

Aerobic Fitness in Children and Young Adults with Primary Ciliary Dyskinesia

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Abstract

Background: Although aerobic fitness is regarded as an overall prognostic measure of morbidity and mortality, its evaluation in the chronic progressive sinopulmonary disease primary ciliary dyskinesia (PCD) has been infrequently and inconsistently reported. Here we assessed peak oxygen uptake (VO_{2peak}) in a large well-characterized cohort of PCD patients, and explored whether VO_{2peak} was associated with parameters of pulmonary function, self-reported physical limitations, and physical activity level.

Methods: VO_{2peak} , spirometry, diffusing capacity, whole-body plethysmography, and nitrogen multiple breath inert gas washout (N_2 MBW) were assessed in a cross-sectional, single-occasion study of clinically stable children and young adults with PCD. We used a questionnaire including self-reported physical limitations in everyday life or in vigorous activities, and estimation of weekly hours of strenuous physical activity. VO_{2peak} in PCD patients was compared with that in matched, healthy control subjects and a national reference.

Results: Forty-four PCD patients aged 6–29 years exhibited reduced VO_{2peak} compared to healthy controls ($P < 0.001$) and the national reference. VO_{2peak} was abnormal (z-score < -1.96) in 34% of PCD patients. Spirometric values, RV/TLC, and indices of N_2 MBW were significantly abnormal, but VO_{2peak} only correlated with FEV_1 and DL_{CO}/V_A . VO_{2peak} correlated with complaints of moderate or significant limitations in vigorous activities ($P = 0.0001$), exhibited by 39% of PCD patients.

Conclusion: One-third of PCD patients exhibited substantially lower aerobic fitness than healthy subjects. Aerobic fitness correlated with FEV_1 , DL_{CO}/V_A and self-reported complaints of limitations in vigorous physical activity. These findings are most likely explained by PCD pulmonary disease and its impact on pulmonary function and physical ability. Considering fitness as an important outcome and including regular strenuous physical activity in PCD treatment would probably altogether increase pulmonary clearance, lung function, aerobic fitness, and quality of life, and prevent lifestyle-related diseases.

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Introduction

The peak oxygen uptake (VO_{2peak}) test is widely used to objectively determine a person's aerobic fitness or cardiopulmonary functional capacity, and as a prognostic measure of morbidity and mortality in disease and in health [1], [2]. VO_{2peak} reflects the ability to perform sustained exercise and thus can be used in patients with chronic pulmonary disease, to provide information that can not be obtained from standard pulmonary function tests [1]. In cystic fibrosis (CF), VO_{2peak} is significantly correlated with survival and quality of life [3], [4], leading to recommendations of annual assessment [4].

Primary ciliary dyskinesia (PCD) is a rare autosomal recessive disorder that affects approximately 1 in 20,000 individuals and is characterized by immotile or dyskinetic respiratory cilia [5]. PCD and CF share several features, including impaired clearance of the lower airways that leads to recurrent and chronic pulmonary infections, and inevitably progresses through declining lung

function and bronchiectasis to chronic respiratory failure [6]. PCD causes significant morbidity and impaired quality of life, including limitations of physical activity [7], [8]. In preschool-age children PCD can already impose a serious threat to lung function [9], although there exists a high degree of variation in the course of lung function after diagnosis [10]. Furthermore, the vast majority of children and adolescents receiving regular centralized care still exhibit substantial peripheral airway dysfunction, as reflected in indices of SF_6 multiple breath inert gas washout (MBW) [11].

Pulmonary function is traditionally assumed to be an important independent predictor of disease morbidity, disease control, and severity, and it may have an impact on aerobic fitness. However, it remains unclear whether VO_{2peak} differs between PCD patients with normal spirometry vs. abnormal spirometry, and healthy controls or if it is associated with other measures of pulmonary function. To date, only two small studies have partly investigated these questions using spirometric measures, and have reported contradictory results. Valerio et al. [12] found impaired VO_{2peak}

in PCD patients with FEV₁ below 85% of the predicted value, compared to matched healthy controls: they have also found that male gender, age, and time spent in vigorous activity were independent predictors of aerobic fitness. In contrast, Wells et al. [13] showed no difference in VO_{2peak} between adolescents with PCD and healthy controls.

The primary aim of the present study was to assess VO_{2peak} in a large cohort of children and young adults with PCD and to compare these values with those of healthy subjects. Secondly, we aimed to evaluate the association between VO_{2peak} and a panel of relevant pulmonary function measures, as well as self-reported physical limitations and weekly physical activity.

Part of this study was previously presented in abstract form at the American Thoracic Society (ATS) International Conference 2012, San Francisco (Abstract no. 30080 presented May 21).

Materials and Methods

Ethics Statement

This study was approved by the research ethics committee of The Capital Region of Denmark (J.no. H-1-2010-042 and amendment 2013-37202). Verbal and written consent was obtained from all patients and healthy controls, as well as from parents or guardians on the behalf of the study participants who were <18 years of age.

