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Meta-analysis

Systematic review and meta-analysis: Associations of vitamin D with pulmonary function in children and young people with cystic fibrosis

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SUMMARY

Background: Increasing evidence suggests that vitamin D is associated with pulmonary health, which may benefit children and young people diagnosed with Cystic Fibrosis (cypCF). Therefore, the aim of this systematic review was to evaluate primary research to establish associations between 25OHD and pulmonary health in cypCF.

Methods: Electronic databases were searched with keywords related to CF, vitamin D, children/young people and pulmonary function. Included studies were cypCF (aged ≤21 years) treated in a paediatric setting. The primary outcome was lung function [forced expiratory volume in 1 s (FEV_1% predicted)] and secondary outcomes were rate of pulmonary exacerbations, 25OHD status and growth. Evidence was appraised for risk of bias using the CASP tool, and quality using the EPHPP tool. A Meta-analysis was performed.

Results: Twenty-one studies were included with mixed quality ratings and heterogeneity of reported outcomes. The Meta-analysis including 5 studies showed a significantly higher FEV_1% predicted in the 25OHD sufficiency compared to the deficiency group [FEV_1% predicted mean difference (95% CI) was 7.71 (1.69–13.74) %; $p = 0.01$]. The mean ± SD FEV_1% predicted for the sufficient (≥75 nmol/L) vs. deficient (<50 nmol/L) group was 94.7 ± 31.9% vs. 86.9 ± 13.2%; $I^2 = 0\%$; $\chi^2 = 0.5$; df = 4). Five studies (5/21) found significantly higher rate of pulmonary exacerbations in those who were 25OHD deficient when compared to the sufficient group and negative associations between 25OHD and FEV% predicted. The effects of vitamin D supplementation dosages on 25OHD status (10/21) varied across studies and no study (12/21) showed associations between 25OHD concentration and growth.

Conclusion: This systematic review suggests that 25OHD concentration is positively associated with lung function and a concentration of >75 nmol/L is associated with reduced frequency of pulmonary exacerbations, which may slow lung function decline in cypCF. Future randomised clinical trials and mechanistic studies are warranted.

1. 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. Higher morbidity and mortality rates are principally due to unresolving and unremitting infections that cause progressive lung disease. Malabsorption as a result of pancreatic insufficiency may impair growth velocity and lead to a reduction in fat soluble vitamins concentration, including vitamin D [2,3]. Supplementation with vitamin D is thus advocated for all people with CF [4]. The role of 25-hydroxyvitamin (25OHD), the main circulating form of vitamin D, on bone health and growth has long been recognised [5].

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Evidence in healthy individuals suggests that 25OHD may also be an important determinant of respiratory health [6] and aerobic fitness assessed using cardiopulmonary exercise testing (CPET) [7–9].

CPET is advocated by both the European CF Society [10] and European Respiratory Society [11] as a functional assessment of lung, cardiovascular and muscular health in patients with CF. Furthermore, markers of aerobic fitness and ventilatory function during exercise have been shown to be significant predictors of mortality in CF [12]. Studies have demonstrated pulmonary [13], cardiovascular [13], metabolic [13] and skeletal muscle [14] abnormalities are factors that modulate exercise capacity in children and young people with CF (cypCF). Therefore, it is essential to evaluate evidence investigating 25OHD as a modulator of exercise capacity and fitness.

The prevalence of 25OHD inadequacy, defined as deficiency and insufficiency [15], has been quantified as being from 23% to as high as 95% in people with CF [16]. This is similar to reports of 25OHD inadequacy in other chronic diseases [17,18], but is a higher rate of 25OHD inadequacy than those reported healthy children, teenagers (19–37%) and adults (29%) [19]. 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 [20]. 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 25OHD inadequacy may exert a role in the pathophysiology of CF [20].

To date, one systematic review of randomised control trials has investigated the effects of vitamin D on 25OHD status and respiratory parameters as secondary outcomes in children and adults with CF [21]. Due to their strict study design inclusion criteria, only two small studies reporting 25OHD status [22,23] and none reporting respiratory outcomes in cypCF were included [21]. Given that most cypCF survive into adulthood, the emerging evidence of the importance of 25OHD on pulmonary health and physical growth during childhood and teenage years, and that more research in this field is of epidemiological nature [24–26], this systematic review will explore the evidence from epidemiological and interventional studies. Therefore, the aims are to:

i) Evaluate if 25OHD concentration is positively associated with lung function and aerobic fitness (CPET) in cypCF
ii) Synthesise the reported evidence on the nature and strength of the associations between 25OHD and pulmonary exacerbations in cypCF
iii) Evaluate if vitamin D supplementation improves 25OHD concentration and/or status
iv) Assess whether vitamin D is positively associated with growth (weight centile/SD, height centile/SD and BMI centile/SD), anthropometry [Mid-upper arm circumference (MUAC) and triceps skinfold thickness (TSF) percentiles/SD] and body composition [fat mass (FM) and fat-free mass (FFM)] in cypCF.

2. Methods

We designed a protocol a priori (https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42019134220). Deviations from the original protocol were made as there was no evidence found following literature searches. There were studies investigating the combined effect of vitamin D and physical activity on lung function and physical function outcomes (six-and 12-min walk tests; shuttle tests; 3-min step test; sit-to-stand test and muscle strength). The process and reporting of this systematic review was performed according to SWiM [27] and following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [28].

2.1. Study outcomes

2.1.1. Primary outcomes

Lung function measured by:

- Forced Expiratory Volume in 1 s (FEV_1) in children/0.5 s in infants
- FEV_1% predicted/$FEV_{0.5}$% predicted
- Comparison of FEV_1 to FEV_1% predicted
- Forced Vital Capacity (FVC)

2.1.2. Secondary outcomes

i. Serum/plasma 25OHD status or 25OHD concentration as (nmol/L) or (ng/ml)
ii Frequency of pulmonary exacerbations
iii Number of lower respiratory tract infections
iv Rate of pulmonary decline measured through Lung Clearance Index (LCI) [29].
v. Lung damage and inflammation as measured through lung imaging (HRCT)
vi. Cardio-respiratory fitness measured using: VO_{2peak}, VO_{2max}, peak work capacity and 6 and 12 min walk tests; shuttle tests; 3-min step test; sit-to-stand test and muscle strength, cardiopulmonary exercise testing (CPET)
vii. Sputum analysis (Macrophage counts and colonisation with *Pseudomonas aeruginosa* or other pathogens)
viii Nutritional status: growth, weight, body mass index (BMI), height, body composition (fat mass, fat free mass, muscle mass)

2.2. Eligibility criteria and search strategy

Eligible studies included those measuring plasma/serum 25OHD concentration in cypCF and treated in a paediatric setting and/or aged less 21 years plus those including one or more of the above outcomes. We excluded studies performed in adults diagnosed with CF or not treated in a paediatric setting and studies where results for adults and children are not presented separately in analysis.

Electronic searches with English and Spanish as language restrictions were performed (no restriction-June 2021) using the Cochrane Library, MEDLINE (via EBSCOhost), CINAHL (via EBSCOhost), Journals@ovid full text; ProQuest; PUBMED and Google scholar to identify systematic reviews, Randomised Controlled Trials (RCT) and non-RCT, observational studies, case control studies and letters to the editor. We also examined the reference list of all relevant articles and narrative reviews. The initial search strategy (https://www.crd.york.ac.uk/PROSPEROFILES/134220_STRATEGY_20190502.pdf) identified the following keywords and subject heading searches (MeSH); “Cystic Fibrosis”, “vitamin D”, “paediatrics” (children, adolescents and young people) and “pulmonary function” (including pulmonary exacerbations, lung damage and lower respiratory tract infections). All searches were repeated prior to the final analysis to ensure all eligible studies were included in the final analysis (Feb 2022).

2.3. Study selection, data extraction and quality assessment

Titles and abstracts from the combined searches were screened by two researchers independently (SC, GO). In those cases of disagreement, an independent reviewer (RRI) made the final decision. Screening for studies was completed by one researcher only (SC). Duplicates were removed. The titles and abstracts of the remaining selected studies were inspected based on the eligibility and exclusion criteria, and separated into 'inclusion', and 'exclusion' categories. The full text of each of the studies was evaluated to ensure the studies in the 'inclusion' category matched the eligibility criteria (SC, GO, RRI). Evidence was critically appraised independently by two researchers (SC, GO) employing a standard methodological tool; the Critical Appraisal Skills Programme (CASP) [30] and the Effective Public Health Practice Project (EPHPP) [31,32]. The EPHPP comprises an assessment of: (i) contextual information including the study objectives, study design and the patient's characteristics; (ii) potential selection bias including inclusion and exclusion criteria, clear patient selection and an assessment of validity, reliability and accuracy of techniques used; (iii) outcome measures including reference values; (iv) statistical analyses employed and (v) reporting of results and control for confounding factors. We then applied quality ratings of "strong", "moderate" or "weak" to each study where a quality rating of "strong" meant that the given study met all areas of the EPHPP criteria [31], "moderate" when there was one weak area and "weak" when $\geq$ two weak areas were identified (SC, GO). In case of disagreement a third independent reviewer (RRI) made the final assessment.

For those studies that did not report the correlation coefficient for associations between 25OHD concentration and pulmonary function markers, we contacted the corresponding author first on two occasions, giving two weeks in between emails to allow for some time to reply. If that failed, we then repeated the process by contacting the last or most senior author and then the second author. If no response was received after these three attempts, the article was included; however, the data was not analysed as this would have been unavailable.

