbA_{1c} equal to or greater than 8% (52). It is important to note that there is debate about the reliability of HbA_{1c} as a diagnostic tool for CF-related diabetes (53–56). However, similar results have been observed in our cohort using glucose levels. In summary, findings support that people with CF exhibit impaired muscle O_2 utilization during maximal exercise when compared to healthy counterparts.

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Sildenafil and Exercise Intolerance in Cystic Fibrosis

Recently, we have demonstrated that four weeks of treatment with sildenafil can improve exercise capacity in patients with CF (12). In order to further understand the observed improvements, we have explored the effects of sildenafil on each of the components that influence O_2 consumption during exercise. Considering that no changes in pulmonary function and no improvements in ventilatory efficiency were observed after the sub-acute treatment with sildenafil, no changes in O_2 gas exchange across the lungs were expected. With respect to O_2 transport and delivery, treatment with sildenafil in CF resulted in a moderate increase in CO during exercise that may be associated with systemic vasodilation, optimization of left ventricular contractility, and/or reductions in cardiac afterload that are common effects of PDE-5 inhibitors(57). Additionally, following four weeks of treatment with sildenafil, individuals with CF exhibited a better chronotropic response to exertion, with only three of the fifteen patients (20%) having a post-treatment CRI lower than 0.80. However, as others have previously reported (58), sildenafil did not promote significant changes in the HR response to exercise in our CF population.

On the contrary, O_2 extraction during maximal exertion improved to the value similar to the one with the control group following treatment with sildenafil in people with CF. Similar results was observed in the active muscle, where we observed an enhanced balance between O_2 delivery and utilization, especially at the highest energetic demand. This improvement was previously inferred after observing a significant decrease in peak RER following the four weeks of sildenafil, supporting an improved skeletal muscle O_2 utilization (12).

Sildenafil is known for its systemic vasodilation; however, it is also able to modify CFTR expression and activity as well as improve and/or restore calcium homeostasis in the sarcoplasmic reticulum, enhancing skeletal muscle function (8, 59, 60). In support of these additive pharmacological effects, a significant improvement in post-treatment VO_2 response time was observed in the present study, suggesting a faster adaptation to the O_2 demand, despite no changes in peak power output. Thus, present findings support that a sub-acute treatment with sildenafil was able to increase skeletal muscle O_2 utilization during maximal exercise in people with CF. Considering the financial burden imposed by the current CF modulator therapies, sildenafil may represent a more affordable treatment strategy in the fight against CF .

Reserve Capacity during Exercise in Cystic Fibrosis

The evaluation of the reserve capacity may help in understanding the relative contributions of the different mechanisms that contribute to exercise intolerance (27). We examined reserved capacity through the assessment of the change in VO_2 and the response of the subcomponents affecting O_2 consumption in the CF and the control groups as well as before and after sildenafil treatment in CF (Figure 2). Our results demonstrate that both hemodynamic (CO: CF, 1.5±0.5 *vs.* controls, 1.8±0.5 fold change, p=0.140) and chronotropic (HR: CF, 0.8±0.3 *vs.* controls, 0.9±0.3 fold change, p=0.407) responses during exercise were similar between individuals with CF and controls. However, a significant change in O_2ER was identified from rest to exertion in people with CF when compared with the control group (CF, 1.3±0.7 *vs.* controls, 2.1±0.9 fold change, p=0.006). Four weeks of

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treatment with sildenafil led to significantly (p=0.032) increase O_2ER in the CF group (1.7±0.1 fold change), while no changes (p≥0.520) were identified in CO or HR. Based on these pre and post-treatment findings, it is conceivable that impaired skeletal muscle oxidative metabolism plays a significant role in exercise intolerance in CF and results in poor O_2 extraction as observed in our reserve capacity analysis Moreover, sildenafil has therapeutic potential to improve muscle perfusion during exercise and improve O_2 extraction in people with CF.

Conclusion

In summary, individuals with mild to moderate CF exhibit reduced peripheral O_2 extraction during exercise that likely contributes to the observed exercise intolerance. The findings of the present study provide support for the existence of impaired O_2 utilization by the active skeletal muscle, especially when exposed to a period of high exertion. In addition, four weeks of treatment with sildenafil improves the capacity of the muscle to utilize O_2 more efficiently during exercise, satisfying the high metabolic demand required during more intense exercise. Taken together, findings of the present study highlight the importance of targeting skeletal muscle O_2 utilization to improve exercise tolerance in CF, and the potential therapeutic role of sildenafil to improve exercise capacity in CF.

