The impact of plasma 25-hydroxyvitamin D on pulmonary function and exercise 1 physiology in cystic fibrosis: a multicentre retrospective study.

The impact of plasma 25-hydroxyvitamin D on pulmonary function and exercise physiology in cystic fibrosis: a multicentre retrospective study

Running title: Relationship between vitamin D and respiratory health in cystic fibrosis

Raquel Revuelta Iniesta[1,2] Adam J. Causer[3,4], Irantzu Arregui-Fresneda[4], Gary Connett[6], Mark I. Allenby[4], Thomas Daniels[4], Mary P. Carroll[4], Don S. Urquhart[7] and Zoe L. Saynor[4,5]

[1] School of Sport and Health Sciences, University of Exeter, United Kingdom

[2] Child Life and Health, University of Edinburgh, Edinburgh, United Kingdom

[3] Department for Health, University of Bath, United Kingdom.

[4] Wessex Cystic Fibrosis Unit, University Hospital Southampton Foundation NHS Trust, United Kingdom.

[5] School of Sport, Health and Exercise Sciences, University of Portsmouth, United Kingdom.

[6] National Institute for Health Research, Southampton Biomedical Research Centre, Southampton Children's Hospital, United Kingdom.

[7] Department of Paediatric Respiratory and Sleep Medicine, Royal Hospital for Sick Children, Edinburgh, United Kingdom.

Corresponding author:
Dr Raquel Revuelta Iniesta
Work telephone: +44 (0) 1392 724928
Email: r.revuelta-iniesta@exeter.ac.uk

Abstract

Background: 25-hydroxyvitamin D (25OHD) may exert immunomodulatory effects on respiratory health, which may translate to improvements in exercise physiology. Thus, we aimed to investigate whether plasma 25OHD is associated with lung function and aerobic fitness in people with cystic fibrosis (pwCF).

Methods: A multi-centre retrospective review of pwCF (>9 years old) attending the Royal Hospital for Sick Children (Edinburgh) or Wessex CF-Unit (Southampton) was performed between July 2017 to October 2019. Demographic and clinical data were collected. Plasma 25OHD measured closest in time to clinical cardiopulmonary exercise testing (CPET) and/or spirometry (forced expiratory volume FEV_1% predicted) was recorded. Pancreatic insufficiency was diagnosed based on faecal elastase of <100 µg/g. We performed multiple-regression analysis with aerobic fitness outcomes [peak oxygen uptake (VO_2peak)] and FEV_1% predicted as primary outcomes.

Results: Ninety pwCF [mean±SD age: 19.1±8.6 years, 54 (60%) children, 48 (53%) males and 88 (98%) Caucasian] were included. 25OHD deficiency and insufficiency was 15 (17%) and 44 (49%) respectively. 25OHD deficiency and insufficiency was significantly associated with pancreatic insufficiency ($\chi 2(4.8)$; $p = 0.02$). Plasma 25OHD was not significantly associated with FEV_1% predicted [$R^2 = 0.06$; $p = 0.42$; 95%; CI (-0.09 - 0.19)] or VO_{2peak} [$R^2 = 0.04$; $p = 0.07$; 95% CI (-011 - 0.005)] in all pwCF. However, 25OHD was significantly associated with both FEV_1% [$R^2 = 0.15$; $p = 0.02$; 95% CI (1.99 - 2.64)] and VO_{2peak} [$R^2 = 0.13$; $p = 0.05$; 95% CI (-0.26 – (-0.005)] in the paediatric cohort.

Conclusion: We showed that 25OHD is associated with improved lung function and aerobic fitness in children and adolescents with CF. Mechanistic and high-quality prospective studies including both lung function and aerobic fitness as primary outcomes are now warranted.

Keywords: cardiopulmonary exercise testing, cystic fibrosis, lung function, ventilation, vitamin D.

Introduction

Cystic fibrosis (CF) is an autosomal-recessively inherited multisystem disorder that affects 1 in 3000 newborn Caucasians children with slightly lower prevalence noted in other ethnic groups[1]. CF affects a number of body systems and is associated with gastrointestinal, hepatobiliary, sinopulmonary and bone disease. Morbidity and mortality are principally due to unresolving and unremitting infections that cause progressive lung disease. However, malabsorption as a result of pancreatic insufficiency may impact growth, as well as consequent reduction in fat soluble vitamin levels[2]. Supplementation with vitamin D is thus advocated for all people with CF (pwCF)[3]. The role of 25-hydroxyvitamin (25OHD), the main circulating form of vitamin D, on bone health has long been recognised[4]. Recently, evidence in healthy individuals suggests that 25OHD may also be an important determinant of respiratory health[5] and aerobic fitness[6; 7; 8]; however, whether it may be associated with improved pulmonary function in pwCF has yet to be investigated.

The prevalence of 25OHD inadequacy, defined as deficiency and insufficiency[9], has been quantified as being from 23% to as high as 95% in pwCF[10]. This is similar to reports of vitamin D inadequacy in other chronic diseases[11; 12], but higher rates of vitamin D inadequacy than those seen in the general children and adolescent (19 – 37%) and adult population (29%)[13]. The active form of 25OHD, 1,25 dihydroxyvitamin D (1,25OHD), has both anti-inflammatory and anti-microbial properties that are explained by its role in the downregulation of pulmonary pro-inflammatory responses and the upregulation of both anti-inflammatory cytokines and antimicrobial peptides activity in response to respiratory pathogens[14]. Furthermore, 1,25OHD may reduce airway resistance by regulating smooth muscle excitation-contraction via intracellular calcium ion (Ca^{2+}) release and Ca^{2+} sensitisation. Therefore, it is biologically plausible that 25(OH)D insufficiency may exert a role in the pathophysiology of CF[14]; however, data in regard to the impact of plasma 25(OH)D on pulmonary function in pwCF are sparse[15; 16; 17].

Cardiopulmonary exercise testing (CPET) is advocated by both the European CF Society[18] and European Respiratory Society[19] as a functional assessment of lung, cardiovascular and muscular health in pwCF. Furthermore, markers of aerobic fitness and ventilatory function during exercise have been shown to be significant predictors of mortality in CF[20]. Studies have demonstrated pulmonary[21], cardiovascular[22], metabolic[23] and skeletal muscle[24] abnormalities are factors that modulate exercise capacity in pwCF. However, the effect of 25OHD as a modulator of exercise capacity has not, to our knowledge, been investigated.

The aim of the study was therefore to use a multicentre retrospective cohort of children and adults with CF to determine whether 25OHD concentration was associated with lung function and aerobic fitness. The present analysis was also designed to investigate; (i) the prevalence of vitamin D deficiency and insufficiency in this cohort of pwCF; (ii) the association between 25OHD concentration (nmol/L) and prescribed vitamin D supplementation (IU); (iii) differences in 25OHD in pwCF classified by lung disease severity and aerobic exercise function. We hypothesised that lower plasma 25OHD would be significantly and negatively associated with lung function and aerobic fitness in pwCF.

