IMPAIRED AEROBIC FUNCTION IN PATIENTS WITH CYSTIC FIBROSIS DURING RAMP EXERCISE

Zoe Louise Saynor[a,b], Alan Robert Barker[a], Patrick John Oades[b], Craig Anthony Williams[a,b]

Running Title: NIRS ramp exercise response in paediatric CF

[a] Children's Health and Exercise Research Centre, Sport and Health Sciences, University of Exeter, Exeter, Devon, UK.

[b] Royal Devon and Exeter NHS Foundation Trust Hospital, Exeter, Devon, UK.

*Correspondence to: C.A. Williams, PhD, Children's Health and Exercise Research Centre, Sport and Health Sciences, University of Exeter, St. Luke's Campus, Heavitree Road, Exeter, EX1 2LU, UK.
Tel: +44 (01392) 724890
Email: c.a.williams@exeter.ac.uk
Fax: +44 (01392) 724726

ABSTRACT

Purpose: This study aimed to document the matching of muscle O_2 delivery-to-O_2 utilisation in young patients with cystic fibrosis (CF) patients from muscle deoxygenation (HHb) dynamics during ramp exercise. **Methods:** Ten patients with stable, mild-to-moderate CF (12.7 ± 2.8 y) and 10 healthy controls (CON; 12.8 ± 2.8 y) completed a combined ramp and supramaximal cycling test to determine maximal O_2 uptake ($\dot{V}O_{2max}$). Changes in gas exchange and ventilation, heart rate and *m. vastus lateralis* HHb (near-infrared spectroscopy) were assessed. Δ[HHb]-work rate and Δ[HHb]-$\dot{V}O_2$ profiles were normalised and fit using a sigmoid function. **Results:** Aerobic function was impaired in CF, indicated by very likely reduced fat-free mass normalized $\dot{V}O_{2max}$ (mean difference, ±90% CI: -7.9 $mL \cdot kg^{-1} \cdot min^{-1}$, ±6.1), very likely lower $\dot{V}O_2$ gain (-1.44 $mL \cdot min^{-1} \cdot W^{-1}$, ±1.12) and a likely slower $\dot{V}O_2$ mean response time (11 s, ±13). An unclear effect was found upon the absolute and relative WR (-14 W, ±44 and -0.7 %PPO, ±12.0, respectively) and the absolute and percentage (-0.10 $L \cdot min^{-1}$, ±0.43 and 3.3 %$\dot{V}O_{2max}$, ±6.0) $\dot{V}O_2$ corresponding to 50% Δ[HHb] amplitude, respectively, between groups. However, arterial oxygen saturation (SpO_2) was very likely lower in CF (-1%, ±1) and demonstrated moderate-to-very large relations with parameters of aerobic function. **Conclusion:** Young patients with mild-to-moderate CF present with impaired aerobic function during ramp incremental cycling exercise. Because the rate of fractional O_2 extraction during ramp cycling exercise was not altered by CF, yet SpO_2 was lower, the present findings support the notion of centrally mediated oxygen delivery to principally limit the aerobic function of pediatric patients with CF during ramp incremental cycling exercise.

Keywords: Near-infrared spectroscopy, aerobic function, exercise testing, exercise limitation, lung disease, paediatrics.

INTRODUCTION

Paragraph 1: Cystic fibrosis (CF) is a complex multiorgan genetic disease, expressed as a disruption in the CF transmembrane conductance regulator (CFTR) protein. In conjunction with its clinical presentation, reduced aerobic fitness (typically determined as maximal oxygen uptake ($\dot{V}O_{2max}$)), is commonly observed in both adult (10) and paediatric patients (17). Reduced aerobic fitness is of clinical relevance in patients with CF, given its association with longevity (25,28), quality of life (6) and risk of hospitalisation (26). Key parameters of aerobic function (i.e., $\dot{V}O_{2max}$, $\dot{V}O_2$ gain, gas exchange threshold (GET), and $\dot{V}O_2$ mean response time (MRT)) (37) have not, however, been comprehensively documented in CF. Moreover, no previous studies have used a valid protocol (30) to obtain a 'true' measure of $\dot{V}O_{2max}$ in this population. Identifying the limiting factor(s) impairing aerobic function in CF will facilitate the development of more effective strategies to improve longevity and quality of life in this aging patient population.

Paragraph 2: Because the body's upper limit for $\dot{V}O_2$ use is determined by the maximal cardiac output ($\dot{Q}$), arterial $\dot{V}O_2$ content, fractional distribution of $\dot{Q}$ to the exercising muscles, and the ability of the skeletal muscle to extract O_2 (35), simultaneous measurements at the central (cardiorespiratory) and peripheral (skeletal muscle) levels are required to understand the dynamic matching of O_2 delivery-to-O_2 utilisation during exercise. Previous studies in CF have, however, largely neglected this complex interaction and based inferences on investigations of isolated organ systems (e.g.8,16,20).

Paragraph 3: As a result, debate remains regarding the relative importance of central and peripheral mechanisms to explain the reduced $\dot{V}O_{2max}$ in patients with mild-to-moderate CF. Expression of CFTR in the human skeletal muscle (19) suggests an intrinsic myocyte metabolic abnormality, which may be specific to CF (e.g.7,8) or a consequence of chronic respiratory disease (30,36). In addition, there is evidence to support a central limitation to

exercise through a reduction in stroke volume (SV) (27) and, presumably, muscle O_2 delivery (29), in pediatric patients with CF. Although a compensatory increase in muscle O_2 extraction may be expected to occur in the presence of reduced muscle O_2 delivery, this was not observed in a previous study (29). However, further confirmation of this response is warranted because inferences at the skeletal muscle level were based upon indirect, interlinked mathematical calculations.

Paragraph 4: To further understand how disease pathophysiology alters the O_2 delivery-to-O_2 utilisation relationship during exercise, near-infrared spectroscopy (NIRS) can provide valuable, non-invasive insight into peripheral O_2 extraction. Specifically, the profile of the deoxyhemoglobin ([HHb]) signal has been used to describe O_2 extraction dynamics during ramp exercise, which in turn permit inferences regarding blood flow within the microcirculation of exercising muscle (4,9,23,24,25). Although the[HHb] profile during ramp exercise has been used to describe the effect of trained status (4,21) and ageing (11,21), there are no data documenting the influence of disease on the [HHb] response to incremental exercise. If the supply of blood to the active muscle during exercise is impaired in CF, as would be indirectly inferred from previous reports of a reduced SV (27), an increased rate of fractional O_2 extraction for a given $\dot{V}O_2$ would be expected (9).

Paragraph 6: The purpose of the present study was twofold; *1)* to characterise the four key parameters of aerobic function in pediatric patients with mild-to-moderate CF and *2)* to characterize the dynamic adjustment of NIRS-derived leg muscle [HHb] during ramp exercise. It was hypothesised that: *1)* aerobic function would be impaired in CF, as evidenced by a reduced $\dot{V}O_{2max}$, slower $\dot{V}O_2$ MRT, earlier occurrence of the GET and shallower $\dot{V}O_2$ gain and, furthermore, that *2)* patients with CF would be characterized by more rapid [HHb] dynamics during ramp exercise (i.e., leftward shift), which will correlate with impaired parameters of aerobic function.