FEV_1% predicted was defined as normal ($\geq$85%), mild (70%–84%), moderate (50%–69%) and severe (<50%) CF lung disease [33]. 25OHD status was defined as deficiency (<50 nmol/L), insufficiency (50 < 75 nmol/L) and sufficiency ($\geq$75 nmol/L) [15]. Median and ranges were calculated from the correlation coefficients and from mean or medians of the eligible studies to summarise all data. Confounding variables, particularly pancreatic function, known to impact pulmonary function and nutritional status [34] were extracted from each study.

2.4. Meta-analysis

A meta-analysis investigating the impact of 25OHD status (deficient vs. sufficient) on pulmonary function (FEV_1% predicted) was performed. Mean and standard deviation (SD) data for FEV_1% predicted were extracted and if medians and ranges were provided in the included studies, these were converted to sample means and SD [35]. 25OHD expressed in ng/mL were converted to nmol/L by multiplying by $\times$2.5 [36]. These were combined in a meta-analysis using the statistical software RevMan5® to synthesise an overall effect size (ES) and 95% confidence interval, and the associated Forrest plot.

A certainty assessment (GRADE) was used to estimate the "importance" and "certainty" of the meta-analysis (SC, RRI). In cases of disagreement, RRI made the final decision [37]. Certainty was defined as the confidence that the true effect is within a particular range and was based on 8 domains. Domains that decrease the certainty of the evidence were scored as -1 each; (i) risk of bias; (ii) inconsistency defined as the percentage of the study heterogeneity (I^2) attributable to variability in the true treatment effect and classified as low <40%, moderate 30–60%, substantial 50–90% or significant 75–100% with $p < 0.05$; (iii) indirectness, (iv) imprecision and (v) publication bias. Domains that increased the certainty of the evidence were classified as $+1$; (i) dose response gradient, (ii) large effect size and (iii) effect of plausible residual confounding.

3. Results

3.1. Study selection and characteristics

The flowchart for the selection and inclusion of articles is shown in Fig. 1 (PRISMA). Twenty-one studies met the eligibility criteria. Of these, 14 (66.6%) were retrospective cohort studies [24,38–47], 2 (9.5%) were randomised control trials [23,48], 2 (9.5%) were cross-sectional studies [49] and 1 (4.7%) was a series of audits (retrospective study) [50].

The majority of the studies investigated pulmonary function of cypCF as either primary or secondary outcome. All studies had a population that was specific to this review (children and young people with CF). A total of 3171 participants aged between 0 and 20 years old were included across the 21 studies, with individual sample sizes ranging from 15 to 597. Geographical location of the studies were: North America 10 (47.6%), Europe 7 (33.3%), South America 2 (9.5%), Asia 1 (4.7%) and Australia 1 (4.7%). Twenty studies (95.2)% were written in English and one (4.7%) in Spanish [39]. The methods used to analyse 25OHD concentration were reported in 16 (76.2%) studies; however, only 3 (14.2%) studies reported intra assays coefficient of variation, which ranged between 8.7 and <10% (Tables 1–4) (see Fig. 1).

3.2. Risk of bias

The risk of bias using the CASP tool of each study is summarised in Fig. 2. Six (28.6%) studies were assessed as 'high' risk of bias, 12 (57.1%) 'moderate' risk of bias, and 3 (14.3%) 'low' risk of bias. The majority of 'high' ratings were due to studies failing to present confidence intervals and other inferential statistics given for the "results" section (n = 10; 47.6%) and no consideration to confounding factors through stratification or regression analysis in the 'worth continuing' section (n = 7; 33.3%).

3.3. Quality of evidence

Through using the EPHPP methods, the evidence was highly variable with 6 (28.6%) studies being classified as 'strong', 5 studies (23.8%) being classified as 'moderate', and 10 (47.6%) studies being classified as 'weak'. The main issues identified were:

I Lack of blinding for both the assessor and the participants (n = 2 out 2)
II Results missing or inappropriately reported (n = 12)
III The process of accounting for confounding variables through regression analysis or stratification (n = 12)
IV Accounting for withdrawals and dropouts (n = 6)
V Failure to report time taken between blood 25OHD measurements and outcomes measured (n = 18)

3.4. Primary outcome; associations between 25OHD concentration and lung function

Fifteen studies (71.4%) investigated the relationship between 25OHD concentration and pulmonary function (Table 1) using

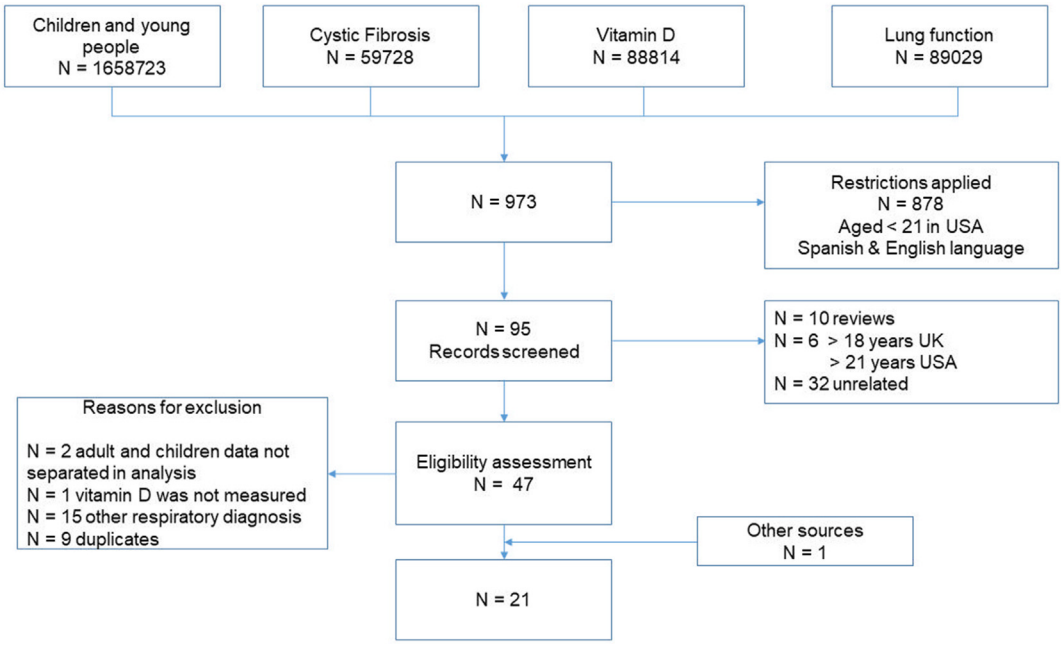

Fig. 1. Flow diagram of the studies searched and included in this systematic review.

FEV_1% predicted (n = 15; 100%), FCV% predicted (n = 8; 53.3%) and FEV_1%/FVC % (n = 1; 4.7%) as either primary or secondary outcome. Of these, 7 studies (46.7%) reported the methods used to assess pulmonary function [24,26,38,42–44,51]. Five out of 15 (33.3%) studies investigated associations of 25OHD status (sufficiency vs. deficiency) and FEV_1% predicted in cypCF and a Meta-analysis was performed based on this data. The remaining 10 studies (66.7%) were excluded due to being too heterogeneous in either the study design, outcome measures, methods used and/or statistical analysis. Therefore, these results are presented narratively.

The Forrest plot depicting the studies included in the Meta-analysis is shown in Fig. 3. All the studies showed a significant effect of higher FEV_1% predicted in the 25OHD sufficiency compared to the deficiency group [FEV_1% predicted mean difference (95% CI) was 7.71 (1.69–13.74) %; $Z = 2.51$; $p = 0.01$]. The mean ± SD FEV_1% predicted for the sufficient vs. deficient group was 94.7 ± 31.9% vs. 86.9 ± 13.2% respectively. There was no evidence of heterogeneity ($I^2 = 0\%$; $\chi^2 = 0.5$; df = 4); however, there was variation in the 95% CI of all studies included in the meta-analysis and a "low certainty" and "not important" attainment was obtained (Fig. 4), which is attributed to the low number of studies included in the analysis and the serious risk of bias, inconsistency and imprecision obtained from one of the studies (Fig. 2).

Eleven out of 15 studies (Table 1) [22,26,38,40,41,44,47,49,51,52], not included in the Meta-analysis, explored the relationship between 25OHD and FEV_1% predicted. Of these, 6 (54.6%) found a positive significant correlation between these two variables and 5 out of these 6 (83.3%) accounted for pancreatic function in their statistical analysis. Five out of 11 (45.4%) did not find significant correlations (correlation coefficient (*r*): Median 0.085; ranged (0.0004–0.62). Unfortunately, 4 out of these 11 (36.4%) studies did not report the correlation coefficients and/or statistical significance of the associations and one study did not account for pancreatic function in their analysis. Of the studies that investigated 25OHD and FVC % predicted, 6 (40.0%) explored the relationship between 25OHD concentration and FVC % predicted and 3 (20.0%) compared the differences between FVC % predicted with data stratified by 25OHD status [deficiency (median (range); 89.4 (75–89.4) %; insufficiency 102.6 (94.7–110.6) %; sufficiency 95.9 (87.7–90.9) %]. Three studies (50%) found a positive relationship between 25OHD concentration and FVC %, whilst the remaining 3 did not find significant correlations (correlation coefficient (*r*): Median 0.03; ranged (−0.01 to 0.63). Of note, the latter 3 studies did not report the data. No differences in FVC % predicted were found between the 25OHD groups in either of the two studies (100%). Finally, no statistically significant differences were found between FEV_1%/FCV % ratio with the data stratified as deficient vs. insufficient vs. sufficient 25OHD status in 8, 12 and 16 year olds (1 study; 100%) [45].