Materials and Methods

Study design and population

A multi-centre retrospective study was performed. Eligibility for the study were people with CF age > 9 years old and attending the Royal Hospital for Sick Children (Edinburgh) or Wessex CF-Unit (University Hospital Southampton) between July 2017 – October 2019 (pre-COVID-19 pandemic and prior to the widespread availability of highly effective CFTR-modulator therapy, Kaftrio®) for lung function and/or cycle-based CPET. The diagnosis of CF was based on NICE criteria[3], which includes elevated [sweat chloride] (>60 mmol/L^{-1}) and a compatible genotype. Demographic (age, gender and ethnicity) and clinical data (diagnosis, blood results and CF-related comorbidities) were collected from medical notes and electronic patient record. Pancreatic insufficiency was diagnosed based on faecal elastase of <100 µg/g[25; 26]. This study was exempted from requiring National Health Service (NHS) ethical approval as data are retrospective and non-identifiable. The study was considered under the category of service evaluation and permission was obtained from NHS Quality Improvement.

Spirometry

Spirometry was performed (3500 MicroLab Spirometer MK8, CareFusion, CA, USA) to ATS/ERS standards[27]. Forced expiratory volume in 1 second (FEV_1) % predicted and forced vital capacity (FVC) were determined as the highest of the three consistent (≤ 5% variability) manoeuvres[28; 29]. FEV_1% predicted was defined as normal (≥85%), mild (70-84%), moderate (50-69%) and severe (<50%) CF lung disease[30].

Anthropometric and bone mineral density data

Stature using a stadiometer and weight on calibrated scales were recorded at each CPET visit. Body mass index (BMI) was calculated for adults and World Health Organisation (WHO) reference ranges[31] were used to define underweight (<18.5 Kg/m^2), normal weight (≥ 18.5 to ≤ 24.9 Kg/m^2), overweight (≥ 25 to ≤29.9 Kg/m^2) and obese (≥ 29.9 kg/m^2) categories. UK-WHO BMI *Z*-scores were calculated for children using the LMSgrowth

program®[32] and nutritional status was classified as underweight (-2.0 SD or ≤ 2.3rd centile), normal weight (> -2.0 to ≤1.05 SD or > 2.0nd to < 85th centile), overweight (> 1.05 to ≤1.63 SD or >85th to ≤95th centile) and obese (1.63 SD or >95th centile)[31].

Dual energy X-Ray absorptiometry is ideally performed in pwCF every 1-3 years. Measurements of lumbar spine bone mineral density (LS-BMD) *Z*-score closest to CPET or spirometry were collated from the EPR.

Vitamin D and associated bone markers data

Plasma 25OHD, parathyroid hormone (PTH), calcium, phosphate and magnesium concentrations, measured closest in time to CPET or spirometry, and vitamin D supplementation (IU) were noted from EPR. The rationale for this was to have the most representative plasma 25OHD at the time of CPET and spirometry as plasma 25OHD half-life of ranges between 15 – 45 days[33]

Both centres used liquid chromatography-tandem MS technique for 25OHD and Immulite 2000 Intact PTH technique for PTH. The immediate CV (%) for the assays were ≤8.9 and 5.7% respectively([34 – 35]). Plasma 25OHD was classified by season as synthesising (1st April – 30th September) and non-synthesising periods (1st October – 31st March)[36]. 25OHD status was defined according to the Endocrine Society (25-50 nmol/L: deficiency; >50 ≤ 75 nmol/L: insufficiency; >75 nom/L: sufficiency/optimal)[9].

CPET

Participants performed CPET on an electromagnetically braked cycle ergometer (Edinburgh: Ergoline Viasprint 200, Ergoline, Blitz, Germany; Wessex: Lode Corival, Groningen, The Netherlands) using an exhaustive incremental protocol. Work rate was increased by 10 – 30 W/min, whilst maintaining a cadence at 60 – 80 rpm. Pulmonary gas exchange and ventilation were measured throughout exercise using a face mask, turbine system and metabolic cart, which was calibrated in line with manufacturer's instructions (Edinburgh: CareFusion UK, Basingstoke, England; Wessex: K5, COSMED, Rome, Italy). Specifically, breath-by-breath measurements of oxygen uptake (VO_2), carbon dioxide production (VCO_2), minute ventilation (V_E), and respiratory exchange ratio (RER) were made. Heart rate (HR) was also monitored continuously by a 12-lead electrocardiogram, blood pressure was measured at rest and every 3 minutes during exercise and transcutaneous oxygen saturation (SpO_2%) was measured by a pulse oximeter placed on the right ear or on the index or middle finger.

Peak aerobic fitness (VO_{2peak}) was taken as the highest 30 second mean and was expressed relative to body mass ($mL \cdot Kg^{1} \cdot min^{-1}$). Additional variables of interest from the CPET included measurement of VO_2 at the gas exchange threshold (GET), expressed as a % predicted VO_{2max} and RPE. Criteria for accepting a maximal CPET were either

the occurrence of a true VO_{2max}, a plateau in VO_2 despite increasing workload, or were based on HR attainment, maximal exercise ventilation and RER data.[19]

Gas exchange threshold (GET) is the point at which ventilation increases at a faster rate than oxygen uptake (VO_2) and reflects the point at which anaerobic metabolism begins to predominate with exponentially increasing CO_2 production and accumulation of fatigue-related metabolites including lactate.[36] These effects on musculoskeletal and respiratory mechanisms serve to limit exercise capacity after GET. The measurement of VO_{2max} at GET thus may tell us about fitness, conditioning and adaptation to exercise.[36] Breathing reserve was defined as $\left(BR = \frac{MVV - VEpeak}{MVV} x\ 100\right)$ where maximal voluntary ventilation (MVV) was estimated using the equation $(35\ x\ FEV_1)$.[37] BR is the percentage of an individual's expected MVV that remains at maximal exercise.[38] Ventilatory equivalents for oxygen (V_E/VO_2) and CO_2 (V_E/VCO_2) were measured. These represent the minute respiration required per unit of oxygen uptake or CO_2 elimination and provide information on a subject's ventilator efficiency.

Statistical analysis

The Statistical Package for Social Sciences (version 21; IBM-SPSS for Windows Statistics) was used to analyse all data. Parametric tests and means (±SD) were used for normally distributed data and non-parametric test with median and interquartile range (IQR) for non-normally distributed data. Descriptive statistics were used to evaluate lung function status and the prevalence of plasma 25OHD deficiency and insufficiency. Correlations between plasma 25OHD and the variables vitamin D supplementation (IU), FEV_1% and GET were performed using Spearman's correlation. The Mann-Whitney test was used to compare plasma 25OHD between pwCF from Edinburgh versus Southampton, males versus females, children versus adults and seasonality. A series of analysis of variance (ANOVA) and Tukey's post-hoc *t*-tests were conducted to establish differences between 25OHD status categories (deficiency/insufficiency, sufficiency and optimal) in the following outcome variables; VO_2 peak, maximal ventilatory equivalent for oxygen (V_E/VO_{2peak}) and CO_2 (V_E/VCO_{2peak}). Hierarchical multiple-regression analysis with $\dot{V}O_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$) and FEV_1% predicted as primary outcomes was performed after adjusting for patient's characteristics (age, BMI, LS-BMD *Z*-score and VO_2max % predicted) for the full cohort and cohort subgroups (geographical location and age classification). Factors were tested one at the time and only those that reached a relaxed significance of 0.1 were included in the conditional model. $P < 0.05$ was considered statistically significant. In order to meet the assumptions of the linear model, non-normally distributed variables were converted to normally distributed using the log10 function. No a priori sample size estimation was performed (convenient sample).