MATERIALS and METHODS

Paragraph 7: Study participants. Ten young patients (9 males (Table 1)) with stable mild-to-moderate CF disease (CF) regularly partaking in school and/or extracurricular physical activity were recruited from outpatient clinics at the Royal Devon and Exeter NHS Foundation Trust Hospital. CF inclusion and exclusion criteria are detailed elsewhere (30). Ten healthy age- and gender- matched control participants (CON) were recruited from the local area (Table 1). Neither group presented with any contraindications to exhaustive exercise, and CON were free from any pulmonary conditions. Ethics approval was granted by the South West NHS Research Ethics Committee. Informed written consent and assent were obtained from parents/guardians and patients, respectively. Details concerning the CF patients' disease severity and clinical profile were obtained by their clinician (Table 2). All CF maintenance medications were continued as usual throughout the study.

Paragraph 8: Anthropometry and pulmonary function. Body mass (Seca 220; Vogel & Halke, Hamburg, Germany) and stature (Seca 220; Vogel & Halke, Hamburg, Germany) were measured to the nearest 0.01 kg and 0.01 m, respectively. Pubertal maturity was determined using Tanner staging (34). Skin folds measured to the nearest 1 mm on the right-hand side of the body at the tricep and subscapula regions (Harpenden; British Indicators, Burgess Hill, UK) were used to estimate fat-free mass (FFM) (32). Forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1) were assessed using flow-volume loop spirometry (MicroMedical MicroLoop 3535, Numed, Sheffield, UK). The best of three consistent (< 5% variability) exhalations was documented and expressed as a percentage of predicted reference data (33).

Paragraph 9: Exercise testing. Participants arrived to the laboratory in a rested state, ≥ 2 h postprandial and having refrained from caffeine for ≥ 2 h. A maximal cardiopulmonary exercise test (CPET) was performed on a cycle ergometer (Lode Excalibur or Lode Corival, Groningen, The Netherlands) using a single session, combined ramp incremental and supramaximal CPET protocol, which has been validated in healthy children (1) and children with CF (30). This protocol, the reproducibility of which has recently been documented in young CF patients (32), involved an exhaustive ramp incremental (10-25 $W \cdot min^{-1}$) cycling test with a subsequent supramaximal (110% peak power output (PPO)) test to exhaustion to verify $\dot{V}O_{2max}$. After a 3-min warm-up period (20 W cycling), participants completed the incremental ramp test while cycling at a cadence of approximately 70-80 rpm until volitional exhaustion, defined as a drop in cadence ≥ 10 rpm for five consecutive seconds, despite strong verbal encouragement. Five-minute active recovery (20 W cycling) and 10-min passive seated recovery then preceded the S_{max} verification test, which involved a 3-min warm-up (20 W cycling) before a "step" transition to a constant work rate (WR) equivalent to 110% PPO. Upon voluntary exhaustion, 5-min active recovery (20 W cycling) completed the CPET assessment.

Experimental measures

Paragraph 10: Before each test, the metabolic cart (Metalyzer 3B Cortex, Biophysik, Leipzig, Germany) was calibrated using gases of known concentration and a 3-L calibration syringe (Hans Rudolph, Kansas City, MO) was used to calibrate the turbine volume transducer. Breath-by-breath changes in pulmonary gas exchange and ventilation were measured and averaged to 15-s time bins, with the highest 15-s stationary average from the ramp or S_{max} representing $\dot{V}O_{2max}$ (11). The GET in absolute terms and expressed as a percentage of $\dot{V}O_{2max}$ was non-invasively identified (1) and confirmed through visual

identification of the ventilatory equivalents for $\dot{V}O_2$ and carbon dioxide output ($\dot{V}CO_2$). The $\dot{V}O_2$ mean response time (MRT) was determined using the time from the onset of ramp exercise to the intersection point between baseline $\dot{V}O_2$ and a backward extrapolation of the slope of $\dot{V}O_2$ as a function of time (37). Regression of the linear portion of the $\dot{V}O_2$ response versus power output was used to determine the functional $\dot{V}O_2$ gain ($\Delta\dot{V}O_2/\Delta WR$). Equation 1 was used to determine oxygen pulse ($\dot{V}O_2$/peak heart rate (HR_{peak})).

$\dot{V}O_2/HR_{peak}$ (mL·beat^{-1}) = $\dot{V}O_2$ (L·min^{-1}) x 1000 mL / HR (beats·min^{-1}) *Equation 1.*

Paragraph 11: ***Near-infrared spectroscopy.*** HHb dynamics from the *m. vastus lateralis* were noninvasively measured using NIRS (Portamon, Artinis Medical Systems). This system has previously been used in children (21) and consists of an emission probe, with three light sources emitting two wavelengths of light (760 and 850 nm) and a photon detector. The intensity of incident and transmitted light was recorded continuously at 10 Hz and used to estimate [HHb]. Since the NIRS-derived [HHb] signal does encompass contribution from intramyocyte myoglobin and does not solely reflect the microcirculatory compartment [vascular (Hb) deoxygenation] (18), the changes in muscle HHb should be considered to represent [Hb+Mb]. The wireless emitter-detector unit was placed over the *m. vastus lateralis*, midway between the greater trochanter and lateral epicondyle of the femur. The area of interrogation was initially cleaned and shaved and, after marking of the placement area, the device was secured with tape (KinesioTex®) and a dark elastic bandage, to minimize extraneous light interference with the near-infrared signal.

Paragraph 12: ***Additional measures****:* HR was measured on a beat-by-beat basis using the ECG-derived R-R interval (PhysioFlow, PF-05, Manatec Biomedical, Paris, France).

Fingertip arterial oxygen saturation ($SpO_{2\%}$) was recorded via pulse oximetry (NONIN, Avant 4000, NONIN Medical Inc., USA). Subjective ratings of perceived exertion (RPE) and dyspnoea (RPD) were determined upon exhaustion using the pictorial children's effort rating table (P-CERT) and the 0-10 category ratio (CR-10) scale, respectively, the methodology for which is described elsewhere (30).

HHb modelling procedures

Paragraph 13: Muscle [HHb] data were interpolated to 1-s intervals and averaged data (15-s) for the entire test were subsequently normalized to the total amplitude of the response (%Δ[HHb]), such that 0% represented steady-state values observed during the period of baseline (20 W) cycling and 100% represented the highest average (i.e., $\Delta[HHb]_{peak}$) (4,11). The response was then expressed as a function of absolute and relative PPO and $\dot{V}O_{2max.}$ Preliminary statistical analyses (GraphPad Prism, GraphPad Software, San Diego, CA) revealed that the sigmoid provided a superior fit to the HHb response when compared with bilinear or hyperbolic curve fitting procedures (data not reported). The Δ[HHb] response to incremental ramp cycling exercise was therefore described using a sigmoidal model (Equation 2) in line with previous studies (4,9,21) as follows:

$$y = f_0 + A / (1 + e^{-(c+dx)}) \qquad \textit{Equation 2.}$$

where f_0 represents baseline [HHb], A the amplitude of the response, d the slope of the sigmoid, c the constant that is dependent on d and c/d the value corresponding to 50% of the total amplitude, respectively.