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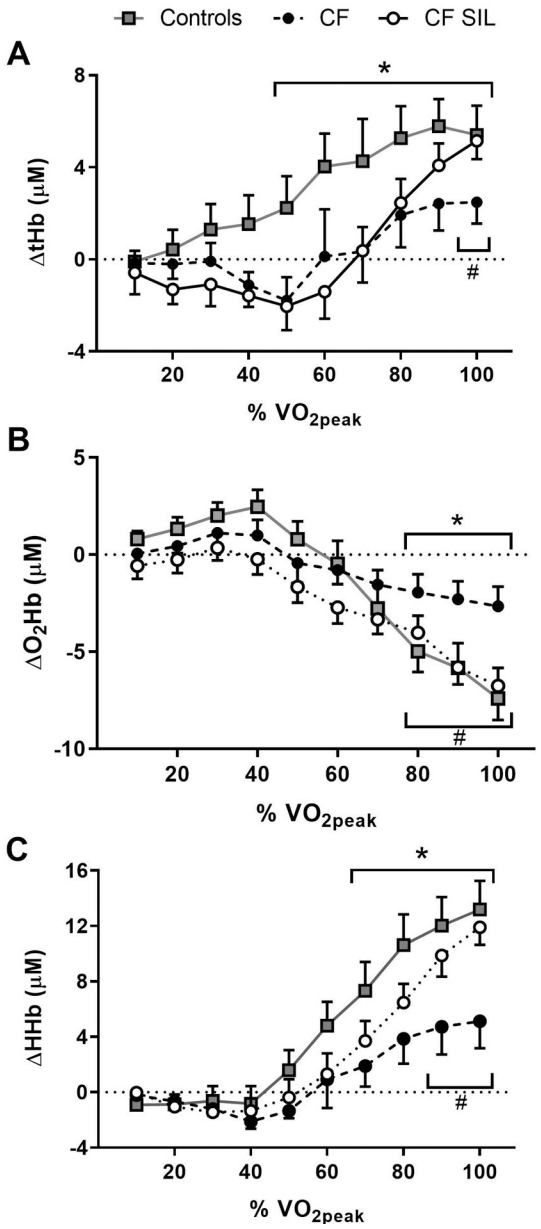

Figure 1. Skeletal muscle O_2 utilization during exercise in patients with cystic fibrosis before and after four weeks of sildenafil and in controls.

Change in total hemoglobin (ΔtHb; panel A), oxygenated hemoglobin (ΔO_2Hb; panel B) and deoxygenated hemoglobin (ΔHHb; panel C) in healthy controls (*grey squares*; n=14), patients with CF before (*black circles;* n=14) and following four weeks of sildenafil (SIL, *white circles;* n=11). Values are mean ± SEM. *Significantly different between patients with CF and controls. #Significantly different (p<0.05) between baseline and four weeks of treatment with sildenafil in patients with CF.