We followed the STROBE guidelines for the presentation of our data[39].

Results

Demographics and clinical characteristics

Ninety pwCF were included in this study. Demographic and clinical characteristics are presented table 1. Of these, 100% had plasma 25OHD and spirometry. Furthermore, 78 (87%) had performed a CPET between July 2018 and Oct 2019. The median (IQR) length of time between the assessment of plasma 25OHD and spirometry was 66 (21-335) days. The Edinburgh cohort differed from the Southampton group by being significantly younger [13.2(12.1 – 14.2) vs. 21.9(15.7 – 29.57) years], having lower BMI [(18.4 (16.8 – 20.2) Kg/m^2 vs. (20.9 (18.7 – 23.6) Kg/m^2] and higher FEV_1% predicted [93.0 (82.5 – 100.2)% vs. 70.5 (56.8 – 87.4)%; $p < 0.01$ for all. The BMI and lung function differences likely pertain to the noted age difference and cohorts were otherwise similar.

Thirty-one (34%) pwCF were vitamin D sufficient. Whilst, the prevalence of vitamin D deficiency and insufficiency was 15 (17 %) and 44 (49%), respectively. Of those who were sufficient, 25 (81%) had vitamin D supplementation [median (IQR) 1600 (900 – 2840)] IU/day prescribed and of those who were deficient and insufficient, 59 (100%) had vitamin D supplementation [median (IQR) 1600 (800 – 1600) vs. 850 (800 – 1600), respectively] IU/day prescribed and in total 84 (93%) pwCF were receiving 1400 (800-1780) IU/day of vitamin D. Thirty-four (40%) pwCF received vitamin D_3, 4 (5%) vitamin D_2 and 46 (55%) was either not recorded or unknown. There was no statistical significant differences in either the 25OHD concentration [U (116.5); p=0.3] or vitamin D supplementation [(U(127.5); p=0.6)] between the synthesising [65.5 (62.0 – 71.75) nmol/L; 950 (650 – 1675 IU)] and non-synthesising periods [60.5 (53.5 – 76.0) nmol/L; 60.5 (53.5 – 76.0)]. A weak positive correlation was found between plasma 25OHD and vitamin D supplementation ($r = 0.23$; $p = 0.06$). Children had significantly higher plasma 25OHD [65.5 (54.0 – 84.6) nmol/L] than adults [63.0 (46.5 – 82.5) nmol/L]; U(698); $p = 0.03$, similar vitamin D doses [children 1000 (800 – 1650) vs. adults 1100 (800 – 1600) IU/day] and there was a statistical significant association between pancreatic insufficiency and 25OHD deficiency and insufficiency ($\chi^2(4.8)$; $p = 0.02$).

The majority of the pwCF cohort had normal FEV_1 42 (47%) with 21 (23%) having mild, 18 (20%) moderate and 9 (10%) severe lung disease. Figure 1 shows 25OHD concentration in the full cohort (figure 1 left) and in children and adults with CF with data stratified by lung function status (FEV_1 % predicted) (figure 1 right). Table 3 shows the characteristics of the pwCF with data stratified by age (paediatric vs. adults) and plasma 25OHD status.

Between group differences of aerobic exercise and ventilatory function during CPET are presented in Figure 2. There was no significant differences in the prevalence of ventilatory limitation ($\dot{V}E$ / MVV ≥85%) amongst groups in the paediatric ($p = 0.31$) or adult ($p = 0.27$) cohorts; however, there was significantly reduced ventilatory efficiency ($\Delta\dot{V}E/\Delta\dot{V}CO2 \geq 35$) in children with 25OHD deficiency and insufficiency (56%) compared to their insufficient (12% ; $p = 0.03$), but not sufficient (40% ; $p = 0.66$), counterparts.

Factors contributing to lung function (FEV_1 %) and aerobic performance expressed as $VO_{2\ peak}$

Plasma 25OHD was not significantly associated with FEV_1% function [$R^2 = 0.06$; $\beta = 0.05$; $p = 0.42$; 95% CI (-0.09 to 0.19)] or VO_{2peak} [$R^2 = 0.04$; $\beta = -0.19$; $p = 0.07$; 95% CI (-011 to 0.005)] in the overall pwCF cohort. Table 4 shows hierarchical multiple regression analysis of the children's CF cohort (Edinburgh). Interestingly, 25OHD significantly predicted both FEV_1% [$R^2 = 0.15$; $\beta = 0.3$; $p = 0.02$; 95% CI (1.99 to 2.64)] and VO_{2peak} [$R^2 = 0.13$; $\beta = -0.36$; $p = 0.05$; 95% CI (-0.26 to (-0.005)] and BMI *Z*-score and age (years) significantly predicted FEV_1% [$R^2 = 0.31$; $\beta = 0.33$; $p = 0.01$; 95% CI (0.001 – 0.002)] and VO_{2peak} [$R^2 = 0.28$; $\beta = -0.39$; $p = 0.03$; 95% CI (0.11 to 2.01)] respectively (table 3). In contrast, 25OHD of the Southampton pwCF cohort predicted neither FEV_1% [$R^2 = 0.01$; $\beta = 0.1$; $p = 0.5$; 95% CI (-0.12 to 0.26)] nor VO_{2peak} [$R^2 = 0.38$; $\beta = -0.19$; $p = 0.17$; 95% CI (-0.92 to 0.16)]. Finally, data was stratified by age (paediatrics vs. adults), 25OHD was not a significant predictor of FEV_1% [children: $R^2 = 0.03$; $\beta =$; $p = 0.26$; 95% CI (-0.27 to 0.08) vs. adults: $R^2 = 0.003$; $\beta = -0.05$; $p = 0.75$; 95% CI (-0.26 to 0.19)] or VO_{2peak} [children: $R^2 = 0.03$; $\beta = -0.18$; $p = 0.21$; 95% CI (-0.13 to 0.03) vs. adults: $R^2 = 0.02$; $\beta = -0.13$; $p = 0.46$; 95% CI (-0.10 to 0.05)].