Study subjects

Children and young adults, (6–29 years of age) with documented PCD from the National Danish PCD center were eligible for the study. Healthy age-, gender- and BMI-matched non-atopic subjects with normal spirometry were included as controls. Subjects were excluded if they were unable to cooperate with the exercises or pulmonary function testing, e.g., due to mental or physical disability or known cardiovascular disease. PCD diagnosis was based on characteristic clinical symptoms [6], abnormal low nasal nitric oxide (nNO) measurement [14], repeated high-speed video microscopic recordings of abnormal ciliary beat pattern and/or frequency, and transmission electron microscopy analysis of ciliary ultrastructure according to previously published guidelines [5].

Study design

We performed a cross-sectional, single-occasion, and single-center case-control study. In the following order, the PCD patients performed N₂ MBW, spirometry, whole-body plethysmography, single-breath diffusing capacity for carbon monoxide (DL_{CO}) and VO_{2peak} test. The matched healthy controls performed only spirometry and VO_{2peak}. All tests for all participants were scheduled to be performed on a single occasion. Visits were postponed in the event of concurrent self-reported pulmonary exacerbation, or FEV₁ decrease of more than 10 percentage points compared to the last visit at which the patient was considered clinically stable.

Methods

Peak oxygen uptake (VO_{2peak}). The VO_{2peak} test was performed as described by Godfrey [15] with step increments of 10, 15, or 20 watts (W) per minute based on standing height. To achieve optimal test duration, the initial workload was determined according to the patient's heart rate while warming up [1]. A valid peak test was defined by continuous objective signs of exhaustion during verbal encouragement from the test leader, combined with at least one of the following criteria: respiratory exchange ratio (RER)>1.00 at test termination [16], or maximal heart rate

(HR_{max}) >85% of age-based predicted maximum (208-0.7 × age) [17]. O₂ and CO₂ concentrations were analyzed using a mass spectrometer (Amis 2000, Innovision, Odense, Denmark). VO_{2peak} was calculated as ml/kg/min using the procedure described in the in supporting information, Text S1. We also calculated the ventilatory reserve (VR) reflecting ventilatory capacity, and the ventilatory equivalent of CO₂ (V_E/VCO₂) reflecting efficiency of ventilation. VR <15% or V_E/VCO₂ >40 were considered abnormal and to be positive signs of ventilatory limitation during the test [18]. Reference values of VO_{2peak} were derived from comparable assessments in 937 healthy Danish children and young adults (426 males and 511 females) (Physical activity – Prevention and treatment) [19], [20] and this reference material was evaluated and compared with the group of matched healthy controls.

Pulmonary function tests. Spirometry, whole-body plethysmography, and DL_{CO} measurement were performed using Jaeger Master Screen Pro (CareFusion, Hochberg, Germany) according to ATS and ERS recommendations [21], [22], [23]. The “all-ages” reference equations were used for spirometry [24]. For children, the reference equations of Koopman et al. [25] were used for DL_{CO} and Zapletal [26] for whole-body plethysmography, except plethysmographic specific airway resistance (sRaw) for which we used the reference equations of Kirkby et al. [27]. For adults (>18 years), we used the reference equations of Cotes et al. [28] and Quanjer et al. [29] for DL_{CO} and whole-body plethysmography, respectively.

N₂ MBW was performed using Exhalyzer D (Eco Medics AG, Duernten, Switzerland), which was completed prior to any tests requiring forced expiratory maneuvers [30] and at least one hour before the VO_{2peak} test. We calculated indices of N₂ MBW, i.e. the Lung Clearance Index (LCI) and the normalized phase III slope indices S_{cond} and S_{acin} [30]. Pre-reviewed normative data was used as reference material [31].

Questionnaire. There is currently no validated PCD-specific instrument to assess quality of life or physical activity. Therefore, we selected and combined validated questions from the St George's Respiratory Questionnaire (SGRQ) [32], Cystic Fibrosis Questionnaire (CFQ-R) [33], Sino-Nasal Outcome Test-22 (SNOT-22) [34], and the Medical Outcomes Study Short Form-36 (SF-36) [35] and finally extracted simple questions about physical activity and limitations useful, particularly, for this study. All, including healthy control subjects, answered questions on the following subjects: physical limitations in activities of every-day-life due to symptoms; subjective judgment of the difficulty performing vigorous activities; and weekly hours spent on physical activities, such as running, cycling, and sports. The specific questions and the scoring system are shown in supplemental material, Text S2.

Analysis

Where appropriate, data regarding all assessed lung function parameters and aerobic fitness are reported as median (range) or mean (SD) of absolute values, values in percent predicted or z-scores. The main outcomes were z-scores for VO_{2peak}, FEV₁, DL_{CO}/V_A, LCI and scores of self-reported physical limitations and level of weekly physical activity. Abnormal lung function and VO_{2peak} was defined as a z score <−1.96, whereas abnormal LCI was defined as z score >1.96. SAS Enterprise guide 4.3 (SAS institute, Cary, North Carolina, USA) and MedCalc© Version 12.3.0. (MedCalc Software, Mariakerke, Belgium) were used for statistical analyses.