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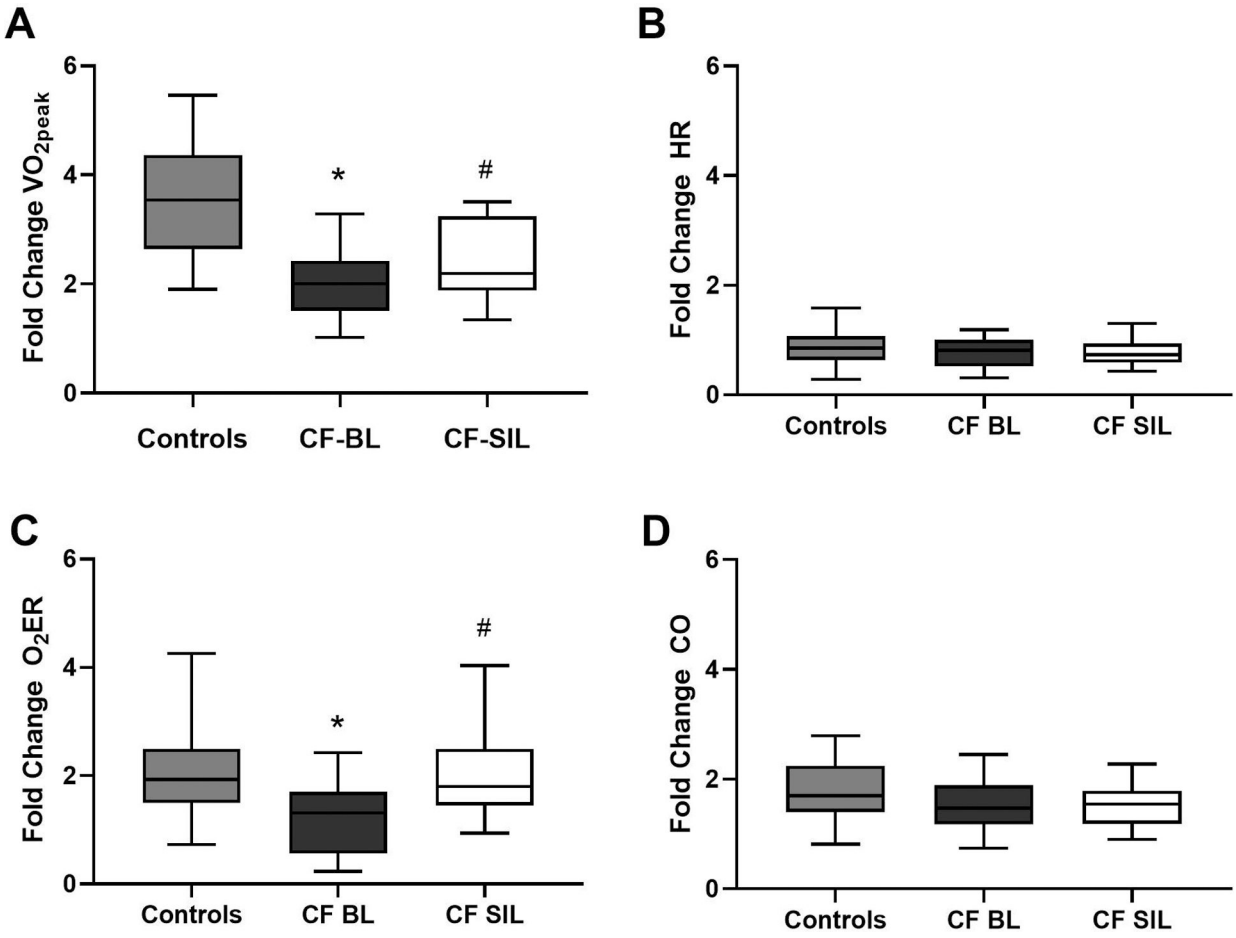

Figure 2. Reserve capacity during exercise evaluated by the increase in O_2 consumption and each component of VO_2.

Box and whiskers plots of the fold change of oxygen consumption (VO_2), heart rate (HR), cardiac output (CO), and peripheral O_2 extraction (O_2ER) from rest to peak exercise independently of resting values. Data representation in healthy controls (control; grey), patients with CF (CF BL; black), and patients with CF after four weeks of sildenafil (CF SIL; white). *Significantly different ($p<0.05$) between patients with CF and controls. #Significantly different ($p<0.05$) between baseline and four weeks of treatment with sildenafil.

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Table 1-

Participant Characteristics and Laboratory Values

Variable	Controls	CF	*p* value
Demographic Characteristics			
N	18	15	-
Sex, m/f	9/9	8/7	0.529
Age, years	27±8	23±11	0.345
Height, cm	165±26	160±13	0.522
Weight, kg	61±12	54±17	0.083
BMI, kg/m	22±5	21±4	0.508
BMI, z-score[#]	−0.7±0.4	-0.9±0.5	0.259
Body fat, %	29±8	28±12	0.712
SBP, mm Hg	114±14	111±13	0.215
DBP, mm Hg	65±8	68±9	0.694
Clinical Laboratory Values			
TC, mg/dL	175±37	135±30	**0.002**
HDL, mg/dL	60±16	41±11	**0.001**
LDL, mg/dL	100±34	74±24	**0.021**
Triglycerides, mg/dL	74±23	94±36	0.059
TC:HDL	3.1±1.1	3.4±0.8	0.348
Glucose, mg/dL	88±7	96±16	0.079
hsCRP, mg/L	1.2±2.2	2.5±4.5	0.344
Hemoglobin, g/dL	14.7±1.6	14.6±1.8	0.876
Hematocrit, %	43.9±4.0	44.1±4.0	0.898
Total blood volume, L	4.3±0.8	3.7±0.9	0.086
RBC volume, L	1.9±0.5	1.7±0.5	0.196
Pulmonary function			
FVC, L	4.6±1.0	3.3±0.9	**0.001**
FEV_1, L	3.7±0.8	2.6±0.8	**<0.001**
$FEF_{25–75}$, L/s	3.6±0.9	2.5±1.3	**0.008**
FEV_1, % predicted	98±9	81±16	**0.001**
FVC, % predicted	97±8	86±8	**0.012**
FEV_1/FVC, %	81±6	77±8	0.198
Exercise Capacity			
VO_2 peak, L/min	2.3±0.8	1.5±0.4	**0.001**
VO_2 peak, mL/kg/min	34.5±7.1	28.2±5.6	**0.019**
VO_2 peak, mL/kg FFM/min	52.4±7.2	41.8±6.2	**<0.001**
VO_2 peak, % predicted	96±16	72±12	**<0.001**
Work peak, watts	204±54	131±40	**<0.001**
VE peak, L/min	94±29	65±19	**0.003**
RER peak	1.18±0.07	1.31±0.15	**0.041**