Discussion

This is the first study to investigate potential associations between abnormal plasma 25OHD concentration upon aerobic fitness and resting and exercise ventilatory function in children, adolescents and adults with CF. Our results show a high prevalence of plasma 25OHD deficiency and insufficiency, and highlights that despite appropriate vitamin D supplementation, total plasma 25OHD was lower in adults than in children, in those with pancreatic insufficiency and there was no seasonal variation. Medium effect sizes suggest that plasma 25OHD abnormalities are associated with aerobic exercise and ventilatory dysfunction in pwCF (Figure 2). Importantly, plasma 25OHD concentration was significantly and negatively associated with lung function (FEV_1 %) and maximal aerobic fitness ($\dot{V}O_{2peak}$) in a childhood CF cohort only. Therefore, we support the hypothesis that lower 25OHD may be associated with poorer lung function and aerobic fitness during an important phase of growth.

Current UK guidelines on vitamin D supplementation for pwCF suggest 400 – 2000 IU/day for infants and 400 – 5.000 IU/day for children (>1 year old) and adults with the aim of achieving a 25OHD concentration of > 50 nmol/L (20 ng/ml)[39]. Despite vitamin D supplementation [1.400 (800 – 1.780) IU/day] meeting current recommendations for pwCF in 86 (95%) of the study cohort, the prevalence of 25(OH)D deficiency and insufficiency (17% vs. 49%) was similar to that previously reported in pwCF elsewhere[10; 40] and in children and adults diagnosed with cancer (33 – 75%), who are either not supplemented or supplemented with lower doses of vitamin D (292 – 464 IU/day)[11], and higher than the general UK population (19 – 37%)[13]. Of note, supplementation in the general population is rare[13]. Children had higher 25OHD concentration than adults, despite being prescribed similar vitamin D doses, and our pwCF cohort did not show any seasonal variation as opposed to healthy individuals who show higher 25(OH)D concentration during the synthesising months (spring and summer) [13]. These findings are in line with most studies performed in the UK and in different latitudes[10; 15; 42]. Seventy-nine percent of the study cohort were pancreatic-insufficient, which impairs absorption of vitamin D even with the use of pancreatic enzymes[42]. Statistically significant associations between pancreatic insufficiency and plasma 25OHD deficiency and insufficiency were noted in our study. Impaired hepatic hydroxylation, which leads to accelerated vitamin D excretion, reduced sunlight exposure due to antibiotic induced photosensitivity or poor health, differences between vitamin D_2 and D_3 bioavailability[13] and low vitamin D binding protein (DBP) concentration may also explain the results found in the present study[43]. Undernutrition has also been associated with low 25OHD status[43], but we did not find any differences between the nutritional status categories. Suboptimal adherence to vitamin D supplements is widely reported; however, it is also plausible that the doses proposed in current vitamin D supplementation guidelines are not enough for pwCF to achieve optimal 25OHD concentration[45]. Daily doses of up to 4.000 IU for 1 – 10 year old and 10.000 IU for > 10 years of age or single doses stratified by age ranging between 100.000 – 600.000 IU have been reported to be successful in achieving and maintaining optimal concentration for over 12 months and reducing patient burden[46].

No statistically significant differences in aerobic fitness (GET) and ventilatory efficiency ($V\dot{V}O_{2peak}$, V_{VE}/VO_{2peak}, V_{VE}/VCO_{2peak}) during exercise amongst children, adolescents and adults with CF was found along the spectrum of plasma 25OHD abnormalities. Nonetheless, a medium effect size (η^2 = 0.07) suggested that plasma 25OHD deficiency may contribute to a reduced $\dot{V}O_{2peak}$ in children with CF (76.8 ± 15.5% predicted), compared to their 25OHD sufficient counterparts (89.4 ± 19.7% predicted), which means this population would be placed in a tertile ($\dot{V}O_{2peak}$ 59-81% predicted) that is at a heightened risk of long-term mortality[20]. The nature of the relationship between 25OHD and exercise capacity is not fully understood. It is biologically plausible that more severely ill

pwCF will have lower 25OHD concentration and both reduced physical activity levels(with less sunlight exposure) and exercise capacity[1; 47], whilst adherence to vitamin D supplements is often suboptimal in pwCF, particularly in adults, due to a significant treatment burden. It is also, therefore possible that 25OHD deficiency and the resultant lowered VO_{2peak} could be due to the effects of poor vitamin D treatment adherence in this particular group.

Consistent with much of the available evidence from studies performed in children and adolescents with CF[15; 16; 17], our study found that plasma 25OHD concentration was significantly associated with lung function (FEV1 %) and aerobic fitness ($\dot{V}O_{2peak}$) in the Edinburgh children's cohort only; however, this was not the case when the cohort was analysed altogether. Importantly, our results showed that nutritional status, measured by BMI *Z*-score, also influenced this relationship indicating that appropriate nutritional status is of paramount importance to maintain lung function in this cohort. It has long been recognised that optimal nutritional status reduces the risk of infection and improves recovery[1]. However, this study suggests that there may be other factors influencing this relationship in adults, including (i) the heterogeneity and complexity of lung disease in pwCF[14; 16]; (ii) slight treatment variations between centres; (iii) the genetic variations in the vitamin D receptor (VDR) expression of lung immune cells and differences in plasma DBP[14; 16]; (iv) severity of CF-associated comorbidities, nutritional status (other than BMI) and dietary intake[1]; (v) time taken between 25OHD sampling and spirometry testing and (vi) regular physical activity or smoking. Of note, this study shows a trend (non-significant) towards lower 25OHD concentration in those with moderate and severe impaired lung function highlighting that 25OHD deficiency and insufficiency may increase the risk of impaired lung function. Likewise more severe pwCF may have lower 25OHD due to their illness, reduced sun exposure and/or poorer dietary intake[17]. It is worth noting that this study was performed before the COVID-19 pandemic and the introduction of triple-combination CFTR-modulator therapy, Kaftrio®, which is licensed for pwCF over the age of 12 in the UK. Eligible pwCF are expected to have improvements in their lung function, longevity, nutritional status and wellbeing[48]. However, real world studies of this exciting new treatment are mandated. The National Institute for Health Research[49] have highlighted the importance of evaluating the consequences of the implementation of this therapy early on and new associated clinical management needs. Together, these data, in addition to the finding that plasma 25OHD is significantly associated with $\dot{V}O_{2peak}$ in children and adolescents (Edinburgh cohort) with CF (Table 4), provide an exciting rationale to include CPET parameters of aerobic fitness and ventilatory function as outcomes in studies investigating the efficacy of vitamin D supplementation[50].