The Mann-Whitney test was used to test the significance of the differences in various parameters between PCDs and healthy controls, and the Chi-square test for the comparison of propor-

tions between these groups. Since VO_{2peak} is directly dependent on body weight and, hence, significantly correlated to BMI, we chose to correct for the latter. The associations between the z-scores for VO_{2peak} , FEV_1 , and DL_{CO}/V_A adjusted for BMI were analyzed with multiple regression analysis, using stepwise forward selection with an entry significance level of 0.05.

We used partial correlations to assess whether our sample size would provide adequate statistical power to show that one covariate (e.g. FEV_1 z-scores) was a significant predictor of VO_{2peak} z-scores when controlling for the other covariates (e.g. DL_{CO}/V_A and BMI, z-scores) using a Type III F test.

To detect a difference of 8.0 ml/kg/min between subgroups of PCD patients, the minimal required sample size per group was 15 assuming a SD of 6.6 ml/kg/min [12], with $\alpha = 0.05$ and $\beta = 0.1$. A two-tailed P value < 0.05 was considered significant.

Results

Patient characteristics

From a total of 108 PCD patients in the Danish PCD cohort, 66 were eligible for the study and 67% (44/66) agreed to participate and were included during the study period. Figure 1 shows the inclusion flowchart. Table 1 shows the baseline and diagnostic characteristics. The gender ratio did not differ significantly from that in the background population ($P = 0.08$). The 18 patients who refused to participate did not differ in baseline demographics or spirometric data (data not shown). All but one patient performed the VO_{2peak} test and the pulmonary function tests on the same day, except one patient who performed the VO_{2peak} test one week later than the other tests due to technical problems. One patient did not complete the questionnaire.

Characteristics of healthy controls

Median age and BMI z-score of the 33 healthy controls were 14.4 years (range, 6.2 to 28.8 years) and, 0.0 (range, -2.2 to 2.8), respectively. The gender ratio (M/F) was 17/16. These values were not significantly different from those in the PCD patient group. Twenty-eight healthy control subjects completed the questionnaire.

Pulmonary function parameters

In the PCD patients, abnormal z-scores were found in 27% (FEV_1), 14% (FVC), 48% (FEF_{25-75}) and 7% (TLC). Diffusion parameters were abnormal in only 7% (DL_{CO}) and 2% (DL_{CO}/V_A), while median N_2 MBW indices were highly increased and abnormal, specifically LCI in 93% (39/42). Table 2 shows the details of pulmonary function in PCDs patients.

As per inclusion criteria, all healthy controls had normal spirometric values with the following median z-scores: FEV_1 , 0.7 (range, -0.8 to 4.4); FVC, 0.9 (range, -1.1 to 3.6), and FEF_{25-75} , -0.1 (range, -2.8 to 3.4).

VO_{2peak}

All patients and healthy controls completed the exercise test and fulfilled the overall criteria for maximal performance. One patient did not reach HR $> 85\%$ of predicted, and RER was below 1.0 in two other patients. The national reference material embraced all healthy control subjects except one (Figure 2). The results are tabulated in Table 3.

VO_{2peak} was significantly reduced in PCD patients compared to in healthy controls (Table 3) and when compared to the national reference material ($P < 0.001$) exhibiting a median VO_{2peak} z-score of -1.00 (range, -3.90 to 0.50) (Figure 2), 34% of PCD patients (15/44) had abnormal VO_{2peak} . Maximal heart rate, test duration,

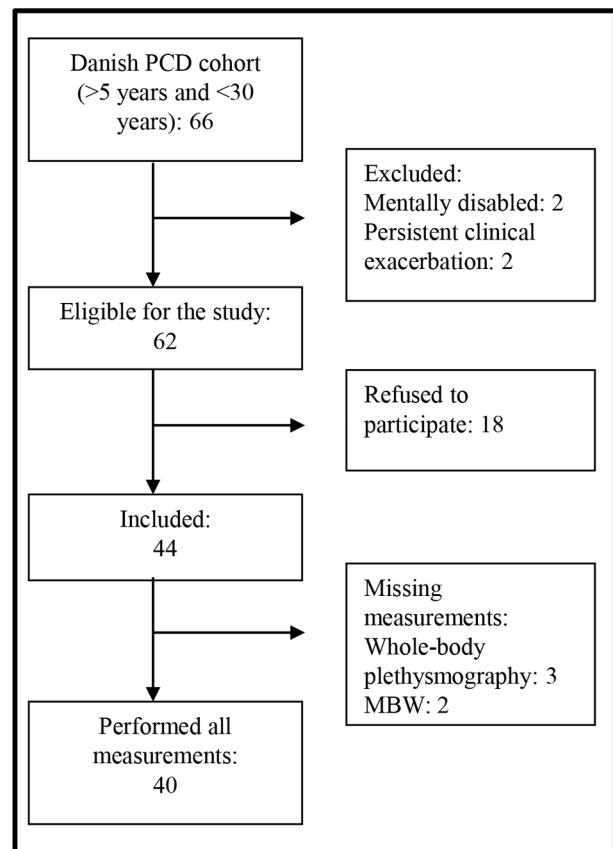


Figure 1. Flow chart showing the inclusion and investigation process.