Paragraph 14: Statistical Analyses. Log-linear allometric models were used to adjust $\dot{V}O_{2max}$ for body size. The log-linear allometric model yielded a scaling exponent close to unity for FFM (b = 1.03), meaning the ratio standard method for normalising $\dot{V}O_{2max}$ was deemed appropriate.

Paragraph 15: Data are expressed as means and standard deviations unless otherwise stated. Independent samples *t*-tests (SPSS v19.0, Chicago, USA) derived *p*-values for subsequent inferential analyses. Inferential statistics, using 90% confidence intervals (CI) and the effect size (ES), were used to derive magnitude-based inferences regarding the true value of the observed effect statistic (15). Facilitated by a published Microsoft Excel® spreadsheet (14), any influence of CF on parameters of the [HHb] response and maximal and submaximal CPET parameters was calculated, using a 90% CI and the ES. Using a smallest worthwhile ES change of 0.2 (5) and the 90% CI, the likelihood that the observed effect was beneficial (e.g. higher $\dot{V}O_{2max}$, faster MRT), trivial or harmful (e.g. lower $\dot{V}O_{2max}$, slower MRT) was reported. The qualitative terms used to inform these decisions were: < 0.5%, 'most unlikely'; 0.5-5%, 'very unlikely'; 5-25%, 'unlikely'; 25-75%, 'possibly'; 75-95%, 'likely'; 95-99.5%, 'very likely'; > 99.5%, 'most likely'. An effect was deemed trivial when the majority (> 50%) of the 90% CI resided between beneficial and harmful. Conversely, an effect was deemed unclear when the likelihood of a beneficial and harmful effect was > 5%.

Paragraph 16: Hopkins' published spreadsheet (14) was also used to determine the 90% CI for Pearson's correlation coefficients to explore the relationship between key parameters of aerobic function (i.e., $\dot{V}O_{2max}$, $\dot{V}O_2$ gain, MRT and the GET) and mechanistically linked parameters of muscle O_2 extraction (e.g., *d* and *c/d* of the [HHb] response) and O_2 delivery (e.g., end-exercise SpO_2% and O_2 pulse) in CF. Cohen's thresholds (4) for small (0.1), moderate (0.3), large (0.5) and very large (0.7) relationships describe the magnitude of correlations.

RESULTS

Paragraph 17: Table 1 presents participants' baseline physical characteristics, with Table 2 detailing the clinical profile of the patients with CF. Body mass index (BMI) was likely higher, whereas FEV_1 (% predicted) was likely lower in CF than CON. Pubertal maturity of both groups were as follows: pre-pubertal (*n* in CF = 3; *n* in CON = 1), circum-pubertal (*n* in CF = 7; *n* in CON = 8) and post-pubertal (*n* in CF = 0; *n* in CON = 1).

Paragraph 18: Maximal and submaximal CPET parameters are presented in Table 3. All participants completed CPET without any adverse events. Ramp PPO was possibly lower in CF and likely lower when expressed relative to body mass. As expected, CF presented with very likely reduced $\dot{V}O_{2max}$, when normalized for both body mass and FFM. Furthermore, the $\dot{V}O_2$ gain was very likely lower and the $\dot{V}O_2$ MRT was likely slowed in CF. The RPD upon exhaustion was most likely higher in CF, respectively.

Paragraph 19: Parameter estimates for normalized muscle [HHb] as a function of absolute and percentage PPO (%PPO) and $\dot{V}O_{2max}$ ($\%\dot{V}O_{2max}$) are compared in Table 4, and the Δ[HHb]-WR profile for two representative CF and CON matched pairs are shown in Figure 1. Any effect of the reduced aerobic fitness in patients with CF upon the slope (*d*) of the Δ[HHb]-WR response was mechanistically unclear when expressed either as a function of absolute and percentage PPO or absolute and percentage $\dot{V}O_{2max}$. Furthermore, the effect of CF upon the absolute and relative WR and $\dot{V}O_2$ corresponding to 50% [HHb] amplitude (*c/d*) was mechanistically unclear.

Paragraph 20: Correlational analyses within the CF group revealed small relationships between patients' $\dot{V}O_{2max}$ and their [HHb] *c/d*, expressed as a function of %PPO (r =0.14, ±0.58) and $\%\dot{V}O_{2max}$ (r = -0.21, ±0.57), respectively. With the exception of the very large relationship between $\dot{V}O_2$ gain and [HHb] *c/d* %PPO (r = 0.70, ±0.36), relationships

between $\dot{V}O_2$ gain and [HHb] *c/d* $\%\dot{V}O_{2max}$ and the GET and MRT with [HHb] *c/d* %PPO and $\%\dot{V}O_{2max}$ were all small. A moderate relationship was observed between $\dot{V}O_{2max}$ and end-exercise $SpO_{2\%}$ (r = 0.33, ±0.51) in CF (Figure 2), however this was small (r = 0.20, ±0.54) in the healthy control group. The relationship between $\dot{V}O_{2max}$ and O_2 pulse was large in CF (r = 0.58, ±0.41; Figure 2) and CON (r = 0.98, ±0.00). Similarly, the relationships between $\dot{V}O_2$ gain and end-exercise $SpO_{2\%}$ and O_2 pulse were moderate (r = 0.40, ±0.49) and large (r = 0.65, ±0.37), respectively in CF, however these were small in CON (r = -0.15, ±0.54 and r = 0.1, ±0.58, respectively). Very large relationships were also evident between the GET and end-exercise $SpO_{2\%}$ (r = -0.88, ±0.16) and O_2 pulse (r = 0.98, ±0.03) in CF. The relationship between the GET and O_2 pulse was very large in CON (r = 0.92, ±0.12); however that with $SpO_{2\%}$ was small (r = 0.1, ±0.55). A moderate relationship (r = 0.39, ±0.49) was also evident between the $\dot{V}O_2$ gain and MRT in CF, however this was small in CON (r = 0.01, ±0.55).

DISCUSSION

Paragraph 22: This is the first study to examine the influence of mild-to-moderate CF on the aerobic function and dynamic adjustments in localized muscle (*vastus lateralis*) fractional oxygen extraction (Δ[HHb]) in pediatric patients during ramp incremental cycling exercise. As expected, CF patients were characterized by impaired aerobic function, as displayed by a very likely reduced body mass or FFM normalized $\dot{V}O_{2max}$, a very likely lower $\dot{V}O_2$ gain, and likely slower $\dot{V}O_2$ MRT. Contrary to the experimental hypothesis, however, this reduced aerobic fitness status did not have a clear effect upon the dynamics of the Δ[HHb] during ramp incremental exercise. Specifically, no clear shift in *c/d* of the [HHb] response was evident when expressed relative to percentage PPO or $\dot{V}O_{2max}$ and relationships with the key parameters of aerobic fitness were small. Indicators of central O_2 delivery were, however,

altered by CF. Specifically, end-exercise $SpO_{2\%}$ was most likely lower and correlated with $\dot{V}O_{2max}$ in the CF group only. Thus, these data show that the observed changes in the aerobic function of pediatric patients with CF during incremental ramp cycling are likely related to alterations in muscle O_2 delivery, with no compensatory adjustment to the dynamics of muscle O_2 extraction within the microcirculation.