Limitations of the study and future directions

A summary of recommendations for future studies is presented in Box 1[51]. The retrospective nature of the present study meant that sampling time between 25OHD measurements and CPET and spirometry was limited to within the same annual review year. However, the half-life of 25OHD has been reported to be within 15-45 days[33] and, therefore, future prospective studies should be designed to sample plasma on the same day (or within 15 days) as CPET and spirometry. An *a posteriori* sample size calculation for multiple regression analysis (with 5 predictors), statistical power ≥ 0.8 and anticipated effect size 0.15 at a α-level ≤ 0.05 indicated that 91 subjects would be required for future studies[52]. Furthermore, the relationship between 25OHD and lung function or aerobic fitness is complex and there might have been confounding factors overlooked in the analysis. BMI and FEV_1% predicted differed between the Edinburgh and Southampton cohorts likely pertain to the noted age difference and cohorts were otherwise similar. Of note, this was accounted for in the statistical analysis. Unfortunately, information on diet, other than vitamin D supplementation, adherence to vitamin D supplementation other than by patient or parent report, smoking, regular physical activity or pulmonary exacerbations was not collected for the purpose of this study. Whilst the present study had a large sample size in comparison to similar studies in groups with CF[23], the sample size of the 25OHD deficient groups was small in both the paediatric ($n = 5$) and adult ($n = 10$) cohorts. $\dot{V}O_{2peak}$ reportedly has a coefficient of variation approximating 9% in children and adolescents with CF[53], whilst our cohort has a standard deviation of 10.3 $mL \cdot Kg^{-1} \cdot min^{-1}$. Therefore, with an α-level set at 0.05 and 80% power, a *post-hoc* power sample size calculation suggests that 35 participants would be required in each group to detect a statistically significant difference in $\dot{V}O_{2peak}$ [52]. Finally, in order to elucidate how 25OHD may influence lung function, aerobic fitness and ventilatory efficiency, future studies should be of prospective nature with a combination of clinical, nutritional and physiological as well as mechanistic outcomes. For instance, 25OHD concentration (and its metabolites) and DBP should be measured in both plasma and alveolar macrophages, obtained from sputum samples, and VDR expression in macrophages.

Conclusion

To conclude, in this preliminary multi-site study, 25OHD deficiency and insufficiency was highly prevalent despite vitamin D doses meeting recommendations. Plasma 25OHD was significantly associated with aerobic fitness and lung function in children with CF from the Edinburgh cohort. Furthermore, medium effect sizes suggest that plasma 25OHD may be associated with ventilatory dysfunction during exercise; however, these findings need to be confirmed by prospective studies with a greater sample size of patients with plasma 25OHD abnormalities in which mechanistic analysis are included.

Box 1. Recommendations for future studies investigating the impact of 25OHD on lung function and aerobic fitness:

- Studies investigating the impact of 25OHD on aerobic fitness (using CPET parameters of aerobic fitness and ventilatory function) and lung function should be of prospective nature and the sampling time between 25OHD and CPET/spirometry within 15 days

- Adherence to vitamin D supplementation, medication, sun exposure, dietary intake, smoking, bone mineral density, body composition (fat mass and fat free mass), pulmonary exacerbations and physical activity levels should be all measured.

- Data should also be stratified by treatment (pre-triple-combination CFTR-modulator therapy vs. traditional therapy) and age groups.

- Control trials and mechanistic studies in which the role of 25OHD on pulmonary health in pwCF that include 25OHD concentration (and its metabolites) and macrophages DBP expression are warranted.

Ethics:

This study was exempted from requiring National Health Service (NHS) ethical approval as data are retrospective and non-identifiable. The study was considered under the category of service evaluation and permission was obtained from NHS Quality Improvement (Royal Hospital for Sick Children, Edinburgh and University Hospital Southampton Foundation NHS Trust)

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Conflicts of Interest

There are no conflict of interest to declare.

Authorship

All authors have made substantial contributions to all of the following: (1) the conception and design of the study (DU, ZS, RRI, AC) or acquisition of data (RRI, AC, DU, IAF, GC, MA, MC) or analysis (RRI, AC) and interpretation of data (DU, ZS, RRI, AC) (2) drafting the article (RRI, AC, DU, ZS) or revising it critically for important intellectual content (all authors), (3) final approval of the version to be submitted (all authors).

Table legends

Table 1. Characteristics of the cystic fibrosis cohort (*n* = 90)

[1]Nutritional status for children was defined as per UK BMI (body mass index) curves (*Z*-scores) and for adults as per WHO (BMI Kg/m^2 reference ranges); underweight (<18.5 Kg/m2), normal weight (≥ 18.5 to ≤ 24.9 Kg/m2), overweight (≥ 25 to ≤29.9 Kg/m2) and obese (≥ 29.9 kg/m2) categories; [2]CFTR (cystic fibrosis transmembrane regulator) genotype class; [3]LS-BMD *Z*-score is a measurement of lumbar spine bone mineral density; [4] 25OHD: 25-hydroxyvitamin D; [5]FEV_1 % predicted: predicted forced expiratory volume in 1 second; [6]VO_{2max} % predicted: maximum oxygen uptake; *Data from the children (<18 years) cohort (*n* = 54); ** Data from the Edinburgh cohort (*n* = 38); ***Data from the Southampton cohort (*n* = 52).

Table 2. Characteristics of the cystic fibrosis cohort (n = 90) with data stratified by geographical location (centre).

[1]Nutritional status for children was defined as per UK BMI (body mass index) curves (*Z*-scores) and for adults as per WHO (BMI Kg/m^2 reference ranges); underweight (<18.5 Kg/m2), normal weight (≥ 18.5 to ≤ 24.9 Kg/m2), overweight (≥ 25 to ≤29.9 Kg/m2) and obese (≥ 29.9 kg/m2) categories ; [2]CFTR (cystic fibrosis transmembrane regulator) genotype class; [3]LS-BMD *Z*-score is a measurement of lumbar spine bone mineral density expressed as standard deviation scores; [4]25OHD; 25-hydroxyvitamin D; [5]FEV_1 % predicted: percentage forced expiratory volume predicted; [6]VO_{2max} % predicted: maximum oxygen uptake; [7]Chi-square test; [8]Mann-Whitney test; [9]Independent-test; *Data from the children (<18 years) cohort (*n* = 54).

Table 3. Participant characteristics with data stratified by plasma 25OHD status and age (<18 or ≥ 18 years)
Data are expressed as means ± standard deviation unless otherwise stated.[1] Kruskal Wallis test performed; [2]*n* = 2 missing. [*]One-way ANOVA, denotes a significantly significant difference with the 25-hydroxyvitamin D [25OHD] sufficient group ($p < 0.05$). CFTR (cystic fibrosis transmembrane regulator) genotype class; BMI, body mass index; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; GET: gas exchange threshold.

Table 4. Hierarchical multiple-regression analysis of the Edinburgh pwCF cohort with FEV_1% predicted and VO_{2peak} as primary outcomes (*n* = 38).

Data are expressed as linear regression (*R* and R^2), the linear regression slope (*B*), standard error (SE), 95% Confidence intervals (95% CI) and *p*-value. FEV_1 %: forced expiratory volume in 1 second; VO_{2peak}; peak aerobic fitness; BMI: body mass index; LP Supine BMD *Z*-scores: lumbar spine bone mineral density *Z*-scores; VO_{2max}: maximum oxygen uptake; 25OHD: 25-hydroxyvitamin-D. All variables were entered one by one and only those with a $p > 0.1$ were entered in the final model.

Figure legends

Figure 1. Plasma 25OHD as per FEV_1 % predicted status in pwCF (left) and with data stratified by age (< 18 years vs. > 18 years) (right).