Two out of 15 studies [22,48] investigated the effects of vitamin D2 and D3 supplementation on pulmonary function assessed using FEV_1% predicted and FCV % using 5000–7000 IU/day or 35,000–50,000 IU/weekly. Simoneau et al. (2016) [48] showed no statistical significant improvements in FEV_1% with either vitamin D2 or vitamin D3 and Pincikova et al. (2017) [22] showed statistically significant improvements in FCV % following vitamin D only (Table 1).

3.5. Associations between 25OHD concentration and pulmonary exacerbations

Table 2 shows that 5 out of 21 (23.8%) studies explored the associations between 25OHD and pulmonary exacerbations as primary or secondary outcome. These focused on comparison of pulmonary exacerbation rates in different 25OHD status groups (n = 5; 100%) [24,39,42,46,53], the associations between 25OHD concentration and number of pulmonary exacerbations (n = 4; 80%) [39,42,46,53] and comparison of type of bacterial colonisation (*pseudomonas aeruginosa, methicillin-sensitive staphylococcus aureus and methicillin-resistant staphylococcus aureus*) between different 25OHD status groups (n = 1; 20%) [46]. All studies found a statistically significantly higher rate of pulmonary exacerbations in those who were 25OHD deficient when compared to the insufficient or sufficient group. Likewise, the insufficient group experienced significantly higher rate of pulmonary exacerbations than the sufficient group. Furthermore, all studies showed that rates of

Table 1
Results and characteristics of all of the studies reporting associations of 25OHD concentration with pulmonary function (FEV_1%, FVC%).

Author	Quality	Risk of Bias	Research Design	Subjects	Method	Outcome of interest	Results
(Chavasse, Francis et al., 2004) England	Weak	High	Retrospective chart review August 1999–April 2001	320 children (median age 9 years) (range 0.9 –18.5 years) 50% female. PI n = 277 (86.5%)	Patients with confirmed CF were measured for 25OHD concentration against healthy British children (Gregory et al., 2000) Accounted for pancreatic sufficiency (PS) and insufficiency (PI) 25OHD concentration measured annually. M: in-house, competitive protein-binding assay following extraction and chromatography of 25OHD on silicic acid (Charing Cross Hospital); CV% N/R Vitamin D supplementation 800–1200 IU/day given to all patients (NHS)	Spirometry was used to measure: FEV_1% predicted FVC % predicted	Median (range) 25OHD concentration 65 (9–190) nmol/L No statistical significant differences in 25OHD concentration between PS (n = 13, median (range) 60 (25 –135) nmol/L) and PS (n = 26, median (range) 72 (9–162) nmol/L), $p > 0.05$ No correlation between 25OHD concentration and pulmonary function (FEV1% predicted FVC % predicted); r = N/R; p = N/R
(Green, Carson et al., 2008) USA	Weak	High	Retrospective cohort study January 2003 to December 2006	262 children aged 4 months to 20 years. PI n = 241 (92%)	25OHD deficiency defined as <30 ng/mL (<75 mmol/L) 3 Protocols used for vitamin D supplementation: Protocol 1 = 50,000IU of ergocalciferol for 8 weeks (as per 2002 CF Foundation statement). Protocol 2 = 50000IU of ergocalciferol twice a week for 8 weeks if protocol 1 unsuccessful (if patients remained deficient) and standard protocol from March 2004 to October 2004. Protocol 3 = 50000IU of ergocalciferol three times a week for 8 weeks Follow ups were completed 2–4 weeks after treatment completion. Standard protocol from October 2004–June 2006 M: 25OHD assays: the Nichols Advantage 25OHD assays; the DiaSorin 25OHD radioimmunoassay kits; liquid chromatography-tandem mass spectroscopy. CV % N/R	Pulmonary function expressed as FEV_1% predicted Confounding variables (BMI, age, sex, PI, and season) M: N/R	Baseline FEV_1% predicted (n 194) 91.8 (20.2–144.8) increased by 10% following supplementation. Higher FEV_1% was associated with higher 25OHD concentration. For each 10% increase in FEV_1% predicted, the 25OHD concentration increased 1.0 ng/mL (r = 0.21, *p = 004;* 95% CI=N/R)
(Green, Leonard et al., 2010) USA	Weak	Moderate	Retrospective chart review Januray 2006 –December 2008	97 paediatric CF patients <21 years old Mean (±SD) 10.9 ± 5.2 PI n = 88 (90.7%)	25OHD deficiency <30 ng/mL M: 25OHD concentration performed by Quest Diagnostics (Chantilly, VA), liquid chromatography–tandem mass spectroscopy; CV % N/R FEV_1% predicted NR Treatment of deficiencies using 50,000 IU D2/ day for 28 days and only cypCF with PI Successful 25OHD concentration >30 ng/mL	FEV_1% predicted Comparison between groups: successfully treated (n = 52) vs. non-successfully treated (n = 45) (post-treatment)	FEV_1% predicted successfully treated (85.0 ± 24.4) vs. non-successfully treated (74.3 ± 25.9); $p = 0.07$ (baseline comparison)
(Grey, Atkinson et al., 2008) Canada	Weak	High	Cross-sectional observational study	81 children with CF Mean ± SD age 12.6 ± 2.9 years PI 100%	Serum 25OHD levels Deficiency defined as <75 nmol/L (Aris et al., 2005) M: Nichols Advantage & the Diasorin RIA CV% N/R	FEV_1% predicted M: N/R FEV1% predicted severity of disease	59/78 patients had mild lung disease (FEV_1 <70%), 16/78 had moderate (FEV_1 40–70%). 3/78 had severe (FEV_1<40%). No correlation was performed/ presented between lung function and 25OHD concentration
(Henderson and Lester 1997) USA	Weak	High	Cohort- Cross-sectional observatory study	54 children Range (4.9 –19.5) years Mean (11.0) years	25OHD and 1,25OHD assessed M: radioimmunoassay and radioreceptor assay kits (Incstar, Stillwater, Minn)	FEV_1% predicted M: Polgar & Promadhat (1971) Brasfield scoring of the chest	No significant correlation between 25OHD concentration and FEV_1% predicted and chest

(*continued on next page*)

Table 1 (*continued*)

Author	Quality	Risk of Bias	Research Design	Subjects	Method	Outcome of interest	Results
				Control cerebral palsy (n = 125) and survivors of childhood cancer (n = 46)	CV% N/R 25OHD deficiency defined as <10 ng/mL and normal as >18 ng/mL	radiographs (1979) Both measured within 3 months of 25OHD and 1,25-diOHD concentration No information on pancreatic function	radiography r = N/R; $p > 0.05$
(Loukou, Moustaki et al., 2020) Greece	Strong	Low	Retrospective longitudinal study 2012–2016	236 children aged 6–20 years old PI n = 198 (83.9%) Mean ± SD 11.3 ± 4.4	25OHD concentration M: direct competitive chemiluminescence immunoassay (CLIA); CV% N/R 25OHD status definition deficient <20 μg/L; insufficient 20–29 μg/L; sufficient ≥30 μg/L	Relationship between 25OHD and lung function Mean, best and D FEV_1% predicted Mean, best and D FVC % predicted Mean, best and D-FEF 25–75% M: Spirometry *Best: best value of the year; Mean: mean value of the year; value of the year D: value recorded concurrently with 25OHD* Variables controlled for (pancreatic function status, liver involvement, CFRD, BMI Z-score, isolation of Pseudomonas and Staphylococcus in cultures, and treatment with CFTR modulators)	D-FEV_1%; $R^2 = 0.25$; 95% CI (0.06–0.44); $p = 0.01$ Best- FEV_1%; $R^2 = 0.17$; 95% CI (0.01–0.33); $p = 0.034$ Mean- FEV_1%; $R^2 = 0.13$; 95% CI (−0.02 to 0.29); $p = 0.10$ D-FVC%; $R^2 = 0.18$; 95%CI (0.03 −0.33); $p = 0.018$ Best-FVC%; $R^2 = 0.10$; 95% CI (−0.03–0.24); $p = 0.10$ Mean-FVC%; $R^2 = 0.10$; 95% CI (−0.02–0.24); $p = 0$ 0.10 D- FEF25-75%; $R^2 = 0.28$; 95% CI (−0.04 to 0.60); $p = 0.09$ Best-FEF25-75%; $R^2 = 0.28$; 95% CI (−0.01 to 0.58); $p = 0.07$ Mean- FEF25-75%; $R^2 = 0.23$; 95% CI (−0.05 to 0.52); $p = 0.10$ PI, liver involvement and *pseudomonas* were statistically significantly associated (negatively) with lung function (D-FEV_1%, Mean FEV_1% & D-FEF25-75%, Best-FEF25-75%) and BMI *Z*-score was statistically significantly (positively) associated with all outcome measures
(Revuelta-Iniesta, Causer et al., 2021) UK	Strong	Low	Multi-centre retrospective study July 2017 to October 2019	90 patients with CF included both adults and children >9 years of age. 54 children included Median (IQR) 16.60 (913.0–25.4) years PI n = 41 (79.9%)	Plasma 25OHD concentration M: Liquid chromatography-tandem mass spectrometry technique; CV% 8.9 25OHD status definition: deficient <50 nmol/L; insufficient ≥50 ≤ 75 nmol/L and sufficient >75 nmol/L (Endocrine Society, Holick et al., 2011)	FEV_1% predicted M: Spirometry to ATS/ERS standards (Spirometry as per (3500 MicroLab Spirometer MK8; MicroMedical) FVC% predicted comparison between 25OHD concentration defined as deficient <50 nmol/L; insufficiency 50 < 75 & sufficiency ≥75 nmol/L M: FVC was determined as the highest of the three consistent (≤5% variability) manoeuvres following British Thoracic Society Guidelines for the measurement of respiratory function 1994; ATS, ERS (Miller et al., 2005) Confounding variables (liver function, pancreatic function, CFTR genotype class, ethnicity, age, LS-BMD *Z* scores, BMI *Z* score, VO_{2max} % predicted)	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)] FVC% predicted: deficiency (89.4 ± 17.8); insufficiency (94.7 ± 10.1) & sufficiency (87.7 ± 15.4); $p = 0.29$; $\eta = 0.06$