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oxygen pulse, and maximum workload corrected for body weight (W_{max}/kg) were each also significantly lower in PCD patients (Table 3). VO_{2peak} did not differ between male and female PCD patients ($P = 0.18$).

Among the PCD patients VR was decreased ($< 15\%$) in 27% (12/44) and V_E/VCO_2 was increased (> 40) in 16% (7/44). V_E/VCO_2 was significantly increased in the total group of PCD patients, indicating ventilatory limitation during the test. However, of the patients with an abnormal VO_{2peak} (z-score < -1.96), only 20% (3/15) showed reduced VR, and increased V_E/VCO_2 was only seen in 27% (4/15). Desaturation ($Sp_{O_2} < 90\%$) or bronchial obstruction (decline of $> 12\%$ in FEV_1) did not occur in any patients during or after the exercise test.

VO_{2peak} and association with lung function parameters

The question at hand was whether reduced aerobic fitness was related to impaired lung function. Assuming partial correlations of 0.72 for FEV_1 z-scores and 0.65 for DL_{CO}/V_A and $\alpha = 0.05$, the power exceeds 0.99 for both FEV_1 z-scores and DL_{CO}/V_A making the model applicable for further analysis. When adjusting for BMI z-scores, multiple regression analysis using stepwise forward selection, showed that VO_{2peak} was significantly associated with FEV_1 z-scores (β -coefficient = 0.41 z-scores, 95% CI: 0.12–0.70 z-scores; $P = 0.01$) and DL_{CO}/V_A (β -coefficient = 1.53, 95% CI: 0.24–2.82; $P = 0.02$). For the overall model: Adjusted $R^2 = 0.32$; $F_{3,40} = 7.01$, $P < 0.01$. There was no significant difference in VO_{2peak} between PCD patient subgroups with normal and low (z-score < -1.96) FEV_1 (Figure 3). Moreover, VO_{2peak} was not

Table 1. Demographic and diagnostic characteristics of patients with primary ciliary dyskinesia.

	Median (range)
Demographics	
N	44
Gender (M/F)	17/27
Age in years, median (range)	14.8 (6.5 to 29.7)
BMI in kg/m ² , median (range)	19.9 (13.7 to 39.0)
BMI z-score, median (range)	-0.1 (-1.8 to 3.1)
Situs inversus	14
Diagnostic tests	
Typical clinical symptoms	44
nNO performed	44
Low level of nNO [§]	42
Cilia beat pattern performed	42
Immotility	16
Asynchrony	26
Not performed	2
Electron microscopy performed	39
Outer Dynein Arm (ODA) defect	11
Inner Dynein Arm (IDA) defect	1
ODA + IDA defect	7
Radial spoke defect	6
Transposition defect	2
Peripheral microtubule defect	6
Central microtubule defect	2
Normal with Hydin mutation [57]	4
Missing or not performed	5
Gram-negative infections	
Chronic PSA [†]	4
Intermittent PSA*	6
Chronic XA [§]	1

[§]Median (range) nNO = 26 (4 to 190); [†]Chronic PSA = chronic infection with *Pseudomonas Aeruginosa*, defined as more than 50% of positive airway cultures the previous year; *Intermittent PSA = intermittent infection with *P. Aeruginosa*, defined as at least one positive culture in the last year; [§]Chronic AX = chronic infection with *Achromobacter xylooxidans*, defined as more than 50% of positive airway cultures the previous year.
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associated with whole-body plethysmographic measures (data not shown) or any N₂ MBW indices (scatter plots in Figure S1). VO_{2peak} did not even differ between patients above or below the 3rd quartile of N₂ LCI z-scores.

Self-reported physical activity questionnaire

In the responses to the questions on physical limitations, 34% of patients (15/43) reported being moderately to highly limited, 44% (19/43) slightly limited, and 21% (9/43) not limited at all by sinopulmonary symptoms in activities of everyday-life. In addition, 39% (17/44) of the patients reported moderate to severe limitations in performing vigorous activities, while 30% (13/43) reported only slight difficulties, and 30% (13/43) denied having any difficulties at all.

VO_{2peak} was significantly lower in patients reporting severe limitations in performing vigorous activities compared to in

Table 2. Baseline pulmonary function in patients with primary ciliary dyskinesia.