Paragraph 23: This study is unique for a number of reasons. It is the first to use a validated protocol (30) to document 'true' $\dot{V}O_{2max}$ in young patients with CF. Consistent with earlier reports (18), $\dot{V}O_{2max}$ in patients with CF was very likely lower than that of CON in this study when normalized for both body mass and FFM. But importantly, the present results are robust because this is the first to include a supramaximal $\dot{V}O_{2max}$ verification phase (30) within the CPET protocol, thereby removing the issue of previous studies, where aerobic fitness status may have been underrepresented because of invalid verification criteria (30).

Paragraph 24: Secondly, this study presents, for the first time, the four key parameters of aerobic function (37), allowing a comprehensive assessment of aerobic fitness in this patient group. Of the additional key parameters, patients with CF presented with a very likely reduced $\dot{V}O_2$ gain and a likely slowed $\dot{V}O_2$ MRT, with no clear effect on the GET. While slower pulmonary $\dot{V}O_2$ kinetics have been documented in patients with CF during constant-load, moderate intensity cycling (13), the present study extends these findings to the $\dot{V}O_2$ response at the onset of ramp incremental cycling. Although no clear influence upon the GET was evident, the functional gain during the moderate-intensity region of ramp exercise was very likely lower in CF, reflecting either an apparently greater skeletal muscle efficiency or impaired muscle O_2 consumption. Shallower $\Delta\dot{V}O_2/\Delta WR$ slopes during exercise have previously been reported in patients with CF (22), congenital heart disease (12) and juvenile dermatomyositis (12). However, steeper $\Delta\dot{V}O_2/\Delta WR$ responses have also been observed in young patients with CF (12). It would be misleading to interpret the reduced $\Delta\dot{V}O_2/\Delta WR$ in

the present findings as enhanced aerobic efficiency, particularly given the impairment in other parameters of aerobic function (i.e., $\dot{V}O_{2max}$ and MRT). Given the moderate relationship between the $\dot{V}O_2$ gain and MRT in CF, the lower $\Delta\dot{V}O_2/\Delta WR$ slope may be related to the slower pulmonary $\dot{V}O_2$ kinetics, such that the rise in $\dot{V}O_2$ was not sufficiently rapid to respond to the work rate increments during the CPET.

Paragraph 25: To our knowledge, this study is the first to report the Δ[HHb] dynamics during ramp exercise in pediatric patients with CF. While pulmonary $\dot{V}O_2$ increased linearly with increasing work rate following an initial time lag, muscle Δ[HHb] (reflecting the ratio of muscle blood flow to muscle O_2 utilisation (Table 4; Figure 1)) increased in a nonlinear manner. This response was well characterized using a sigmoid function relative to WR and $\dot{V}O_2$ in both groups in the current study, which is consistent with previous reports in children and young and old adults during ramp cycling exercise (4,11,21).

Paragraph 26: Contrary to the study hypothesis, the Δ[HHb] dynamics during ramp exercise were similar between CF and CON in the present study. That is, no clear effect of CF upon either the absolute and relative WR and $\dot{V}O_2$ corresponding to 50% Δ[HHb] amplitude was observed. This is despite previous reports that aerobic fitness status has an effect upon the dynamic balance between O_2 supply and demand and, consequently, the sigmoidal pattern of [HHb] (4,21). Boone *et al.* (4) previously demonstrated that a higher aerobic fitness is associated with a rightward shift of the [HHb] sigmoidal response (relative to %PPO) in healthy adults and that the response correlated with parameters of aerobic fitness (i.e., $\dot{V}O_{2max}$ and the GET). The purported mechanism for this rightward shift in the [HHb] response was attributed to a higher oxidative capacity and/or altered muscle fiber distribution. The rate of fractional oxygen extraction has been shown to be influenced by training status and enhanced O_2 delivery in trained versus untrained healthy girls (21). Because $\dot{V}O_{2max}$ was meaningfully reduced in patients with CF in the present study, a more rapid increase in

Δ[HHb] during ramp exercise would be expected. However, a previous study (11) comparing older (approximately 70 yr) and younger (approximately 25 yr) healthy adults, which observed alterations when expressed relative to absolute power output, did not observe any age-related differences in Δ[HHb] response dynamics when expressed as a function of %PPO, despite a reduced $\dot{V}O_{2max}$ in the older participants (30 vs. 49 $mL \cdot kg^{-1} \cdot min^{-1}$).

Paragraph 27: Some caution may, however, be applied when considering the present findings to suggest that there are *no* differences between the Δ[HHb] response of healthy and CF children and adolescents. Inter-patient differences (Figure 1) in the Δ[HHb] response suggest that the interpretation that the rate of muscle O_2 extraction is unaltered by CF may be too simplistic. When the distribution of the 90% CI for the effect of CF on the ΔHHb *c/d* was expressed relative to percentage $\dot{V}O_{2max}$, the majority of the 90% CI distribution favoured a reduced rate of extraction (leftward shift: 9%; trivial: 22%; rightward shift: 69%). The unclear statistical outcome is, therefore, likely to reflect the large inter-patient variability present for this outcome (see Figure 1). Indeed, inter-patients differences are not improbable given the complex nature and varied clinical presentation of CF disease, meaning further comment on the responses shown in Figure 1 may be of clinical interest. Patient A, who has a left shift on the Δ[HHb] response, is a physically mature boy with few complications and excellent lung function. In contract, patient B (also male), whose Δ[HHb] response is shifted to the right has poorer lung function, a worse chest x-ray score, nutritional concerns, and complications including CF-related liver disease and impaired glucose tolerance. This is reflected in patient B having received 28 d of intravenous antibiotics, which signifies treatment intensification, within the preceding year. However, despite patient B's poorer clinical profile, his $\dot{V}O_{2max}$ is markedly higher than that of patient A (49.4 vs. 32.6 $mL \cdot kg^{-1} \cdot min^{-1}$), which may have played a role in causing the rightward shift in the Δ[HHb] response

dynamics. However, it should be noted that we only found a small relationship between $\dot{V}O_{2max}$ and [HHb] *c/d* in the present study.

Paragraph 28: Interestingly, the present findings of unaltered [HHb] response dynamics for the CF group in the present study are in line with a previous report by Rosenthal *et al.* (29), who observed similar O_2 extraction dynamics during exercise in young patients with CFR and their healthy counterparts, despite presenting with impaired aerobic function. When considered in reference to the Fick equation, these data therefore suggest that the impaired aerobic function characterising young patients with CF is caused by a reduction in O_2 delivery. Altered cardiac function (3,16,27) and an inability to augment SV during exercise (29), which are likely to reduce central O_2 delivery, have previously been documented in patients with CF, and the most likely lower $SpO_{2\%}$ in the present study provides further support. Although it has been propositioned that patients with CF can achieve apparently "normal" cardiac output in the presence of reduced SV during exercise, through elevated HR (16,20), this compensation only seems viable at submaximal exercise intensities, as both CF and CON had similar HR responses at maximal exercise in the present study. In accordance with this findings, a reduced (approximately 24%) estimated SV (derived using respiratory mass spectroscopy) at maximal exercise in young patients with CF coupled with a similar HR response to healthy controls has previously been documented (29).