(McCauley, Thomas et al., 2013) USA	Strong	Moderate	Retrospective Longitudinal study 2000–2012	130 children with CF aged 6–18 years Excluded PS cypCF	25OHD concentration 25OHD status defined as deficient <20 μg/L; insufficient 20–29 μg/L; sufficient ≥30 μg/L (Cystic Fibrosis Foundation recommendations) M: Liquid chromatography/tandem mass Spectrometry; CV % NR	FEV_1% predicted FVC predicted FEV_1%/FVC % M: Spirometry American Thoracic Society Guidelines, ATS, ERS (Miller et al., 2005)	In 16-year-olds, a 10 μg/L 25OHD was associated with a FEV_1 5.5% increase ($p < 0.04$, r = N/R, 95% CI = 0.5–10.5). FEV1% predicted 8 years old: deficient (104); insufficient (91 ± 27); sufficient (107 ± 18); $p = 0.410$ 12 years old: deficient (88 ± 17); insufficient (89 ± 19); sufficient (95 ± 15); $p = 0.418$ 16 years old: deficient (87 ± 15); insufficient (88 ± 18); sufficient (100 ± 18); $p = 0.072$ FVC% predicted 8 years old: deficient (96); insufficient (110 ± 6); sufficient (109 ± 14); $p = 0.647$ 12 years old: deficient (94 ± 13); insufficient (95 ± 17); sufficient (98 ± 14); $p = 0.750$ 16 years old: deficient (98 ± 13); insufficient (96 ± 20); sufficient (106 ± 15); $p = 0.145$ FEV_1%/FVC % 8 years old: deficient (0.90); insufficient (0.74 ± 0.26); sufficient (0.85 ± 0.10); $p = 0.3$ 12 years old: deficient (0.82 ± 0.13); insufficient (0.83 ± 0.07); sufficient (0.86 ± 0.07); $p = 0.397$ 16 years old: deficient (0.77 ± 0.09); insufficient (0.80 ± 0.07); sufficient (0.82 ± 0.08); $p = 0.442$
(Norton, Page et al., 2015) Canada	Strong	Moderate	Cohort- Retrospective chart review 2010–2011	96 children with CF age 1–18 years (mean age = 9 years) 2010 PI n = 74 (90.2%) 2011 PI n = 80 (91.9%)	Vitamin D supplementation 25OHD concentration defined as deficient <20 μg/L; insufficient 20–29 μg/L; sufficient ≥30 μg/L (Cystic Fibrosis Foundation recommendations) M: N/R	FEV_1% predicted Confounding variables (residence ≤52nd C° > 52nd C°, pancreatic enzyme, steroids, CFRD, CF-related hospital admissions >1 day & no days)	2010 FEV_1% predicted (Mean ± SD); (100 ± 39); $p > 0.05$; r = N/R 2011 FEV_1% predicted (97 ± 26); $p = 0.029$; positively associated with 25OHD concentration r = N/R
(Ongaratto, Rosa et al., 2018) Brazil	Weak	Moderate	Retrospective study July 2013–March 2015	37 children and adolescents with CF. Mean ± SD age 11 ± 5.58 years. Range 1–20 years PI n = 35 (94.6%)	25OHD concentration 25OHD status defined as per Cystic Fibrosis Foundation and Endocrine Society: deficiency <20 ng/mL; insufficiency 20–29.9 ng/mL & sufficiency ≥30 ng/mL M: N/R Patients were stratified into two groups; sufficiency vs. hypovitaminosis (deficient and insufficient) All subjects received routine oral CF-specific vitamin supplementation as per Cystic Fibrosis Foundation (birth to 12 months: 400–500 IU/day D3; >12 months to 10 years: 800 to 1000 IU/day D3; >10 years: 800 to 2000 IU/day D3)	Comparison of lung function between 25OHD hypovitaminosis (deficiency & insufficiency) vs. sufficiency FEV_1% predicted FVC M: Spirometry was performed at the routine follow-up outpatient clinic in subjects aged over 5 years Confounding variables NR	FEV_1% (Mean ± SD): Hypovitaminosis (75.33 ± 27.13) Sufficiency (85.67 ± 31.53); $p = 0.354$; r = N/R 95% CI = N/R) FVC% (Mean ± SD): Hypovitaminosis (75 ± 6.16) Sufficiency (90.94 ± 11.2); $p = = 0.717$; r = N/R, 95% CI=N/R)
(Pincikova, Paquin-Proulx et al.,	Moderate	Moderate		16 children with CF PI n = 14 (87.5%)	3 groups: vitamin D2 supplementation, vitamin D3 supplementation or control.	FEV_1% predicted FVC % predicted	Positive moderate correlation between 25OHD concentration

(*continued on next page*)

Table 1 (*continued*)

Author	Quality	Risk of Bias	Research Design	Subjects	Method	Outcome of interest	Results
2017) Sweden			Randomised pilot control trial April 2010 May 2011		Dose for 3 months and 2 months wash out <16 years old 35,000 IU/week (5000 IU/day) ≥16 years old 50,000 IU/week (7143 IU/day). M: N/R	M: N/R Confounding variables NR for analysis	and FEV_1% predicted at 1 month of wash out ($p = 0.042$; $r = 0.62$) Positive moderate correlation between 25OHD concentration and FVC % predicted at 1 month of wash out ($p = 0.036$; $r = 0.63$) FVC% predicted (mean ± SD) Control group: baseline (89.3 ± 15.0); 3 months (89.7 ± 6.1); 5 months (92.8 ± 7.3) Vitamin D2: baseline (87.8 ± 24.9); 3 months (87.3 ± 16.4); 5 months; (84.0 ± 27.9) Vitamin D 3: baseline (83.2 ± 23.8); 3 months (99.5 ± 13.5); 5 months (87.0 ± 25.1) $p < 0.05$ (comparison with baseline)
(Sexauer, Hadeh et al., 2015) USA	Moderate	Moderate	Retrospective study	N = 597 Paediatric (<18 years) n = 271 PI n = 231 (85.2%) Mean ± SD 12.4 ± 3.3	25OHD concentration (ng/ml) M: One centre and Quest performed 25OHD assays via liquid chromatography tandem mass spectrometry (LC–MS/MS) technology. Labcorp performed assays using the DiaSorin 25OHD radioimmunoassay kit.	FVC % predicted FEV_1% predicted M: Wang et al., 1992 and Hankinson et al., 1999) Confounding variables (season, sex, pancreatic function, age, age at diagnosis, genotype, vitamin D supplementation, BMI, pathogens, CFRD, ABPA, bone disease, steroids, history of meconium ileus	Correlation between 25OHD concentration and FEV_1% predicted ($r = 0.11$; $p = 0.076$) Correlation between 25OHD and FVC% predicted ($r = -0.01$; $p = 0.89$)
(Simoneau, Sawicki et al., 2016) USA	Moderate	Moderate	Non-blinded randomised control trial April 2012–June 2013	50 children age 6–21 years with CF PI 100%	Compare vitamin D2 dose of 50,000 IU twice weekly for 8 weeks vs. vitamin D3 50,000 IU weekly M: Liquid chromatograph tandem mass spectrometry (AB Sciex, FosterCity, CA) with external quality control through DEQAS assessment. CV % N/R	To achieve 25OHD concentration >30 ng/mL Secondary outcomes FEV_1% predicted M: N/R	FEV_1% predicted changes ($p = 0.56$): Vitamin D2 (mean ± SD) baseline (86.2 ± 28.4) follow up (86.9 ± 27.2); $p = 0.76$; 95% CI N/R Vitamin D3 (mean ± SD) baseline (83.0 ± 24.9) follow up (85.5 ± 26.1); $p = 0.26$; 95% CI N/R
(Timmers, Stellato et al., 2019) The Netherlands	Strong	Low	Retrospective study January 2012 June 2016	190 CF patients above the age of 6 PI 100%	Vitamin D supplementation 10–50 μg (400 –2000 IU) for all ages (Sinaasappel et al., 2002) 25OHD status deficient (<50 nmol/L); sufficient (≥50 ≤ 75 nmol/L) & high sufficient (>75 nmol/L) as per European Union guidelines. Serum 25OHD concentration M: Electrochemiluminescence sandwich immunoassay, CV% 8.7%	FEV_1% predicted FVC % predicted Reference: Global Lung Function Initiative reference values (Quanjer et al., 2012) M: N/R	Linear mixed effect regression including age, sex, BMI *Z*-score, IgG, CFLD, CFRD, corticosteroid use and season FEV_1% predicted $r^2 = 0.06$; 95% CI (0.01–0.10); $p = 0{\cdot}018$ FVC % predicted $r^2 = 0{\cdot}05$; 95% CI (0.01–0.80); $p = 0{\cdot}017$ Relationship between 25OHD and pulmonary function (FEV_1% and FVC % predicted). Each 20 nmol/L increase of serum 25OHD increased FEV_1% by 1.12% (95% CI 0.2–2.04) and

							FVC% by 0.9% (95% CI 0.16 −1.64)
(Wani, Nazir et al., 2019) India	Weak	High	Retrospective cohort January–December 2016	62 children with CF < 15years Median (IQR) 11.4 (6.01 −14.47) PI n = 53 (85.4%) PS n = 9 (14.6%)	Vitamin D supplementation 400–800 IU/day. 25OHD status defined as deficiency (<12 ng/mL); insufficiency (≥12 < 20 ng/mL) and sufficiency in D sufficient (≥20 ng/mL) as per Global Consensus recommendations (2016) 25OHD concentration M: Liquid chromatography; CV % N/R	Comparison of lung function (FEV_1% predicted) between 25OHD status M: N/R Confounding variables NR	FEV_1% predicted 25OHD deficient [(0.75 (0.717 −0.777%)] vs. sufficient [(0.82 (0.74–0.92%)]; $p < 0.05$ FEV_1% predicted 25OHD insufficient [(0.79 (0.77 −0.79%)] vs. sufficient [0.82 (0.74–0.92)]; $p > 0.05$].