Functional parameters	% of predicted		z-score	
	Median	Range	Median	Range
Spirometry (N = 44)				
FEV ₁	84.9	52.9 to 112.8	-1.2	-4.1 to 1.3
FVC	97.0	72.0 to 115	-0.2	-2.6 to 1.5
FEF ₂₅₋₇₅	58.2	21.8 to 120.0	-1.9	-4.5 to 1.1
FEV ₁ /FVC	88.0	67.3 to 105.3	-1.4	-3.0 to 0.9
Diffusion capacity (N = 44)				
DL _{CO}	94.1	73.2 to 135.4	-0.4	-2.3 to 2.3
DL _{CO} /V _A	95.8	74.3 to 128.0	-0.3	-2.1 to 2.5
Multiple breath washout, N₂ (N = 42)				
LCI _{N2}	157.1	98.0 to 255.3	12.2	-0.4 to 28.9
S _{cond}	344.4	92.3 to 636.2	6.4	-0.2 to 14.1
S _{acin}	220.1	61.2 to 812.4	2.7	-0.9 to 17.3
FRC _{N2, L}	122.7	78.4 to 190.6	2.4	1.2 to 5.9
Whole-body plethysmography (N = 41)				
sRaw (age <18 years), (N = 23)	109.8	74.2 to 495.2	20.4	0.9 to 6.2
FRC	107.4	78.2 to 155.2	0.4	-1.4 to 5.0
RV	140.2	63.7 to 211.2	1.8	-1.7 to 5.2
TLC	105.0	77.3 to 131.5	0.5	-2.6 to 4.8
VC	92.5	64.8 to 162.6	-0.7	-3.5 to 5.6
RV/TLC z-score	134.7	79.9 to 189.6	1.5	-1.3 to 5.6

FEV₁: Forced Expiratory Volume in 1 s. FVC: Forced Vital Capacity. FEF₂₅₋₇₅: Forced Mid Expiratory Flow. DL_{CO}: Single Breath diffusing capacity of the lung for carbon monoxide. DL_{CO}/V_A: Diffusing Capacity corrected for Alveolar Volume. MBW, N₂: Nitrogen Multiple Breath Washout LCI: Lung Clearance Index. S_{cond}: Conductive Airways ventilatory heterogeneity. S_{acin}: Acinar airways ventilatory heterogeneity. FRC: Functional Residual Capacity. sRaw: specific airway resistance. RV: Residual Volume. TLC: Total Lung Capacity. VC: Vital Capacity.
doi:10.1371/journal.pone.0071409.t002

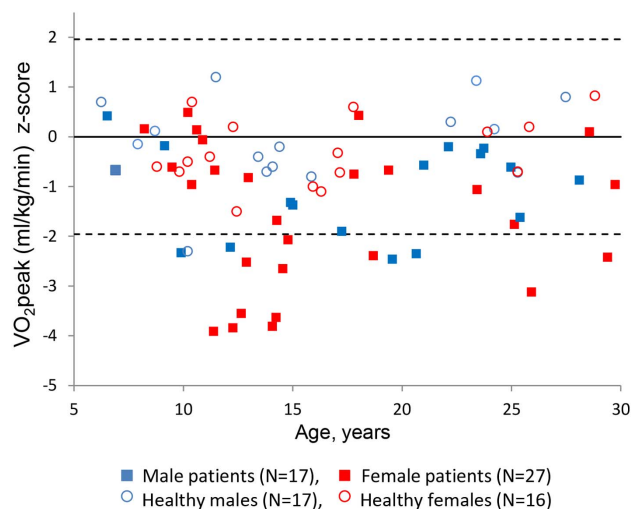


Figure 2. VO_{2peak} z-scores in 44 Danish PCD patients and 33 healthy control subjects according to age distribution. Solid line indicates a z-score of 0, and dashed lines indicate z-scores ±1.96.
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Table 3. Exercise test results in 44 PCD patients and 33 healthy subjects.

	PCD patients (N = 44)		Healthy subjects (N = 33)	
	Median	Range	Median	Range
VO _{2peak} , ml/kg/min	37.9***	19.4 to 51.0	44.4	30.6 to 57.6
VO _{2peak} , ml/kg/min % predicted	83.7**	48.9 to 105.7	95.7	66.2 to 120.1
VO _{2peak} , ml/kg/min, z-scores	-1.00***	-3.9 to 0.5	-0.3	-2.3 to 1.2
V _E , L/min (BTPS)	65.9	38.6 to 163.1	93.8	40.7 to 185.5
FR, min ⁻¹	47	25 to 61	47.9	29.2 to 66.5
VT, L	1.5	0.8 to 3.0	1.9	0.9 to 3.8
RER	1.14***	0.95 to 1.26	1.23	1.05 to 1.36
HR _{max} , bpm	192*	160 to 212	196	180 to 210
Min. S _{p,O₂} , %	97	90 to 100	97	94 to 100
Test duration, min.	6.1***	4.1 to 10.0	7.50	5.30 to 11.10
O ₂ pulse, % predicted	84.7**	50.4 to 108.2	95.9	62.9 to 126.0
W _{max} , watt	135	70 to 330	210	60 to 420
W _{max} /kg	3.1***	1.5 to 4.5	3.9	2.8 to 6.1
VR, %	27	-11.3 to 54.5	28.5	1.4 to 50.0
V _E /VCO ₂ , %	36.0**	26.6 to 48.8	33.2	26.5 to 43.4
AT, % VO _{2peak}	57.5	29.5 to 74.4	61.3	39.0 to 75.4

VO_{2peak}: Peak Oxygen uptake. V_E: peak Minute Ventilation. RF: Respiratory Frequency. VT: Tidal Volume. RER: Respiratory Exchange Ratio. HR_{max}: maximal Heart Rate. Min S_{p,O₂}: oxygen saturation. W_{max}: Maximal work load. W_{max}/Kg: watts per kilogram. VR: Ventilatory Reserve (1-(Minute Ventilation/Maximal Voluntary Ventilation)*100). V_E/VCO₂: Ventilatory equivalent for CO₂. AT, %VO₂: Anaerobic Threshold (AT) % of pred.