FEV_1 % predicted status: Normal > 85%; Mild ≥ 70 to 84%; Moderate 50 to 69%; Severe < 50%; *Kruskal-Wallis test

Figure 2. Parameters of aerobic fitness and ventilatory function amongst groups with cystic fibrosis and 25-hydroxyvitamin D sufficiency (>75 nmol/L), insufficiency (50-75 nmol/L) or deficiency (<50 nmol/L). Figure 2a represents the paediatric cohort and figure 2b the adult cohort.

$\dot{V}_E$, pulmonary minute ventilation; $\dot{V}O_2$, pulmonary carbon dioxide production $\dot{V}O_2$, pulmonary oxygen uptake. Ventilatory drive ($\Delta V_E/\Delta VCO_2$ L/min). A series of analysis of variance (ANOVA) and Tukey's post-hoc t-tests were conducted to establish differences between 25OHD status categories (deficiency/insufficiency, sufficiency and optimal) in the following outcome variables; F: F-statistic is this ratio, F = variation between sample means / variation within the samples; *p* values and η^2: effect size.

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666 Table 1. Characteristics of the cystic fibrosis cohort (n=90)

Patient's characteristics		
	N	%
Children <18 years	54	60
Male/Female	48/42	53/47
Nutritional status[1]		
Undernutrition	5	6
Healthy range	72	80
Overweight	10	11
Obese	3	3
Ethnicity		
Caucasian	88	98
Asian	2	2
25OHD status (nmol/L)	86	95
Deficiency/insufficiency <50	17	20
Sufficiency 51 - 75	43	50
Optimal >75 -170	26	30
CFTR genotype class[2]		
Class I-III	76	84
Class IV-V	14	16
Pancreatic insufficiency	71	79
	Median	IQR
Age, y	16.60	13.0 – 25.4
Weight Kg	52.30	39.80 – 62.90
Weight Z-score*	-0.05	(-0.90) - 0.58
Height cm	160.0	150.3 – 171.0
Height Z-score*	-0.10	(-0.92) - 0.50
BMI kg/m^2	19.82	17.96 – 22.91
BMI Z-score*	-0.05	(-0.52) - 0.42
Vitamin D_3 (IU)	900	800 – 1760
LS-BMD Z-score[3]	-0.75	(-1.47) – (-0.10)
	Mean	±SD
25OHD (nmol/L)[4]	64.58	17.74
PTH (pmol/L)**	4.59	2.27
AlkPhosphatase (U/L)**	254.13	74.56
Mg (mmol/L)	0.82	0.06
Ca (mmol/L)	2.38	0.08
Phosphate (mmol/L)	1.36	0.20
HbA1c (mmol/mol)***	40.86	12.64
FEV % predicted[5]	78.17	19.91
VO_2max % predicted[6]	88.81	22.05
Total Fat Mass %*	28.84	6.30

667 [1]Nutritional status for children was defined as per UK BMI (body mass index) curves (*Z*-scores) and for adults as per WHO
668 (BMI Kg/m^2 reference ranges); underweight (<18.5 Kg/m2), normal weight (≥ 18.5 to ≤ 24.9 Kg/m2), overweight (≥ 25 to
669 ≤29.9 Kg/m2) and obese (≥ 29.9 kg/m2) categories; [2]CFTR (cystic fibrosis transmembrane regulator) genotype class; [3]LS-
670 BMD *Z*-score is a measurement of lumbar spine bone mineral density; [4] 25OHD: 25-hydroxyvitamin D; [5]FEV_1 % predicted:
671 predicted forced expiratory volume in 1 second; [6]VO_{2max} % predicted: maximum oxygen uptake; *Data from the children
672 (<18 years) cohort (*n* = 54); ** Data from the Edinburgh cohort (*n* = 38); ***Data from the Southampton cohort (*n* = 52).

673

Table 2. Characteristics of the cystic fibrosis cohort with data stratified by location (n=90)

Patient's characteristics					
	Edinburgh		**Southampton**		
	N	**%**	**N**	**%**	***P* value**
Children <18 years	38	100	16	30.8	-
Gender					0.4[7]
Male	18	47.4	30	57.7	
Female	20	52.6	22	42.3	
Nutritional status[1]					0.6[7]
Undernutrition	1	2.6	4	4.7	
Healthy range	33	6.8	39	75	
Overweight	3	7.9	7	13.5	
Obese	1	2.6	2	3.8	
Ethnicity					-
Caucasian	36	94.7	52	100	
Asian	2	5.5	0	0	
25OHD status (nmol/L)	34	89.5	52	100	0.008[7]
Deficiency/insufficiency <50	2	5.9	13	25	
Sufficiency 51 - 75	24	70.6	20	38.5	
Optimal >75 -170	8	23.5	19	36.5	
CFTR genotype class[2]					0.4[7]
Class I-III	32	84.2	41	78.8	
Class IV-V	6	15.8	10	19.2	
Pancreatic insufficiency	29	76.3	42	80.8	0.8[7]
	Median	**IQR**	**Median**	**IQR**	
Age, y	13.2	12.1 – 14.3	21.9	15.7 – 29.6	<0.01[8]
Weight Kg	39.8	35.2 – 49.0	58.0	48.9 – 65.0	<0.01[8]
Height cm	155.2	146.1 – 165.8	167.0	156.6 – 173.0	<0.01[8]
BMI kg/m^2	18.4	16.8 – 20.2	20.9	18.7 – 23.6	<0.01[8]
Vitamin D_3 (IU)	1200	800 - 1900	900	800 – 1600	0.2[8]
LS-BMD *Z*-score[3]	-0.4	-0.9 – 0.2	-1.1	-1.8 – (-0.25)	<0.01[8]
	Mean	**±SD**	**Mean**	**±SD**	
25OHD (nmol/L)[4]	66.0	17.7	69.3	4.4	0.2[9]
Mg (mmol/L)	0.82	0.06	0.79	0.08	0.1[9]
Ca (mmol/L)	2.36	0.09	2.35	0.09	0.5[9]
Phosphate (mmol/L)	1.36	0.19	1.08	0.23	<0.01[9]
FEV_1 % predicted[5]	91.79	11.31	70.57	20.03	<0.01[9]
VO_2max % predicted[6]	89.51	23.53	88.31	21.13	0.8[9]

[1]Nutritional status for children was defined as per UK BMI (body mass index) curves (*Z*-scores) and for adults as per WHO (BMI Kg/m^2 reference ranges); underweight (<18.5 Kg/m2), normal weight (≥ 18.5 to ≤ 24.9 Kg/m2), overweight (≥ 25 to ≤29.9 Kg/m2) and obese (≥ 29.9 kg/m2) categories ; [2]CFTR (cystic fibrosis transmembrane regulator) genotype class; [3]LS-BMD *Z*-score is a measurement of lumbar spine bone mineral density expressed as standard deviation scores; [4]25OHD; 25-hydroxyvitamin D; [5]FEV_1 % predicted: percentage forced expiratory volume predicted; [6]VO_{2max} % predicted: maximum oxygen uptake; [7]Chi-square test; [8]Mann-Whitney test; [9]Independent-test; *Data from the children (<18 years) cohort (*n* = 54).