ABPA: allergic bronchopulmonary aspergillosis; BMI: body mass index; BMI Z score: BMI standard deviation; CF: Cystic fibrosis; CFLD: Cystic fibrosis related liver disease; CFRD: Cystic fibrosis related diabetes; CI: confidence interval; CV%: percentage coefficient of variation; FEV_1% predicted: forced expiratory volume in 1 s expressed as percentage; FVC: forced vital capacity; IQR: interquartile range; M: Method; N/R = Not reported in the study; SD: standard deviation; 1,25OHD; 1,25 dihydroxyvitamin D; 25OHD: 25-hydroxyvitamin D; *- included both adult and children, but only paediatric data was used for this review; SD: standard deviation.

pulmonary exacerbations negatively correlated with 25OHD concentration, all studies used vitamin D supplementation with a range of dosages (400–2000 IU/day) and 25OHD concentration was not a basal level in any study. Due to the heterogeneity of the variables and data of these studies, we were unable to calculate descriptive statistics to summarise the results.

3.6. Effects/impact of vitamin D supplementation on 25OHD status in cypCF

Table 3 summarises 9 out 21 (42.8%) studies reporting 25OHD concentration following vitamin D supplementation. Of these, 1 (11.1%) [51] described 25OHD concentration at one time point and 8 studies (88.9%) investigated the impact of vitamin D supplementation on 25OHD concentration using either observational methodology (n = 7; 77.8%) [39–41,45,50,52,54] or randomised control trials (n = 2; 22.2%) [22,23]. Fifty % (n = 4) studies demonstrated a statistically significant improvement in 25OHD concentration following vitamin D supplementation (Fig. 5). The dosages ranged from 1400 IU/day oral intake to 100,000–600,000 IU administered intramuscularly in a single dose ("*stoss therapy*"); however, most study protocols used 50,000 IU either administered orally once, twice or three times per week. The highest increase in 25OHD concentration resulted from "*stoss therapy*" one month post-supplementation, which increased from 49.6 ± 12.9 to 94.8 ± 41.0 nmol/L and remained above insufficiency (>50 nmol/L) [15] for 12 months (64.6 ± 20.0 nmol/L) [55]. In contrast, 50% studies found a non-statistically significant increase in 25OHD concentration with vitamin D supplementation administered orally and using dosages of 2000 IU–7153 IU/day. A study [41] reported a downtrend in those who were only exposed to sunlight (Table 3; Fig. 5). No data was reported on the following: length of time spent outdoors, time of the day, location, mode of UVB exposure, use of sunscreen and vitamin D intake from food sources in any of the studies and only 5 out of 9 (55.6%) studies controlled for pancreatic sufficiency and none controlled for compliance.

3.7. Relationship between 25OHD concentration and secondary outcomes (markers of growth and aerobic fitness) in cypCF

Twelve out of 21 studies (57.1%) explored either the relationship between 25OHD concentration and measurements of growth (n = 7; 75.0%) [38,40,46,47,51,52,54] or compared growth measurements with data stratified by 25OHD status (n = 5; 41.7%) [41–43,46,53] (Table 4). Of these, 9 (75.0%) used BMI *Z*-score as a primary or secondary outcome, 4 (33.3%) used height (3 converted it into height *Z*-score and 1 used height in cm), 4 (33.3%) used weight (3 converted it into weight *Z*-score and 1 used weight in Kg) and 1 (8.3%) used skinfold thickness.

None of the 12 studies reported a statistically significant association between BMI *Z*-score (n = 5; 100%), height Z-score (n = 3; 100%), skinfold thickness (n = 1; 100%) and 25OHD concentration in a time frame between 0 and 3 years; however, only 2 studies reported correlation coefficients [40,53] and the methods used to measure growth [54]. Likewise, no study found statistical significant differences in BMI Z-score (n = 6; 100%) and height *Z*-score (n = 1; 100%) between deficient, insufficient and sufficient 25OHD groups. Finally, three out of 4 studies (75.0%) reported no statistically significant relationship between weight *Z*-score and 25OHD concentration; whilst one study performed over 4 years (25%) found a negative association between these two variables ($r = -0.79$; $p = 0.002$).

Eleven out of 12 studies (91.7%) looking at 25OHD and growth parameters reported pancreatic function status. Of these, 3 out of 12 studies (25.0%) accounted for pancreatic function in their

Table 2
Studies reporting associations between 25OHD concentration and number of pulmonary exacerbations and/or infections in paediatric cystic fibrosis patients.

Author	Quality	Risk of Bias	Research Design	Subjects	Method	Outcome	Results
(González Jiménez, Muñoz Codoceo et al., 2015) Spain	Moderate	Moderate	Multicentre retrospective study Nov 2012–April 2014	377 children with CF aged <21 years Median (IQR) 8.9 (N/R)	25OHD concentration 25OHD status defined <30 ng/mL insufficient (Cystic Fibrosis Foundation recommendations 2012) M: N/R Chronic pulmonary exacerbation identified by bacterial colonisation from sputum. Chronic infection defined as >2 positive colonisations in <2 months and from the same bacteria Vitamin D supplementation dosage Median (IQR) 900 (66–1600) IU/day (Tangpricha et al., 2012)	Prevalence of chronic pulmonary infection Correlation between 25OHD concentration and number of pulmonary bacterial colonisations Comparison of 25OHD concentration between patients without pulmonary colonisations vs. number of colonisations (1, 2 & 3)	61% patients identified as having chronic pulmonary infection Correlation between 25OHD concentration and number of positive bacterial colonisations r = −0.16; $p < 0.01$ No colonisation 30 (95% CI 10 −52); 1 colonisation 25 [9–45]; 2 colonisation; 23 [5–46]; 3 colonisations 16 [10–30] ng/mL; $p = 0.0004$ *(app. values)*
(McCauley, Thomas et al., 2014) USA	Strong	Moderate	Retrospective Longitudinal study 2000–2012	130 children with CF aged 6–18 years	25OHD concentration 25OHD status defined as deficient <20 μg/L; insufficient 20–29 μg/L; sufficient ≥30 μg/L (Cystic Fibrosis Foundation recommendations 2012) M: Liquid chromatography/tandem mass spectrometry Pulmonary exacerbations were defined as hospitalisations, identified from the longitudinal record by inpatient pulmonary function tests. Data stratified by age groups; 6–10, 11–14 & 15–18 years) Data on vitamin D supplementation dosage N/R, but followed Cystic Fibrosis Foundation's recommendation (birth to 12 months: 400–500 IU/day D3; >12 months to 10 years: 800 to 1000 IU/day D3; >10 years: 800 to 2000 IU/day D3)	PE	The rate of PE for the 25OHD deficient group (aged 15–18 years) was 13 per 10 patient-years (95% CI, 6–31); the insufficient and sufficient group was 4.3 per 10 patient-years (95% CI 2–8) ($p = 0.041$ and $p = 0.035$ respectively). No statistical significant differences were found in the other age groups.
(Ongaratto, Rosa et al., 2018) Brazil	Weak	Moderate	Retrospective study July 2013–March 2015	37 children and adolescents with CF. Mean ± SD age 11 ± 5.58 years. Range 1–20 years	25OHD concentration 25OHD status defined as per Cystic Fibrosis Foundation and Endocrine Society: deficiency <20 ng/mL; insufficiency 20–29.9 ng/mL and sufficiency ≥30 ng/mL M: N/R Patients were stratified into two groups; sufficiency vs. hypovitaminosis (deficient and insufficient) Data on vitamin D supplementation dosage N/R. All subjects received routine oral CF-specific vitamin supplementation as per Cystic Fibrosis Foundation (birth to 12 months: 400 −500 IU/day D3; >12 months to 10 years: 800 to 1000 IU/day D3; >10 years: 800 to 2000 IU/day D3)	Primary outcome: PE episodes over 2 years and post-dosing period. Correlation between 25OHD concentration and PE PE defined as per criteria: signs and symptoms of exacerbation (fever, increased cough, change in volume or consistency of sputum, decreased appetite, weight loss and/or change in physical examination) and/or reduction in the lung function parameters of at least 5–10%, associated with the use of systemic antibiotics (Goss et al., 2007)	PE over 2 years: 25OHD sufficient group had median (range) 2 (0.5–4.5) PE and the deficient group 4.5 [3 −8] PE; $p = 0.007$ PE post-dosing period: 25OHD sufficient group had 0 (0–1) PE and the deficient group 2 [1,2] PE; $p = 0.002$ Correlation between the number of PE over a 2 year period and lower 25OHD correlation ($p = 0.004$); r = N/R Correlation between higher PE and lower 25OHD concentration in the period post-dosing ($p = 0.008$); r = N/R
(Simoneau, Bazzaz et al., 2014) USA	Weak	Moderate	Retrospective chart review January 2009–December 2011	148 children under 12 years of age with CF (10 months–12 years) Mean ± SD age 82.7 ± 40.8 months	25OHD status defined as: deficiency <25OHD 20 ng/mL; insufficiency ≥20 ≤ 29.9 ng/mL; sufficiency ≥30 ng/mL M: BCH Clinical Laboratory using liquid chromatography–tandem mass	Relationship of 25OHD concentration and *Pseudomonas* culture positivity, inflammatory markers (CRP, IgE, IgG) Independent variables: age, sex, genotype, BMI (or w/l),	Subjects aged 6–12 years with positive *Pseudomonas* culture were significantly more likely to be vitamin D insufficient/deficient (n = 86 OR 3.2; 95% CI (1.1–9.4);