*Patients with PCD vs. Healthy subjects (*P*<0.05), **Patients with PCD vs. Healthy subjects (*P*<0.01), ***Patients with PCD vs. Healthy subjects (*P*<0.001). doi:10.1371/journal.pone.0071409.t003

patients without any limitations (*P*=0.001). VO_{2peak} was also lower in patients who reported that they were highly limited by sino-pulmonary symptoms in everyday-life compared to patients who were not being limited at all (*P*=0.04).

Responses to questions on weekly physical activity revealed that 30% (13/43) of patients reported performing less than three hours of physical training, while only 16% (7/44) spent more than seven hours every week. VO_{2peak} was significantly correlated with both limitations in everyday activities (*P*<0.01) and vigorous activities

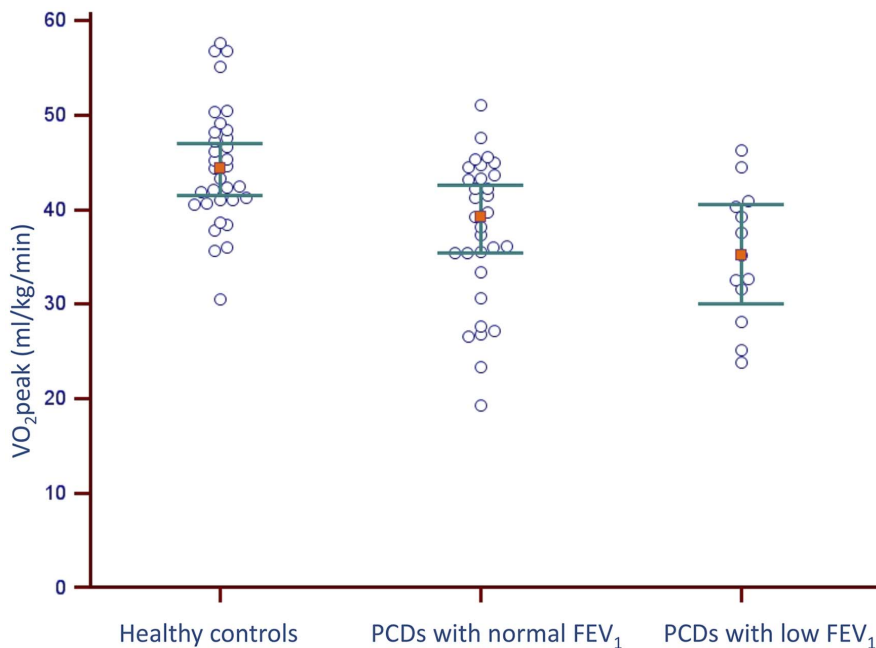


Figure 3. VO_{2peak} in PCD patients with normal FEV₁ and reduced FEV₁ (<-1.96 z-score) compared to healthy subjects. Dots are single participants. Red squares are median values with error bars of 95% CI. doi:10.1371/journal.pone.0071409.g003

($P < 0.0001$). VO_{2peak} was not correlated with self-reported weekly physical activity ($P = 0.23$). None of the healthy controls reported any limitations in physical abilities. Eight of the healthy control subjects reported being physical active less than 5 hours a week, which was not associated with VO_{2peak} .

Discussion

To our knowledge, this is the most comprehensive study to assess VO_{2peak} as well as other common and more sophisticated lung function measures in a large well-characterized group of patients with PCD. We believe that measurement of VO_{2peak} is of utmost relevance for the overall assessment of physical capacity and well-being. As observed in CF, the chronic nature of PCD and the pulmonary impairment has an indisputable effect on the desire and ability to perform physical activity, depending on disease stage. However, little is known about this subject, which prompted us to investigate the potential extent and degree of impairment in aerobic fitness in this rare disease entity.

Children and young adults with PCD, ranging in age from 6–30 years, exhibited significantly lower aerobic fitness than a matched control group. Surprisingly, as many as one third of the PCD patients exhibited significantly and markedly reduced VO_{2peak} compared to the national reference material. This reduction was associated with lower FEV_1 z-scores and DL_{CO}/V_A , but not with gender, age, or any other lung function parameters, including LCI by N_2 MBW. VO_{2peak} was also clearly associated with self-reported level of limitations due to difficulties performing vigorous physical activity.

Our finding of reduced aerobic fitness in PCD is consistent with a recently published smaller study by Valerio et al. [12]. They assessed VO_{2peak} in 10 children with PCD and a matched group of eight healthy subjects, and found that VO_{2peak} was significantly reduced in the total group compared to healthy controls; however this reduction was specifically only found in those patients with a reduced FEV_1 of below 85% of the predicted value. It was not possible to deduce the proportion of patients burdened by abnormal VO_{2peak} z-score. In contrast, Wells et al. [13] performed a study specifically investigating muscle function and metabolism, they reported VO_{2peak} in 10 patients with PCD, 20 matching healthy controls, and 20 CF patients, but did not find reduced VO_{2peak} in patients with PCD compared to in healthy controls or in CF patients.