Paragraph 29: Although it has been hypothesized that patients limited by O_2 delivery during exercise would present with a compensatory increase in O_2 extraction at the local level (9), both this study and the previous study by Rosenthal *et al.* (9), using respiratory mass spectroscopy, observed no augmentation of O_2 extraction in the face of inadequate O_2 delivery during exercise (29). Although the previous authors could not determine the cause of this because no direct peripheral measurements were made, it was suggested that muscle metabolic issues resulting from chronic bronchial sepsis may contribute. Importantly, the

present study utilising NIRS corroborates the observations of Rosenthal *et al.* (29) using a more direct measurement technique.

Paragraph 31: The relationships between parameters of aerobic function and mechanistic parameters indicative of O_2 delivery and extraction further emphasize the importance of O_2 delivery in explaining the impaired aerobic function in young patients with CF. Although the relationships between key parameters of aerobic function and the rate of peripheral fractional O_2 extraction were small, stronger relationships with parameters of O_2 delivery were evident. Furthermore, while $SpO_{2\%}$ did not correlate with FFM normalized $\dot{V}O_{2max}$ in healthy controls, a moderate correlation with end-exercise $SpO_{2\%}$ was observed in patients with CF, along with a large relationship between $\dot{V}O_{2max}$ and the O_2 pulse. The relationships between $\dot{V}O_2$ gain and end-exercise $SpO_{2\%}$ and O_2 pulse were also moderate and large, respectively. Similarly, very large relationships were evident between the GET and end-exercise $SpO_{2\%}$ and O_2 pulse.

Paragraph 32: There are several limitations of NIRS, which must be acknowledged. First, measurements are restricted to a specific area of interrogation over a, in this case, single heterogenous and superficial muscle, which may not represent whole body skeletal muscle blood flow responses. However, the muscle deoxygenation response measured in the superficial and deeper muscle fibres using NIRS has been shown to reflect muscle oxygenation as measured using phosphorous quenching derived microvascular O_2 partial pressure within the same region of muscle (18). Although inter-site variation in the [HHb] response cannot be directly rectified, the device was secured to the same anatomical region for all participants to eradicate inter-individual regional differences within the *m. vastus lateralis*. Although the influence of adipose tissue at the area of interrogation was not directly determined, in line with recommendations, responses were standardized to the total [HHb] amplitude to provide a physiologic normalization (4). Finally, the generalizability of these

findings should be viewed in light of the small sample of Northern European patients with CF recruited for this study.

Paragraph 33: To conclude, this was the first study to examine the influence of mild-to-moderate CF on key parameters of aerobic function in pediatric patients. As expected, pediatric patients presented with impaired aerobic fitness compared with that of their healthy counterparts. Specifically, $\dot{V}O_{2max}$ and the $\dot{V}O_2$ gain were very likely reduced and the MRT likely slowed. However, in contrast to the study hypothesis, NIRS derived [HHb] dynamics during ramp cycling exercise were similar between CF and CON. The present findings support the notion of centrally mediated oxygen delivery principally limiting the aerobic function of young patients with CF during ramp incremental cycling exercise.

ACKNOWLEDGMENTS / CONFLICTS OF INTEREST

Paragraph 34: The authors would like to thank the participants who volunteered to be involved and are grateful for the ongoing support from the CF team at the Royal Devon and Exeter NHS Foundation Trust Hospital. This study was funded by a grant from the Royal Devon and Exeter NHS Foundation Trust. The authors report no conflicts of interest. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

REFERENCES

1. Barker AR, Williams CA, Jones AM, Armstrong N. Establishing maximal oxygen uptake in young people during a ramp cycle test to exhaustion. *Br J Sports Med* 2011;45(6)498-503.
2. Beaver WL, Wasserman K, Whipp BJ. A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol* 1986;60(6):2020-2027.
3. Benson LN, Newth CJ, DeSouza M, Lobraico R, Kartodihardjo W, Corkey C, Gilday D, Olley PM. Radionucline assessment of right and left ventricular function during bicyle exercise in young patients with cystic fibrosis. *Am Rev Respir Dis* 1984;130:987-992.

4. Boone J, Koppo K, Barstow TJ, Bouckaert J. Pattern of deoxy[Hb+Mb] during ramp cycle exercise: influence of aerobic fitness status. *Eur J Appl Physiol* 2009;105:851-859.
5. Cohen J. *Statistical power analysis for the behavioural sciences.* Hillsdale (NJ): Lawrence Erlbaum Associates; 1988.
6. de Jong W, Kaptein AA, van der Schans CP, Mannes GP, van Aalderen WM, Grevink RG, Koëter GH. Quality of life in patients with cystic fibrosis. *Pediatr Pulmonol* 1997;23:95-100.
7. de Meer K, Jeneson JAL, Gulmans VAM, van der Laag J, Berger R. Efficiency of oxidative work performance of skeletal muscle in patients with cystic fibrosis. *Thorax* 1995;50:980-983.
8. Divangahi M, Balghi H, Danialou G, Comtois A, Demoule A, Ernest S, Haston C, Robert R, Hanrahan JW, Radzioch D, Petrof BJ. Lack of CFTR in skeletal muscle predisposes to muscle wasting and diaphragm muscle pump failure in cystic fibrosis mice. *PLoS Genet* 2009;5(7):e1000586.
9. Ferreira LF, Koga S, Barstow TJ. Dynamics of noninvasively estimated microvascular O_2 extraction during ramp exercise. *J Appl Physiol* 2007;103:1999-2004.
10. Freeman W, Stableforth DE, Cayton RM, Morgan MD. Endurance exercise capacity in adults with cystic fibrosis. *Respir Med,* 1993;87(7):541-549.
11. Gravelle BMR, Murias JM, Spencer MD, Paterson DH, Kowalchuk JM. Adjustments of pulmonary O_2 uptake and muscle deoxygenation during ramp incremental exercise and constant-load moderate-intensity exercise in young and older adults. *J Appl Physiol* 2012;113:1466-1475.
12. Groen WG, Hulzebos HJ, Helders PJ, Takken T. Oxygen uptake to work rate slope in children with a heart, lung or muscle disease. *Int J Sports Med* 2010;31:202-206.
13. Hebestreit H, Hebestreit A, Trusen A, Hughson RL. Oxygen uptake kinetics are slowed in cystic fibrosis. *Med Sci Sports Exerc* 2005;37(1):10-7.
14. Hopkins WG. A spreadsheet for deriving a confidence interval, mechanistic inference and clinical inference from a P-value. *Sportscience* 2007;11:16-20.
15. Hopkins WG, Marshall SW, Batterham AM, Hanin J. Progressive statistics for studies in sports medicine and exercise science. *Med Sci Sports Exerc* 2009;41:3-13.
16. Ionescu AA, Ionescu A-A, Payne N, Obieta-Fresnedo I, Fraser AG, Shale DJ. Subclinical right ventricular dysfunction in cystic fibrosis. *Am J Respir Crit Care Med* 2001;163:1212-1218.
17. Keochkerian D, Chlif M, Delanaud S, Gauthier R, Maingourd Y, Ahmaidi S. Breathing pattern adopted by children with cystic fibrosis with mild to moderate pulmonary impairment during exercise. *Respiration* 2008;75(2):170-177.
18. Koga A, Kano Y, Barstwo TJ, Ferreira LF, Ohmae E, Sudo M, Poole DC. Kinetics of muscle deoxygenation and microvascular P_{O_2} during contractions in rat: comparison of optical spectroscopy and phosphorescence-quenching techniques. *J Appl Physiol* 2012;112:26-32.