					spectrometry (AB Sciex, Foster City, CA); CV% NR Bacterial colonisation: defined as having two out of the three cultures positive for the same organism M: Microbiology, method NR Vitamin D supplementation Median (IQR) 800 (400–1000) IU/day	pancreatic insufficiency and FEV_1 Comparison of 25OHD concentration between subjects with bacterial colonisation and without	$p = 0.033$ 25OHD concentration in subjects colonised with *Pseudomonas* was significantly lower than those without this infection; Median (IQR) 27.7 (25.3–33.8) vs. 32.9 (26.5–39.3); $p = 0.021$ No difference in subjects colonised with *methicillin-resistant staphylococcus aureus* and m*ethicillinsensitive staphylococcus aureus* Median (IQR) N/R; p = N/R *P. aeruginosa* was a more common pathogen in the patients who were vitamin D insufficient/deficient (18 of 63) as compared with those who were vitamin D sufficient (11 of 85); $p = 0.018$.
(Wani, Nazir et al., 2019)India	Weak	High	Retrospective cohort January–December 2016	51 children with CF < 15years (PI) Median (IQR) 11.4 (6.01–14.47)	Vitamin D supplementation 400–800 IU/day. 25OHD status defined as deficiency (<12 ng/mL); insufficiency (≥12 < 20 ng/mL) and sufficiency in D sufficient (≥20 ng/mL) as per Global Consensus recommendations (2016) 25OHD concentration M: Liquid chromatography Bacterial colonisations: M: Microbiological cultures of respiratory secretions performed every three months. Pulmonary secretions for cultures retrieved by active coughing or oropharyngeal swab. Broncheoalveolar lavage performed if patients failed to respond to treatment directed at pathogens cultured using the above mentioned methods.	Comparison of PE and bacterial colonisation between 25OHD status Associations between 25OHD status and PE and colonisation (multivariate regression) PE defined as acute or sub-acute worsening of respiratory symptoms severe enough to warrant oral or intravenous treatment with antibiotics. Pulmonary colonisation defined as positive respiratory cultures in the absence of increase in baseline signs and symptoms	PE in 25OHD sufficient 0 (0–1); insufficient 1 (1–1.5) and deficient 1 (1–3.25);$p<0.001$ 25OHD deficiency was positively correlated to female sex ($\chi^2 = 2.483$; $p = 0.001$), PE ($\chi^2 = 0.507$; $p = 0.001$), age at diagnosis ($\chi^{2=}0.335$; $p = 0.016$), bacterial colonisation ($\chi^2 = 0.500$; $p = 0.035$) In multivariate regression analysis, bacterial colonisation and greater number of PE were associated with the highest odds of developing 25OHD deficiency in patients with CF; PE OR 5.12; 95% CI (1.28–20.50); $p = 0.02$; Bacterial colonisations OR 2.9; 95% CI (0.57–14.82); $p = 0.01$

CF: Cystic fibrosis; CI: confidence interval; CV%: percentage coefficient of variation; FEV_1% predicted: forced expiratory volume in 1 s expressed as percentage; FVC: forced vital capacity; IQR: interquartile range; M: Method; N/R = Not reported in the study; OR: odd ratio; PE: pulmonary exacerbations; SD: standard deviation; 1,25OHD; 1,25 dihydroxyvitamin D; 25OHD: 25-hydroxyvitamin D; *- included both adult and children, but only paediatric data was used for this review; SD: standard deviation.

Table 3
Impact of vitamin D supplementation on 25OHD status on children and young people with cystic fibrosis.

Author	Quality	Risk of Bias	Research Design	Subjects	Method	Outcome/exposure	Results
(Brodlie, Orchard et al., 2012) England	Weak	High	A series of audit 2008 and 2010 (retrospective study)	2008 audit 78 PI children aged ≥1 year Median (Range) 10 [1−16] years 2010 audit 72 PI children aged ≥1 years Median (range) 9 [1−17] years 2010 audit 15 PS children Median (range) 3.5 1−14 years	Serum 25OHD concentration M: N/R Audit 2008: PI patients Vitamin D supplementation as per CF Trust Bone Mineralisation Working Group (2007) Audit 2010: PI patients received treatment of 800 IU/day up to 3800 IU/day based on a mass dose calculation Audit 2010: PS patients supplementation protocol N/R	Serum 25OHD status 75−150 nmol/L	A significant increase in median (range) 25OHD from 51.5 (8−91) nmol/L to 72 [26− 72]) nmol/L (P < 0.0001, 95% CI= N/A) 49% of children remained ≤75 nmol/L 2010 audit PS 87% (13/15) <75 nmol/L N = 7 were supplemented with vitamin D
(González Jiménez, Muñoz Codoceo et al., 2015) Spain	Moderate	Moderate	Multicentre retrospective study Nov 2012−April 2014	377 children with CF aged <21 years Median (IQR) 8.9 (N/R)	Serum 25OHD concentration 25OHD status defined <30 ng/mL insufficient & <20 deficient (Cystic Fibrosis Foundation recommendations 2012) M: N/R Vitamin D intake quantified as UI/day; M: NR	Prevalence of 25OHD insufficiency and deficiency Correlation between age and 25OHD concentration Comparison between 25OHD and age groups (>10 years vs. 2−10 years vs. < 2 years)	9% patients did not receive supplementation 91% patients received median (IQR) 900 (666−1600) IU/day 25OHD concentration median (IQR) 26 (20−32.5); range [8−72]) ng/mL 65% insufficient 23% deficient 25OHD insufficiency in pancreatic insufficiency 68% vs. sufficiency 53%; $p = 0.01$ Correlation between age and 25OHD concentration; r = −0.20; $p < 0.001$ 25OHD concentration in >10 years old 25.2 (95% CI 23.6−26.7); 2−10 years old 28.4 (95% CI 26.9−30.0); <2 years old 30.2 (95% CI 27.3−33.2); $p = 0.007$
(Green, Carson et al., 2008) USA	Weak	High	Retrospective cohort study January 2003 to December 2006	262 children aged 4 months to 20 years.	25OHD deficiency defined as <30 ng/mL (<75 mmol/L) 3 Protocols used for vitamin D supplementation: Protocol 1 = 50,000IU of ergocalciferol for 8 weeks (as per 2002 CF Foundation statement). Protocol 2 = 50000IU of ergocalciferol twice a week for 8 weeks if protocol 1 unsuccessful (if patients remained deficient) and standard protocol from March 2004 to October 2004. Protocol 3 = 50000IU of ergocalciferol three times a week for 8 weeks Follow ups were completed 2−4 weeks after treatment completion. Standard protocol from October 2004−June 2006 M: 25OHD assays: the Nichols Advantage 25OHD assays; the DiaSorin 25-OHD radioimmunoassay kits; liquid chromatography-tandem mass	25OHD concentration pre-post vitamin D supplementation Post-treatment protocol 1, 2 & 3 were compared vs. no-treatment group Risk factors for 25OHD deficiency	No treatment group: Median (range) 25OHD pre-treatment 19 [6−29] to post-treatment 24 [6−56] ng/mL; $p > 0.05$ Protocol 1: 33% (7/21) achieved 25OHD concentration >30 ng/mL Pre-treatment; median (range) 25OHD 11 [6−21] ng/mL to post-treatment; 25 [5−69] ng/mL χ^2, $p = 0.80$) (compared to non-treatment group) Protocol 2: 26% (6/23) achieved 25OHD concentration >30 ng/mL Pre-treatment median (range) 22 [7−29] to post-treatment 23 [7−48] ng/mL (χ^2, $p = 0.34$) (compared to non-treatment group). Protocol 3: 43% (61/141)