There may be several explanations for these contradictory findings. Difference in age is one possibility; both previous studies had a lower mean age \pm SD (13.2 ± 2.8 and 13.8 ± 2.3 years, respectively) compared to that in our population (16.9 ± 8.8 years). Differences in gender distribution could also contribute, as there was a higher prevalence of females in our study. However, we did not find any correlation between VO_{2peak} (z-score) and gender or age. Finally, our PCD patients had a median FEV_1 of 84.9%, which was comparable to those of PCD patients in the study by Valerio et al. [12] ($87.5\% \pm 9.6\%$), but somewhat lower compared to those of the patients in the study by Wells et al. [13] ($95.4\% \pm 9.5\%$). Studies in CF patients have demonstrated that reduced FEV_1 is correlated with a low VO_{2peak} [36], although this relationship is non-linear, as CF patients with mild or moderate reduced pulmonary function had normal aerobic fitness compared to CF patients with severely reduced pulmonary function [37].

The mean VO_{2peak} (22.0 ml/kg/min) reported by Valerio et al. [12] is remarkably lower than those in our study (37.4 ml/kg/min) and reported by Wells et al. [13] (41.1 ml/kg/min). There is no clear explanation for this difference. Comparison of results between studies can be difficult due to the clinical heterogeneity

of the different patient cohorts, e.g. degree of bronchiectasis and chronic atelectasis. PCD represents a broad spectrum of disease severity, possibly due to multiple mutations [6]; however we did not have sufficient genetic data available to estimate the heterogeneity of our findings. Methodological differences (e.g. choice of exercise protocol, standard operating procedures, and skills of investigators) [38], [39] as well as crude genetic variations in aerobic performances [40] may explain the differences between and within PCD cohorts.

PCD is characterized by obstructive pulmonary impairment as reflected in spirometric and plethysmographic measurements (Table 2). Hence, it was of particular interest to analyze whether this was reflected by ventilatory measures such as V_E/VCO_2 . As expected, V_E/VCO_2 was higher in the PCD group than in healthy controls, and in line with the finding of Valerio et al. [12]. However, remarkable few patients with abnormal VO_{2peak} demonstrated ventilatory limitation as defined by V_E/VCO_2 and VR.

Although it has been claimed that VO_{2peak} assessment is the most valid measure of metabolic demand during exercise testing [41], W_{max}/kg may also provide additional useful information [42]. We found a significantly reduced W_{max}/kg in PCD patients compared to in healthy controls. Abnormal spirometric parameters have consistently been reported in PCD patients [10], [43], [44], [45]. The present data concerning plethysmographic parameters were quite similar to those reported by Pifferi et al. [45], showing significantly increased airway resistance, FRC, RV and RV/TLC. Interestingly, it has been claimed that these measures show better prediction of abnormalities on imaging by chest high-resolution computed tomography than spirometry [45].

The gas-exchanging capacity (DL_{CO} and DL_{CO}/V_A) is a key measurement in interstitial lung diseases, but has been only scarcely reported in PCD patients [46] and, as expected, only a few patients in our study demonstrated abnormal values (< -1.96 SD). Since loss of lung volume due to chronic atelectasis is a common feature in PCD patients, DL_{CO}/V_A may be a more appropriate parameter than DL_{CO} [45]. DL_{CO}/V_A appeared to correlate with VO_{2peak} , which theoretically seems reasonable, as this functional parameter of the blood-gas interface is an essential factor for sufficient oxygen supply during exercise. However, we do not believe that genuine impairment of pulmonary diffusion plays an important role as a limiting factor for VO_{2peak} in this patient group, since most patients had normal DL_{CO}/V_A values and normal TLC measured by plethysmography. Measurement of DL_{CO} and estimation of V_A during single-breath CO measurement in patients with obstructive disease is problematic, as the uneven ventilation distribution in the short breath-holding time can lead to an incomplete mixing between the inspired gas and the residual gas volume [47]. Additionally, correlation between DL_{CO}/V_A and VO_{2peak} may depend on a physiological phenomenon, since DL_{CO} (at rest) has been correlated to cardiac output (at rest), which is an important parameter inducible by regular training [48], [49].

Notably, more than 30% of the PCD patients reported limitations in both everyday life and vigorous activities with clear association with the VO_{2peak} level. This finding is comparable to studies in CF patients, that have reported similar association between VO_{2peak} and subjective judgment of physical disability [50]. This association indicates good self-awareness of physical ability and limitations among the patients. In contrast, Valerio et al. [12], surprisingly found that hours spent in vigorous physical activity was not associated with VO_{2peak} .

Here we again reported indices of MBW in PCD patients, this time using N_2 as tracer gas instead of SF_6 [11]. We consistently observed severe ventilation distribution inhomogeneity, but we

found no correlation with VO_{2peak} . Interestingly, these parameters did not relate to aerobic fitness even when excessively increased. Further analyses are needed to understand the clinical impact, to compare these observations, and to determine the likely explanation and importance of these findings.