19. Lamhonwah AM, Bear CE, Huan, LJ, Chiaw PK, Ackerley CA, Tein I. Cystic fibrosis transmembrane conductance regulator in human muscle dysfunction causes abnormal metabolic recovery in exercise. *Ann Neurol* 2010;67:802-808.
20. Lands LC, Heigenhauser GJ, Jones NL. Cardiac output determination during progressive in cystic fibrosis. *Chest,* 1992;102:1118-1123.
21. McNarry MA, Welsman JR, Jones AM. Influence of training and maturity status on the cardiopulmonary responses to ramp incremental cycle exercise and upper body exercise in girls. *J Appl Physiol* 2011;110:375-381.
22. Moser C, Tirakitsoontorn P, Nussbaum E, Newcomb R, Cooper DM. Muscle size and cardiorespiratory response to exercise in cystic fibrosis. *Am J Crit Care Med,* 2000; 162(5)1823-1827.
23. Murias JM, Keir DA, Spencer MD, Paterson DH. Sex-related differences in muscle deoxygenation during ramp incremental exercise. *Respir Physiol Neurobiol* 2013; http://dx.doi.org/10.1016/j.resp.2013.08.011.
24. Murias JM, Spencer MD, Keir DA, Paterson DH. Systemic and vastus lateralis muscle blood flow and O_2 extraction during ramp incremental cycle exercise. *Am J Physiol Regul Integr Comp Physiol* 2013;304:R720-R725.
25. Nixon PA, Orenstein DM, Kelsey SF, Doershuk CF. The prognostic value of exercise testing in patients with cystic fibrosis. *N Engl J Med* 1992;327(25):1785-1788.
26. Pérez M, Groeneveld IF, Santana-Sosa E, Fiuza-Luces C, Gonzalez-Saiz L, Villa-Asensi JR, López-Mojares LM. Rubio M, Lucia A. Aerobic fitness is associated with lower risk of hospitalization in children with cystic fibrosis. *Pediatr Pulmonol* 2013, dio: 10.1002/ppul.22878.[Epub ahead of print].
27. Pianosi P, Pelech A. Stroke volume during exercise in cystic fibrosis. *Am J Respir Crit Care Med* 1996;153:1105-1109.
28. Pianosi P, Leblanc J, Almudevar A. Peak oxygen uptake and mortality in children with cystic fibrosis. *Thorax* 2005;60(1):50-54.
29. Rosenthal M, Narang I, Edwards L, Bush A. Non-invasive assessment of exercise performance in children with cystic fibrosis (CF) and non-cystic fibrosis bronchiectasis: Is there a CF specific muscle defect? *Ped Pulmonol* 2009;44:222-230.
30. Saynor ZL, Barker AR., Oades PJ & Williams CA. A protocol to determine valid $\dot{V}O_{2max}$ in young cystic fibrosis patients. *J Sci Med Sport* 2013;16(6):539-544.
31. Saynor ZL, Barker AR, Oades PJ, Williams CA. Reproducibility of maximal cardiopulmonary exercise testing for young cystic fibrosis patients. *J Cystic Fibros* (2013), http://dx.doi.org/10.1016/j.jcf.2013.04.012.
32. Slaughter MH, Lohman TG, Bioleau RA, Horswill CA, Stillman RJ, Vanloan MD, Bemben DA. Skinfold equations for estimation of body fatness in children and youth. *Hum Biol* 1988;60:709-723.
33. Stanojevic S, Wade A, Cole TJ, Lum S, Custovic A, Silverman M, Hall GL, Welsh L, Kirkby J, Nystad W, Badier M, Davis S, Turner S, Piccioni P, Vilozni D, Eigen H, Vlachos-Mayer H, Zheng J, Tomalak W, Jones M, Hankinson JL, Stocks J; Asthma UK Collabative Group. Spirometry centile charts for young Caucasian children: the asthma UK collaborative initiative. *Am J Respir Crit Care Med,* 2009;180(6):547-552.

34. Tanner JM. *Growth at adolescence*, 2nd ed. Oxford, Blackwell Scientific Publications, 1962. p. 1-65.
35. Wasserman K, Hansen JE, Sue DY, Stringer WW, Whipp BJ. *Principles of exercise testing and interpretation*, 4th ed. Philadelphia, Lippincott Williams & Wilkins, 2004. p. 1-244.
36. Wells GD, Wilkes DL, Schneiderman JE, Rayner T, Elmi M, Selvadurai H, Dell SD, Noseworthy MD, Ratjen F, Tein I, Coates AL. Skeletal muscle metabolism in cystic fibrosis and primary ciliary dyskinesia. *Pediatr Res* 2011;69:40-45.
37. Whipp BJ, Davis JA, Torres F, Wasserman K. A test to determine parameters of aerobic function during exercise. *J Appl Physiol*, 1981; 50:217-221.