					spectroscopy. CV % NR		achieved 25OHD concentration >30 ng/mL Pre-treatment 21 [5–29] to post-treatment 19 [6–29] ng/mL (χ^2, $p = 0.22$) (compared to non-treatment group). (r = N/A, 95% CI= N/A)
(Green, Leonard et al., 2010) USA	Weak	Moderate	Retrospective chart review Januray 2006 –December 2008	97 paediatric CF patients <21 years old Mean ± SD 10.9 ± 5.2	Age, gender, exocrine pancreatic status, 25-OHD concentration, BMI percentile and FEV1% predicted. M: 25OHD concentration performed by Quest Diagnostics (Chantilly, VA), liquid chromatography –tandem mass spectroscopy Treatment of deficiencies using 50,000 IU D2/day for 28 days	25OHD status ≥30 ng/mL If pre-treatment 25OHD concentration predicted outcome Subgroup analysis: 25OHD baseline status; 0–19 ng/mL vs. 20 –29 ng/mL 25OHD post-treatment concentration performed within 3–6 months post-vitamin D supplementation (n = 92) 25OHD status seasonal differences (winter, spring, summer & fall)	Linear regression of 147 treatment courses showed that for every one day increase in time to post-treatment follow-up level the difference in pre and post 25OHD concentration decreases by 0.08 ($p = 0.001$) Vitamin D_2 supplementation (n = 111) pre-treatment 4 [4–19] ng/mL to post-treatment 12 (4–168) ng/mL vitamin D_3 (sun exposure) (n = 68) pre-treatment 19 [4–29] ng/mL to post-treatment 15 [4– 49]) ng/mL 41 (74.5%) were 25OHD sufficient after 3 months of vitamin D2 supplementation A higher success rate was seen in those patients who had pre-treatment concentration obtained in winter (n = 33/54) or spring (n = 32/51) compared with summer (n = 11/31) or fall (n = 4/11) ($\chi 2$ $p = 0.04$). Seasonal 25OHD concentration [Median (range)] Winter pre-treatment (25 [7–29] to post-treatment 34 (5 –94) ng/mL Spring pre-treatment (24 [6–29] to post-treatment 35 (13 –310) ng/mL Summer pre-treatment 23 [9–29] to post-treatment 25 (6 –235) ng/mL Fall pre-treatment 24 [5–29] to post-treatment 33 (5–310) ng/mL
(Henderson and Lester 1997) USA	Weak	High	Cohort- Cross-sectional observatory study	54 children Range (4.9 –19.5) years Mean (11.0) years Control cerebral palsy (n = 125) and survivors of childhood cancer (n = 46)	25OHD and 1,25-diOHD assessed M: radioimmunoassay and radioreceptor assay kits (Incstar, Stillwater, Minn) CV% NR 25OHD deficiency defined as <10 ng/mL and normal as >18 ng/mL Vitamin D supplementation 600 IU/day	25OHD and 1,25-diOHD concentration	No statistical significant differences in 25OHD concentration between the vitamin D supplemented CF group (mean ± SE 25.3 ± 3.4) ng/mL vs. non-supplemented CF group (mean ± SE 24.7 ± 2.4) ng/mL; 95% CI NR; p = NR
	Moderate	Moderate		15 children with CF (3 –15 years)	Serum 25OHD concentration M: Serum 25OHD concentration	Serum 25OHD, 1,25OHD concentration	Pre-treatment 25OHD (mean ± SD): 35.4 ± 13.2 ng/mL

(continued on next page)

Table 3 (*continued*)

Author	Quality	Risk of Bias	Research Design	Subjects	Method	Outcome/exposure	Results
(Hillman, James et al., 2008) USA			Double-blinded randomised cross-over controlled trial.	Mean ± SD 9.1 ± 2.3 Median (range) 8.5 [6−13]	Radioimmunoassay (Incstar, Stillwater, MN), intra-assay CV% <10% & inter-assay CV% <12%. Vitamin D supplementation: Multivitamin (A, D, E, K) containing 400 IU of vitamin D and 1600 IU of vitamin D Calcium tablets as calcium carbonate Placebo tablets 4 groups: (P) placebo and 400 IU vitamin D; (Ca) 1g Ca (calcium carbonate) and 400 IU vitamin D; (D) 2000 IU vitamin D and placebo; (Ca + D) 2000 IU vitamin D and 1 g Ca Intervention 6 months each with 3-month washout period		Post-treatment 25OHD (Mean ± SD): 64.8 ± 14.2 ng/mL P (n = 9): 61.9 ± 18.2 Ca (n = 10): 66.9 ± 20.7 D (n = 12): 64.8 ± 14.2 Ca + D (n = 11): 69.1 ± 31.4 $p > 0.05$; 95% CI=N/A)
(Norton, Page et al., 2015) Canada	Strong	Moderate	Cohort- Retrospective chart review in 2010 and 2011	96 children with CF age range 1−18 years Age (mean ± SD) 2010 (n = 82) 8.5 ± 5.1 years 2011 (n = 87) 8.8 ± 5.0 years	25OHD status: adequacy ≥75 nmol/L (≥30 ng/mL) and inadequacy <75 nmol/L (<30 ng/mL) as per CFF standards Vitamin D supplementation (vitamin D_3) 400 IU/day if 25OHD concentration 24−30 ng/mL (60−75 nmol/L) 1000 IU if 25OHHD concentration >24 ng/mL (>60 nmol/L) concentration Vitamin D intake was calculated from all ingested sources (diet and supplementation) M: self-reported intake, N/R	Changes in 25OHD concentration in 2010 and 2011 in supplemented groups Correlation between vitamin D IU intake and 25OHD levels	Based on adequacy status, a dosage of either 400 IU/day or 1000 IU/day vitamin D showed a significant increase of 25-OHD from 88 ± 25 in 2010 to 89 ± 26 in 2011 ($p = 0.03$) Positive correlation between vitamin D intake and 25OHD levels ($r^2 = 0.247$; $p = 0.03$) 50% of supplemented patients reached 25OHD concentration ≥75 nmol/L.
(Oliveria, Matsunga et al., 2019) Brazil	Strong	Moderate	Observational longitudinal research 2015−2017	68 CF children (infants and pre-schoolers) Mean ± SD 22.9 ± 17.3 months 102 without CF or malabsorption Mean ± SD 22.0 ± 11.2 months	25OHD concentration defined as sufficiency >30.0 ng/mL and insufficiency <30.0 ng/mL (Brazilian Pediatric Society and Cystic Fibrosis Foundation) 25OHD concentration assessed twice (0, 6 months) M: e kit LIAISON® 25 OH Vitamin D TOTAL Assay. CV % N/R Vitamin D supplementation as per Brazilian Pediatric Society and the Ministry of Health. CF children with PI were given supplementation according to CF guidelines 2017.	Prevalence of 25OHD insufficiency at 0 and 6 months 25OHD concentration comparison between CF and non-CF at 0 and 9 months Correlation between age, pancreatic sufficiency (PS)/ insufficiency (PI), and 25OHD concentration Factors contributing to 25OHD <30 ng/mL for CF and non-CF at 0 and 6 months (ethnicity, sun exposure, seasonality, sunscreen use, vitamin D supplementation)	<30 ng/mL at 0: 43.6% and at 6 months 30.7% >30 ng/mL at 0 : 56.4 and at 6 months 69.3% 88.2% of PI patients were supplemented with vitamin D at first assessment 100% of PI patients at 6 months At 0: mean ± SD CF 32.8 ± 10.2 vs. non-CF 29.3 ± 9.3; $p = 0.046$ At 6 months: mean ± SD CF 35.6 ± 9.3 vs. 33.5 ± 9.2; $p = 0.2$ No association between PI and 25OHD concentration; r = N/R; p = N/R No correlation age and 25OHD at 0 ($p = 0.827$) and 6 months ($p = 0.133$) assessments; r: N/R At 0 months: Lack of supplementation was the one variable associated to insufficiency: adjusted OR 2.81 (CI 95% 1.38−5.70); $p = 0.004$. At 6 months: Seasonality (winter/fall) was associated to lower 25OHD concentration:

							adjusted OR 3.28 (CI 95% 1.30–8.29); $p = 0.016$
(Pincikova, Paquin-Proulx et al., 2017) Sweden	Moderate	Moderate	Randomised control trial	16 children with CF Mean ± SD N/R	Vitamin D supplementation: Dose for 3 months and 2 months wash out <16 years old 35,000 IU/week (5000 IU/day) ≥16 years old 50,000 IU/week (7143 IU/day)	Primary outcome serum 25OHD concentration at 3 months in nmol/L Aim to reach 25OHD concentration >100 nmol/L	Positive correlation between vitamin D dose and 25OHD concentration ($p = 0.03$; $r = 0.76$) Mean ± SD baseline serum 25OHD in control group (49.0 ± 38.7), D2 group (55.7 ± 16.0) and D3 group (65.0 ± 13.9). 25OHD concentration in control group at 2 months (78.0 ± 23.2) and 3 months (62.5 ± 14.9); $p > 0.05$; 95% CI N/R 25OHD concentration in D2 group at 2 months (79.3 ± 13.4) and 3 months (81.5 ± 10.7); $p = 0.106$; 95% CI NR 25OHD concentration in D3 group increased significantly at 2 months (104.0 ± 17.6) and at 3 months (90.0 ± 4.2);$p<0.05$; 95% CI N/R 100% (n = 9) in both intervention arms had 25OHD >75 nmol/L at 3 months. None of the patients allocated to the D2 group reached the goal of 100 nmol/L. 40% (n = 2/5) in D3 group achieved >100 nmol/L
(Shepherd, Belessis et al., 2013) Australia	Weak	Moderate	Retrospective chart review 2007–2011	142 CF Patients (7–17 years) Median (range) 8 [2–18] years	25OHD deficiency (<75 nmol/L) M: 25OHD analysis; automated Liaison system utilising a chemoluminescent assay; CV % N/R Control group: vitamin D supplementation of 400 IU/day <1 year and 800 IU/day >1 year as per US and Australian guidelines (Aris et al., 2004, 2005 & Green et al., 2008). Vitamin D intervention: intramuscular high single dose 100,000–600,000 IU (stoss therapy) 38 received stoss therapy, 37 were not treated and acted as a control group	Control group 25OHD concentration at annual review and 12 months later 25OHD concentration at time 1, 3 and 12 months post dose (stoss)	The control group did not have a significant increase in (mean ± SD) 25OHD levels: time 0 (59.18 ± 11.9) and at 12 months (64.30 ± 15.17) nmol/L; $p = 0.132$; 95% CI N/R 82.4% of the control group remained vitamin D deficient. Stoss therapy intervention showed a significant increase in (mean ± SD) 25OHD concentration at every stage (1, 3, 6 and 12 months) post dose; time 0 (49.6 ± 12.9); 1 month (94.82 ± 41.0); $p = 0.001$; 3 months (81.54 ± 24.6); $p = 0.0001$, 6 months (92.18 ± 36.5); $p = 0.008$ & 12 months (64.6 ± 20); $p = 0.006$; 95% CI N/R.