The strengths of the present study include the relatively large and well-characterized PCD cohort and the comprehensive panel of pulmonary function measures reflecting almost all pulmonary functional aspects, – including ventilation distribution inhomogeneity, which has not been previously correlated with VO_{2peak} .

To obtain reliable and consistent results, all exercise tests were supervised and conducted by an experienced test leader. Moreover, VO_{2peak} reference values were derived from comparable VO_{2peak} assessments of healthy Danish children and young adults. We proved the robustness of this reference material and the reliability of our test setup by testing a group of healthy subjects with an age range mirroring this material in our laboratory, and we found acceptable agreement between VO_{2peak} levels. Although using a set of pooled data from different age groups and periods as reference may be regarded as a study weakness, it has been documented that VO_{2peak} in healthy subjects is highly repeatable and consistent, both between months [39] and when judged from secular trends provided the tests are performed by highly skilled test leaders using a fixed standard operating procedure for the maximal test [51].

The study was slightly limited by the cross-sectional design and by the use of a non-validated questionnaire on physical limitations and weekly physical or sport activity, which was composed and inspired by questions from previously validated questionnaires. A similar approach was previously used by Pifferi et al. [8], as no validated PCD-specific quality of life questionnaire yet exists. Moreover, activity information derived from self-reported questionnaires is often potentially prone to response bias (e.g. imprecise recall, and influence of social desirability) [52]. However, the questionnaire was completed during the visit but before the exercise test, and in co-operation with the investigator. Corrections and additional information could be provided if necessary to obtain reliable answers. Improved comparison between international studies will require a common validated questionnaire. The BESTCILIA network has been recently developed to provide better diagnostic and treatment tools for PCD, as well as validated PCD-specific quality of life questionnaire, which may satisfy this need [53].

Although we found an association between aerobic fitness and FEV_1 and DL_{CO}/V_A , it is difficult to estimate the degree of direct influence from these measures. Other important limiting factors that were not measured in the present study include maximal cardiac output, blood oxygen carrying capacity, and peripheral limitations, such as mitochondrial enzyme level and capillary density in muscles [48]. It has not been currently known whether other factors like severity of sinusitis may have any influence, and further studies are needed to explore potential complex association between chronic sinopulmonary disease, cardiopulmonary physiology and VO_{2peak} in PCD.

Despite its limitations, we think that this study provides important new information on the rare pulmonary disease entity PCD. The reduced VO_{2peak} observed in the PCD population might have been caused by the burden of chronic respiratory disease and impairment of pulmonary function, which may lead to a sedentary lifestyle with generally low fitness level, and a theoretically reduced and insufficient capacity to increase cardiac

output during vigorous physical activity. This hypothesis is supported by studies in healthy populations that show a sedentary lifestyle to be related to a significantly lower VO_{2peak} [54], [55] and our present findings suggest, that low aerobic fitness may be due to sedentary life style, reflecting the chronic disease. In fact, PCD pulmonary disease might be an additional risk factor of increased morbidity and mortality in these patients since low aerobic fitness is related to increased cardiovascular disease even in otherwise healthy randomly selected children [56]. However, the use of VO_{2peak} as an outcome measure in PCD requires prospective longitudinal studies since longitudinal decline, and not a single VO_{2peak} measurement, might be a better predictor of prognosis, as demonstrated in CF patients [3].

Conclusion

The present study shows that more than one-third of Danish children and young adults with PCD had significantly abnormal VO_{2peak} , which was associated with FEV_1 z-scores and DL_{CO}/V_A , but not with age or gender. The reduced VO_{2peak} may be related to a sedentary lifestyle caused by the chronic pulmonary disease. We believe that regular physical exercise is of great importance to these patients and should be formally implemented in the clinical management of patients with PCD, with the aim of improving mucociliary clearance and prevent lifestyle-related diseases. Further studies are needed to evaluate VO_{2peak} as a prognostic marker of pulmonary morbidity in PCD.

Supporting Information

Figure S1 VO_{2peak} z-scores plotted against indices of N_2 MBW measurements in patients with PCD. **A)** VO_{2peak} Z-scores vs. N_2 LCI. **B)** VO_{2peak} z-scores vs. N_2 S_{cond} . **C)** VO_{2peak} z-scores vs. N_2 S_{acin} . VO_{2peak} : peak oxygen uptake, LCI: lung clearance index, S_{cond} and S_{acin} : normalized phase III slope indices. The dashed red horizontal lines denote the lower limit of normality of VO_{2peak} (mean -1.96 SD). The dashed vertical blue lines denote the upper limits of normal for N_2 LCI, S_{cond} , and S_{acin} . (TIF)

Text S1 Method for VO_{2peak} measurement. (DOC)

Text S2 Self-reported physical activity questionnaire. The included questions and the scoring system. (DOCX)

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Author Contributions

Conceived and designed the experiments: KG FB KGN. Performed the experiments: AM KG BH. Analyzed the data: AM KG FB BH KGN. Contributed reagents/materials/analysis tools: AM KG FB BH KGN. Wrote the paper: AM KG FB BH KGN.

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