Table 3. Participant characteristics with data stratified by plasma 25OHD status and age (<18 or ≥ 18 years)

	Plasma 25-hydroxyvitamin D status				
	>75 nmol/L	**50-75 nmol/L**	**<50 nmol/L**	***p*-value**	***n*[2]**
Paediatrics (<18 years), *n* (%)	17 (34)	28 (56)	5 (10)	-	-
Males, *n* (%)	10 (59)	15 (63)	2 (40)	-	-
CFTR genotype class I-III, *n* (%)	12 (71)	24 (86)	5 (100)	-	-
Pancreatic insufficient, *n* (%)	12 (71)	24 (86)	5 (100)	-	-
Age, y	12.9 ± 1.8	13.7 ± 2.2	13.8 ± 1.3	0.92	<0.01
BMI *Z*-score (median, IQR)[1]	-0.15 (-1.02, 0.60)	0.05 (-0.90,095)	-0.40 (-1.07,0.87)	0.74	-
FEV_1, % predicted	84.1 ± 15.1	91.2 ± 10.9	81.7 ± 24.9	0.23	0.07
FVC, % predicted	87.7 ± 15.4	94.7 ± 10.1	89.4 ± 17.8	0.29	0.06
GET ($mL \cdot Kg^{-1} \cdot min^{-1}$)	22.3 ± 4.6	22.04 ± 4.4	20.7 ± 5.4	0.08	0.1
Plasma 25(OH)D, nmol/L	**101.4 ± 19.4**	**62.3 ± 7.4***	**36.8 ± 14.0***	**<0.01**	**0.75**
Adults (≥18 years), *n* (%)	10 (28)	16 (44)	10 (28)	-	-
Males, *n* (%)	4 (40)	10 (62)	7 (70)	-	-
CFTR genotype class I-III, *n* (%)	9 (90)	16 (94)	10 (100)	-	-
Pancreatic insufficient, *n* (%)[2]	9 (90)	16 (94)	10 (100)	-	-
Age, y	29.8 ±7.8	28.5 ± 8.1	24.3 ± 4.5	0.23	0.08
BMI, kg/m^2	21.5 ± 1.8	23.3 ± 4.0	22.3 ± 3.3	0.41	0.05
FEV_1 % predicted[a]	61.9 ± 12.2	70.1 ± 21.3	63..1 ± 22.6	0.43	0.05
FVC % predicted[a]	76.8 ± 7.8	85.9 ± 14.1	77.5 ± 15.8	0.16	0.11
GET ($mL \cdot Kg^{-1} \cdot min^{-1}$)	19.5 ± 3.9	18.2 ± 4.6	18.6 ± 3.1	0.7	0.02
Plasma 25OHD, nmol/L	**103.3 ± 21.0**	**60.7 ± 5.8***	**29.2 ± 9.1***	**<0.01**	**0.84**

Data are expressed as means ± standard deviation unless otherwise stated.[1] Kruskal Wallis test performed; [2]*n* = 2 missing. *One-way ANOVA, denotes a significantly significant difference with the 25-hydroxyvitamin D [25OHD] sufficient group ($p < 0.05$). CFTR (cystic fibrosis transmembrane regulator) genotype class; BMI, body mass index; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; GET: gas exchange threshold.

Table 4. Hierarchical multiple-regression analysis of the Edinburgh pwCF cohort with FEV_1% predicted and VO_{2peak} as primary outcomes (n = 38).

Variables	***R***	***R^2***	***β***	**SE**	**95% CI**	***P***
			FEV_1% predicted			
25OHD nmol/L	**0.36**	**0.13**	**0.36**	**0.06**	**-0.38 – (-0.03)**	**0.02**
Age (years)	0.42	0.17	0.21	0.06	-0.003 – 0.17	0.18
BMI *Z*-scores	**0.56**	**0.31**	**0.33**	**0.05**	**0.001 – 0.002**	**0.01**
LS-BMD *Z*-scores	0.56	0.31	0.06	0.05	-0.015 – 0.023	0.67
VO_2max % predicted	0.56	0.32	0.05	0.05	-0.001 – 0.001	0.70
			$\dot{V}O_2$peak (mL·kg^{-1}·min^{-1})			
25OHD nmol/L	**0.36**	**0.13**	**-0.36**	**0.06**	**-0.26 – (-0.005)**	**0.05**
Age, y	**0.53**	**0.28**	**-0.39**	**0.46**	**0.11 – 2.01**	**0.03**
BMI *Z*-scores	0.53	0.28	0.04	0.35	-0.65 – 0.82	0.08
LS-BMD *Z*-scores	0.55	0.30	-0.15	1.01	-2.81 – 1.26	0.40
VO_2max % predicted	0.59	0.35	0.23	0.09	-0.07 – 0.28	0.24

Data are expressed as linear regression (R and R^2), the linear regression slope (B), standard error (SE), 95% Confidence intervals (95% CI) and p-value. FEV_1 %: forced expiratory volume in 1 second; VO_{2peak}; peak aerobic fitness; BMI: body mass index; LP Supine BMD *Z*-scores: lumbar spine bone mineral density *Z*-scores; VO_{2max}: maximum oxygen uptake; 25OHD: 25-hydroxyvitamin-D. All variables were entered one by one and only those with a $p > 0.1$ were entered in the final model.

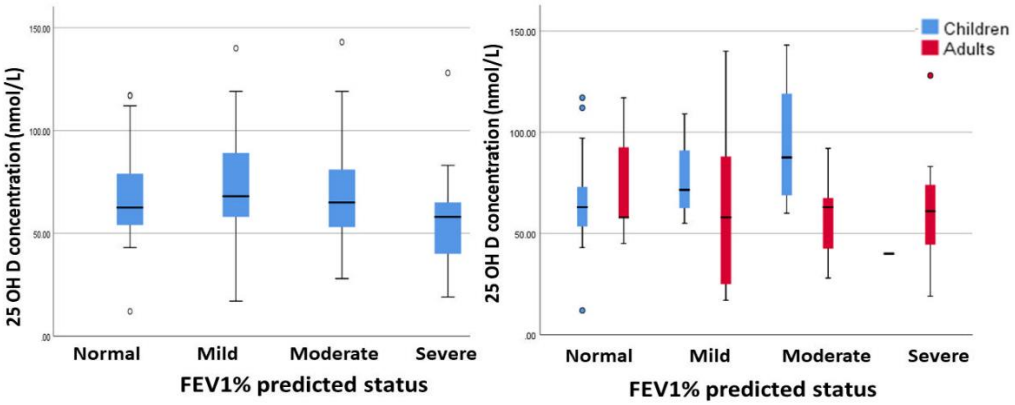

Figure 1. Plasma 25OHD as per FEV_1 % predicted status in pwCF (left) and with data stratified by age (< 18 years vs. > 18 years) (right).