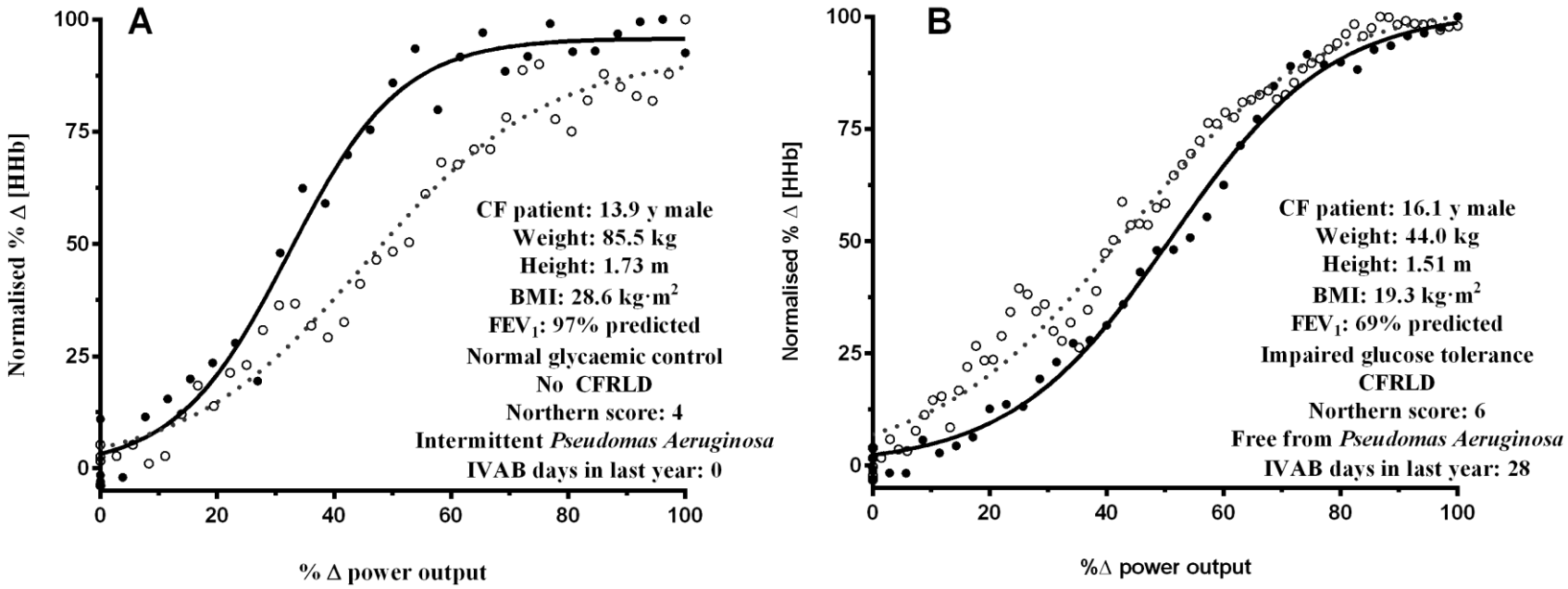
FIGURE LEGENDS

Figure 1. Sigmoid models of normalized muscle deoxygenation (% Δ[HHb]) during ramp incremental exercise as a function of percentage peak oxygen uptake for two representative young patients with CF (●, black circles) of the leftward and rightward response patterns and their healthy age- and gender-matched control participants (dashed line; ○, white circles).

Figure 2. The relationship between $\dot{V}O_{2max}$ and changes in end-exercise $SpO_{2\%}$ and the maximal oxygen pulse in young patients with CF.

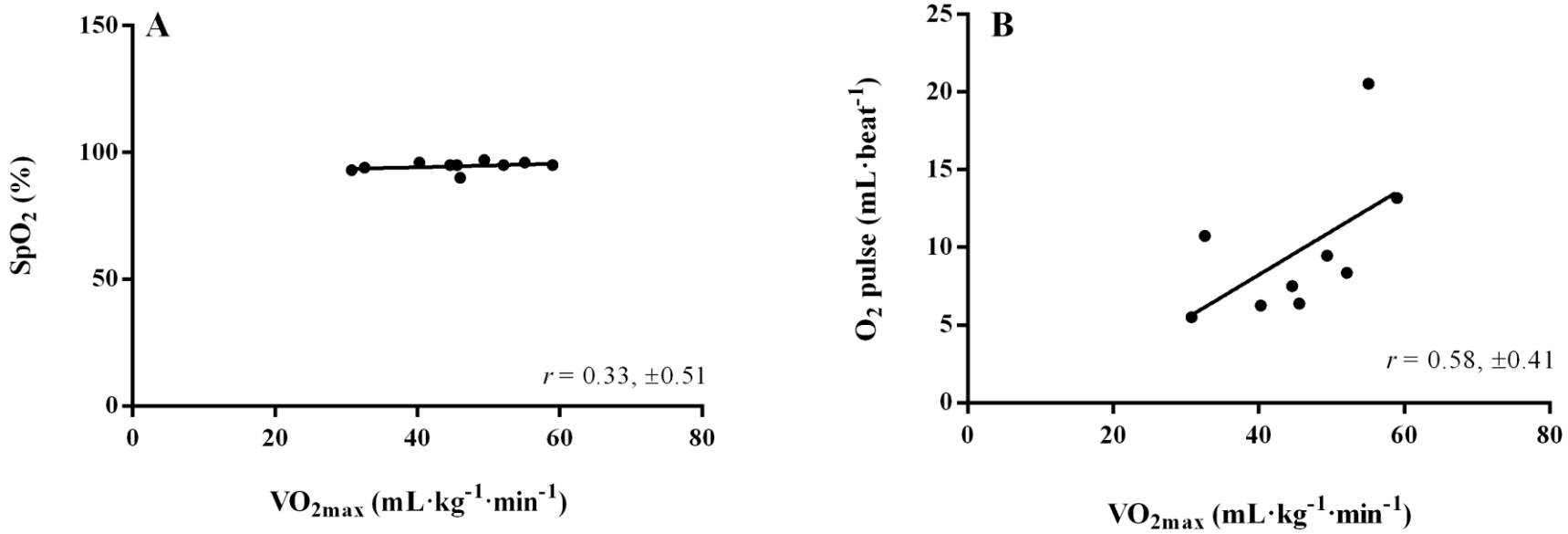
FIGURES

Figure 1



N.B. [HHb], concentration of deoxyhaemoglobin (and myoglobin); CFRD, CF-related diabetes; CFRLD, CF-related liver disease; IVAB, intravenous antibiotics.

Figure 2


A
150
100
50
0
0
20
40
60
80
SpO2 (%)
r = 0.33, ±0.51
VO2max (mL·kg-1·min-1)
B
25
20
15
10
5
0
0
20
40
60
80
O2 pulse (mL·beat-1)
r = 0.58, ±0.41
VO2max (mL·kg-1·min-1)

TABLES

Table 1. Baseline anthropometric and pulmonary function data for young CF patients (*n* = 10, 1 female) and healthy age- and gender-matched controls.

Variable	CF (Mean ± SD)	CON (Mean ± SD)	Change, 90% CL	Inference (in CF)	ES
Age (y)	12.7 ± 2.8	12.5 ± 2.8	0.2, ±2.2	Unclear	0.07
Stature (m)	1.53 ± 0.15	1.58 ± 0.19	-0.05, ±0.14	Unclear	-0.25
Body mass (kg)	53.2 ± 20.0	50.5 ± 17.4	2.7, ± 14.6	Unclear	0.14
BMI ($kg \cdot m^2$)	22.0 ± 4.6	19.5 ± 2.7	2.4, ±3.0	Likely higher	0.60
BSA (m^2)	1.51 ± 0.35	1.48 ± 0.35	0.03, ±0.27	Unclear	0.08
FFM (kg)	42.0 ± 14.6	41.3 ± 14.0	0.6, ±11.1	Unclear	0.04
FVC (L)	3.36 ± 1.30	3.69 ± 1.33	-0.33, ±1.03	Unclear	-0.24
FVC (% predicted (range))	102 ± 14 (79-123)	106 ± 10 (92-125)	-4, ±10	Unclear	-0.28
FEV_1 (L)	2.69 ± 1.12	3.18 ± 1.18	-0.49, ±0.89	Unclear	-0.41
FEV_1 (% predicted (range))	97 ± 22 (66-127)	107 ± 10 (96-129)	-10, ±14	Likely lower	-0.55

Values are means ± SD. ES; Effect size; CI, confidence intervals; BMI, body mass index; FFM, fat-free mass (calculated using the equation of Slaughter *et al.* (32); FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 second. N.B. Parameters of pulmonary function are expressed as a percentage predicted normal using appropriate reference data (33).

Table 2. Baseline clinical characteristics for the young CF patients (*n* = 10, 1 female).