Values are mean ± standard deviation (SD). Bold font indicates statistical significance ($p<0.05$). CF= cystic fibrosis; BMI= body mass index; SBP= systolic blood pressure; DBP= diastolic blood pressure; TC= total cholesterol; HDL= high density lipoproteins; LDL= low density lipoproteins; hsCRP= high sensitive C-reactive protein; RBC= red blood cells; FVC= forced vital capacity; FEV_1= forced expiratory volume in one second; $FEF_{25–75}$= forced expiratory flow at 25–75%; VO_2= oxygen consumption; VE= ventilation; RER: respiratory exchange ratio.

[#]For participants 19 years old or younger.

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Table 2-

Hemodynamics, Chronotropic Response, and Oxygen Content and Utilization at Rest and During Peak Exercise in Patients with Cystic Fibrosis and Control Subjects.

	Controls		CF BL		CF Sildenafil	
Variable	**Rest**	**Peak Exercise**	**Rest**	**Peak Exercise**	**Rest**	**Peak Exercise**
Hemodynamic and Chronotropic Response						
CO, L/min	5.7±1.5	16.2±5.9	5.1±1.2	13.3±3.7	5.2±0.9	14.9±3.5
CI, L/min/m^2	3.3±0.6	9.4±3.0	3.4±1.3	8.3±2.6	3.4±0.9	9.1±2.1
SV, mL	67±21	91±37	57±19	69±25	61±15	75±19
SVI, mL/m^2	38±9	51±15	39±12	47±13	40±9	49±11
EDFR, %	45±6	57±21	47±6	52±19	44±4	49±15
SVRi, dyn.s/cm^5 m^2	2141±297	1029±266	2267±1217	1146±569	1969±501	980±261
SVR, dyn.s/cm^5	1246±204	605±182	1488±566	765±291	1281±222	644±159
SBP, mm Hg	118±15	176±23	111±13	157±27*	112±11	157±28*
DBP, mm Hg	73±9	80±8	68±9	76±9	70±10	74±10
MAP, mm Hg	92±10	119±10	87±10	109±15*	88±10	108±14*
HR, bpm	60±9	183±10	69±10	175±14*	69±10	178±11*
CRI	-	0.95±0.08	-	0.81±0.08*	-	0.85±0.08*
Oxygen Content and Utilization						
SpO_2, %	99±1	98±1	98±1	97±2	98±1	97±2
$S\bar{v}O_2$, %	75±7	32±15	73±5	45±8*	75±6	35±11#
O_2ER, %	24±7	66±13	25±8	52±9*	23±7	62±10#

Values are mean ± standard deviation (SD).

*Statistical significance (p<0.05) differences are denoted by representing differences between the control group and

#the CF group and by a representing differences before and after treatment with sildenafil in the patients with CF. CF= cystic fibrosis; BL= Baseline; CO= cardiac output; CI= cardiac index; SV= stroke volume; SVI= stroke volume index; EDFR=end diastolic filling ratio; SVRi= systemic vascular resistance index; SBP= systolic blood pressure; DBP= diastolic blood pressure; MAP= mean arterial pressure; HR= heart rate; CRI= chronotropic response index; SpO_2= peripheral oxygen saturation; $S\bar{v}O_2$= mixed venous oxygen saturation, O_2ER= peripheral oxygen extraction