FEV_1 % predicted status: Normal > 85%; Mild ≥ 70 to 84%; Moderate 50 to 69%; Severe < 50%; *Kruskal-Wallis test

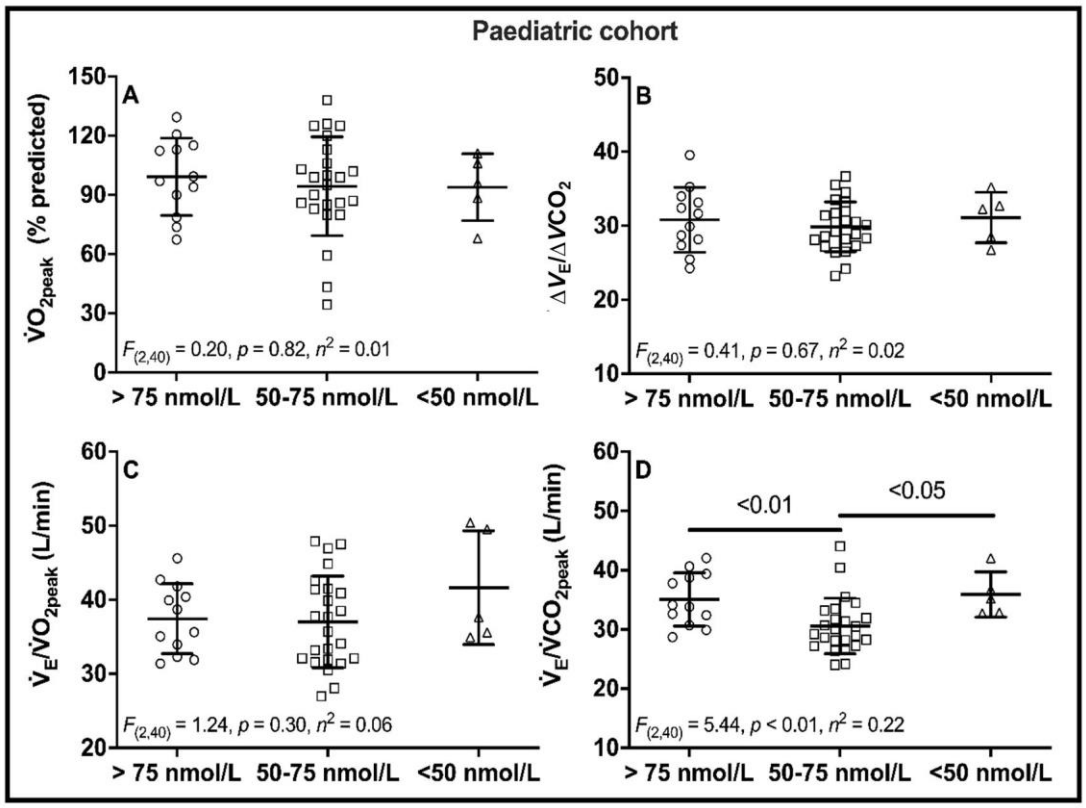

Figure 2a.

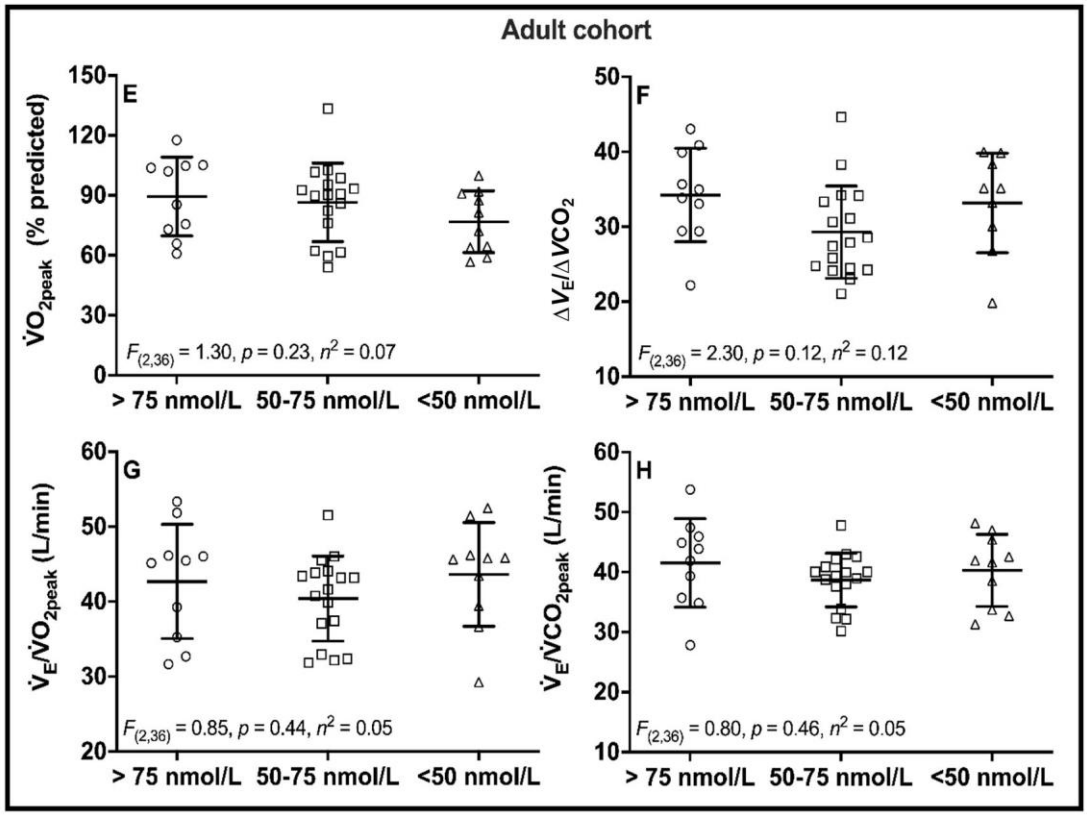

Figure 2b.

Figure 2. Parameters of aerobic fitness and ventilatory function amongst groups with cystic fibrosis and 25-hydroxyvitamin D sufficiency (>75 nmol/L), insufficiency (50-75 nmol/L) or deficiency (<50 nmol/L). Figure 2a represents the paediatric cohort and figure 2b the adult cohort.

$\dot{V}_E$, pulmonary minute ventilation; $\dot{V}O_2$, pulmonary carbon dioxide production $\dot{V}O_2$, pulmonary oxygen uptake. Ventilatory drive ($\Delta V_E/\Delta VCO_2$ L/min). A series of analysis of variance (ANOVA) and Tukey's post-hoc t-tests were conducted to establish differences between 25OHD status categories (deficiency/insufficiency, sufficiency and optimal) in the following outcome variables; F: F-statistic is this ratio, F = variation between sample means / variation within the samples; *p* values and η^2: effect size.