Variable	Value (mean ± SD)	Range
CFTR genotype:	-	-
Homozygote ΔF508 (Class I mutation)	8	-
ΔF508/ 2184delA (Class II Mutation)	1	-
ΔF508/ G55ID (Class III mutation)	1	-
Chronic P. Aeruginosa infection [a]	"chronic," *n* = 2; "intermittent," *n* = 3	"free," *n* = 3 "never," *n* = 2
Shwachman score	81 ± 7	67-91
Northern score [b]	4 ± 1	2-6
Pancreatic insufficient	*n* = 10	
CF-related diabetes	*n* = 3	-
CF-related liver disease	*n* = 3	-
IVABs (days in last year)	10 ± 15	0-42

Values are means ± SD, unless otherwise stated.
CFTR, cystic fibrosis transmembrane conductance regulator; *P. Aeruginosa*; *Pseudomonas Aeruginosa*; Shwachman score - scoring 4 separate aspects of the disease profile; general activity; physical examination; nutritional status; and chest radiographic findings, using the most recent clinical review information. A total of 100 points represents a perfect score of health; IVABs, intravenous antibiotics; [a]According to Leeds Criteria, "chronic", >50% of the preceding 12 months were *P. aeruginosa* culture positive; "intermittent", ≤50% of the preceding 12 months were *P. aeruginosa* culture positive; "never", no growth of *P. aeruginosa* for the previous 12 months, having previously been *P. aeruginosa* culture positive; "free", *P. aeruginosa* has never been cultured.
[b] Provides evidence of radiographic chest findings. Maximum score is 20, with 20 being the most severe.

Table 3. Maximal and submaximal physiologic responses of young patients with CF and healthy age- and gender-matched controls to ramp incremental cycle exercise.

Variable	CF (Mean ± SD)	CON (Mean ± SD)	Change, 90% CI	Inference (in CF)	ES
Maximal exercise parameters	-	-	-	-	-
Absolute $\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	1.93 ± 0.84	2.21 ± 0.79	-0.29, ±0.63	Unclear	-0.34
Relative $\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	36.3 ± 7.6	43.9 ± 5.2	-7.6, ±5.1	Very likely lower	-1.11
$\dot{V}O_{2max}$/FFM ($mL \cdot kg^{-1} \cdot min^{-1}$)	45.5 ± 9.1	53.5 ± 6.4	-7.9, ± 6.1	Very likely lower	-0.96
$\dot{V}_{Emax}$ ($L \cdot min^{-1}$)	84.27 ± 33.07	99.31 ± 39.95	-15.04, ±28.53	Unclear	-0.39
Breathing reserve (%)	20.4 ± 19.9	12.4 ± 16.2	8.0, ±14.1	Unclear	0.42
HR_{max} ($beats \cdot min^{-1}$)	192 ± 11	190 ± 13	2, ±10	Unclear	0.18
$\dot{V}O_2/HR_{max}$ ($mL \cdot beat^{-1}$)	9.78 ± 4.71	11.01 ± 3.39	-1.23, ±3.41	Unclear	-0.28
SpO_2 (%)	95 ± 2	97 ± 1	-3, ±1	Most likely lower	-1.63
Ramp PPO (W)	176 ± 94	205 ± 82	-30, ±69	Possibly lower	-0.32
Relative ramp PPO ($W \cdot kg^{-1}$)	3 ± 1	4 ± 1	-0.7, ±0.5	Likely lower	-0.84
RPE	9 ± 2	10 ± 1	-1, ±1	Unclear	-0.35
RPD	9 ± 2	6 ± 2	3, ±2	Most likely higher	1.46
Submaximal exercise	-	-	-	-	-
$\dot{V}O_2$ at the GET ($L \cdot min^{-1}$)	1.13 ± 0.41	1.20 ± 0.30	-0.07, ±0.28	Unclear	-0.20
$GET_{\%}$ (% of $\dot{V}O_{2max}$)	61.3 ± 10.2	56.7 ± 8.4	4.6, ±7.3	Unclear	0.47
MRT (s)	49 ± 21	38 ± 11	11, ±13	Likely slower	0.63
$\Delta\dot{V}O_2/\Delta WR$ ($mL \cdot min^{-1} \cdot W^{-1}$)	7.62 ± 1.67	9.05 ± 1.17	-1.44, ±1.12	Very likely lower	-0.95

Values are means ± SD.
RPE, RPD and SpO2% were measured at the end of exercise.
$\Delta\dot{V}O_2/\Delta WR$, oxygen cost of exercise (efficiency); GET, non-invasive estimate of the lactate threshold which was verified by the ventilatory threshold; $GET_{\%}$, GET expressed as a percentage of $\dot{V}O_{2max}$; MVV, maximal voluntary ventilation; $\dot{V}O_{2max}$, maximal oxygen uptake; MVV, maximal voluntary ventilation; $\dot{V}O_2/HR_{max}$, maximal oxygen pulse; $\dot{V}_{Emax}$, maximal minute ventilation; $\dot{V}O_2/HR_{max}$, maximal oxygen pulse; $\dot{V}_E/\dot{V}CO_2$-slope, ventilatory drive.

Table 4. Parameter estimates for normalized muscle deoxygenation (Δ[HHb]) as a function of absolute and percentage PPO during ramp incremental cycling and the absolute and normalized ratio of HHb-to-pulmonary oxygen uptake above and below the GET and at exhaustion.

Variable	Parameter expressed function of	CF (*n* = 9) (Mean ± SD)	CON (*n* = 9) (Mean ± SD)	Change, 90% CI	Inference (in CF)	ES
A (%)	PPO	100.1 ± 18.0	96.1 ± 8.1	4.0, ±11.8	Unclear	0.27
d (%·W^{-1})	PPO	0.1 ± 0.1	0.1 ± 0.0	0.0, ±0.1	Unclear	0.40
c/d (W)	PPO	98 ± 52	112 ± 54	-14, ±44	Unclear	-0.25
A (%)	%PPO	100.0 ± 17.8	96.1 ± 8.1	3.9, ±11.7	Unclear	0.26
d (%·%$_{peak}^{-1}$)	%PPO	0.1 ± 0.1	0.1 ± 0.0	0.0, ±0.0	Unclear	0.16
c/d (%$_{peak}$)	%PPO	47.1 ± 17.8	47.7 ± 9.1	-0.7, ±12.0	Unclear	-0.04
A (%)	$\dot{V}O_{2max}$	88.2 ± 10.2	93.6 ± 6.8	-5.4, ±7.2	Likely lower	-0.59
d (%·L^{-1})	$\dot{V}O_{2max}$	13.3 ± 16.4	6.9 ± 4.3	6.3, ±10.4	Unclear	0.48
c/d (L)	$\dot{V}O_{2max}$	1.27 ± 0.51	1.36 ± 0.52	-0.10, ±0.43	Unclear	-0.18
A (%)	%$\dot{V}O_{2max}$	91.6 ± 9.0	93.5 ± 6.8	-1.9, ±6.6	Unclear	-0.23
d (%·%$_{max}^{-1}$)	%$\dot{V}O_{2max}$	0.2 ± 0.1	0.1 ± 0.0	0.0, ±0.1	Unclear	0.38
c/d (%$_{max}$)	%$\dot{V}O_{2max}$	66.9 ± 8.5	63.6 ± 5.7	3.3, ±6.0	Unclear	0.43

Values are means ± SD unless otherwise stated.
Because of technical issues, [HHb] data are presented for nine matched pairs.
A, amplitude of the change in the deoxygenated hemoglobin (Δ[HHb]) response; *c*, constant that is dependent upon *d* and where *c/d* *x*-value corresponding to 50% *A*, respectively; *d*, slope of sigmoid.