CF: Cystic fibrosis; CI: confidence interval; CV%: percentage coefficient of variation; FEV_1% predicted: forced expiratory volume in 1 s expressed as percentage; FVC: forced vital capacity; IQR: interquartile range; M: Method; N/A: non-applicable; N/R = Not reported in the study; OR: Odd ratio; PI: pancreatic insufficiency; PS: pancreatic sufficiency; SD: standard deviation; 1,25OHD; 1,25 dihydroxyvitamin D; 25OHD: 25-hydroxyvitamin D; *- included both adult and children, but only paediatric data was used for this review; SD: standard deviation.

Table 4
Studies reporting associations and/or effects of 25OHD concentration on secondary outcomes (nutritional status and aerobic fitness) and their characteristics.

Author	Quality	Risk of Bias	Research Design	Subjects	Method	Outcome	Results
(Chavasse, Francis et al., 2004) England	Weak	High	Retrospective chart review August 1999−April 2001	320 children Median (range) age 9 (0.9 −18.5) years PI n = 277 (86.5%)	Patients with confirmed CF were measured for 25OHD concentration against healthy British children (Gregory et al., 2000) 25OHD concentration measured annually. M: in-house, competitive protein-binding assay following extraction and chromatography of 25OHD on silicic acid (Charing Cross Hospital); CV% N/R Vitamin D supplementation 800−1200 IU/day to all patients (NHS) M: Growth measurements N/R Accounted for pancreatic sufficiency (PS) and insufficiency (PI)	Correlation between 25OHD concentration and growth (weight *Z* score, height *Z* score)	No correlation between 25OHD concentration and measurements of growth (weight or height *Z*-scores) All values described as mean ± SD Age 1−4: Height *Z* score (−0.29 ± 2.7); Weight *Z* score (−0.34 ± 2.7) Age 5−12: Height *Z* score = −0.49 ± 2.7; Weight *Z* score (−0.24 ± 2.0) Age >13: Height *Z* score (−0.54 ± 2.1); Weight *Z* score (−0.51 ± 2.4) Total: Height *Z* score (−0.4 ± 2.3); Weight *Z* score (−0.3 ± 2.3); r = N/R; *p* = N/R Differences between PS and PI in growth parameters N/R
(Green, Carson et al., 2008) USA	Weak	High	Retrospective cohort study January 2003 to December 2006	262 children aged 4 months to 20 years. Median (range) 9.9 (0.3 −20.0) years PI n = 241 (92%)	25OHD deficiency defined as <30 ng/mL (<75 mmol/L) 3 Protocols used for vitamin D supplementation: Protocol 1: 50,000IU of ergocalciferol once per week for 8 weeks (CF Foundation statement, 2002). Protocol 2: 50,000IU of ergocalciferol twice a week for 8 weeks if protocol 1 unsuccessful (if patients remained deficient) and standard protocol from March 2004 to October 2004. Protocol 3: 50000IU of ergocalciferol three times a week for 8 weeks Follow ups were completed 2−4 weeks after treatment completion. Standard protocol from October 2004−June 2006 M: 25OHD assays: the Nichols Advantage 25OHD assays; the DiaSorin 25-OHD radioimmunoassay kits; liquid chromatography-tandem mass spectroscopy. CV % NR	Associations between BMI percentile categories and 25OHD concentration M: N/R	BMI was not significantly associated with 25OHD concentration BMI percentile = (median (range)) 40.1 (0.01−99.2) (OR r = 0.10; *p* = 0.12) Differences between PS and PI in BMI percentiles N/R BMI percentiles: <5% OR 2.34; 95% CI 0.63−8.67 5%−10% OR 1.32; 95% CI 0.33 −5.23 10%−25% OR 1.15; 95% CI 0.53 −2.49 25%−50% OR 0.67 95% CI 0.34 −1.30
(Green, Leonard et al., 2010) USA	Weak	Moderate	Retrospective chart review Januray 2006 −December 2008	97 paediatric CF patients <21 years old Mean (±SD) 10.9 ± 5.2 PI n = 88 (90.7%)	25OHD deficiency <30 ng/mL M: 25OHD concentration performed by Quest Diagnostics (Chantilly, VA), liquid chromatography−tandem mass spectroscopy Treatment of deficiencies using 50,000 IU D2/day for 28 days Treatment of deficiencies using 50,000 IU D2/day for 28 days and only cypCF with PI Successful 25OHD concentration >30 ng/mL	BMI percentile comparison between successfully vs. non-successfully treated M: N/R	Mean ± SD BMI percentile of the successfully treated (44.8 ± 26.6) and the non-successfully treated (40.8 ± 28.8); *p* = 0.490 95% CI N/R

(Henderson and Lester 1997) USA	Weak	High	Cohort- Cross-sectional observatory study	54 children Range (4.9 −19.5) years Mean (11.0) years Control cerebral palsy (n = 125) and survivors of childhood cancer (n = 46)	25OHD and 1,25OHD assessed M: radioimmunoassay and radioreceptor assay kits (Incstar, Stillwater, Minn) CV% NR 25OHD deficiency defined as <10 ng/mL and normal as >18 ng/mL	Correlation between 25OHD and growth (height *Z* score, weight *Z* scores, skinfold percentiles) M: N/R No information on pancreatic function	No correlation between growth measurements and 25OHD concentration. (r = N/R; *p* = N/R)
(Norton, Page et al., 2015) Canada	Strong	Moderate	Cohort- Retrospective chart review 2010−2011	96 children with CF age 1−18 years (mean age = 9 years) 2010 PI n = 74 (90.2%) 2011 PI n = 80 (91.9%)	Vitamin D supplementation 25OHD status defined as deficient <20 μg/L; insufficient 20−29 μg/L; sufficient ≥30 μg/L (Cystic Fibrosis Foundation recommendations) M: N/R	BMI percentiles M: N/R	25OHD concentration was not associated with BMI percentile 2010 Mean ± SD BMI *Z* score 0.16 ± 0.98; *p* > 0.05 2011 Mean ± SD BMI percentile 0.06 ± 0.98 (r = NR; 95% CI= N/R; *p* > 0.05) Differences between PS and PI in BMI percentiles N/R
(Oliveria, Matsunga et al., 2019) Brazil	Strong	Moderate	Observational longitudinal research 2015−2017	68 CF children (infants and pre-schoolers) Mean ± SD 22.9 ± 17.3 months PI n = 34 (87.2%) PS n = 5 (12.8%) 102 without CF or malabsorption Mean ± SD 22.0 ± 11.2 months	25OHD concentration defined as sufficiency >30.0 ng/mL and insufficiency <30.0 ng/mL (Brazilian Pediatric Society and Cystic Fibrosis Foundation) 25OHD concentration assessed twice (0, 6 months) M: e kit LIAISON® 25 OH Vitamin D TOTAL Assay. Vitamin D supplementation as per Brazilian Pediatric Society and the Ministry of Health. CF children with PI were supplemented as per CF guidelines 2017 Accounted for confounding variables (ethnicity, sex, diagnosis, pancreatic function, clinical history, genotype, sun exposure habits and seasonality and mother's education level)	BMI *Z* scores were calculated using the software WHO Anthro (2011)	BMI *Z* score did not correlate with 25OHD concentration at 0 (*p* = 0.453) and 6 months (*p* = 0.573) assessments; r = N/R No association between PI and 25OHD concentration r = N/R Differences between PS and PI in BMI percentiles N/R
(Ongaratto, Rosa et al., 2018) Brazil	Weak	Moderate	Retrospective study July 2013−March 2015	37 children and adolescents with CF. Mean ± SD age 11 ± 5.58 years; range 1−20 years PI n = 35 (94.6%)	25OHD concentration 25OHD status defined as per Cystic Fibrosis Foundation and Endocrine Society: deficiency <20 ng/mL; insufficiency 20−29.9 ng/mL & sufficiency ≥30 ng/mL M: N/R Patients were stratified into two groups; sufficiency vs. hypovitaminosis (deficient and insufficient) All subjects received routine oral CF-specific vitamin supplementation as per Cystic Fibrosis Foundation (birth to 12 months: 400−500 IU/day D3; >12 months to 10 years: 800 to 1000 IU/day D3; >10 years: 800 to 2000 IU/day D3) M: Weight and height measured using stadiometers and digital scales & light clothing. Data analysed using the Anthrosoftware (WHO 2009) and AnthroPlus (WHO 2011)	Comparison of BMI Z score and height for age (H/A) Z-scores in sufficient vs. deficient. Confounding variables N/R	Median (IQR) BMI Z score 25OHD sufficient 0 (-1-0.50) vs. deficient group −1 (-1 to 0); *p* = 0.141 Median (IQR) H/A *Z*-score sufficient −1 (-1 to 0) vs. deficient group −1 (-1 to 0); *p* = 0.232

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Table 4 (*continued*)

Author	Quality	Risk of Bias	Research Design	Subjects	Method	Outcome	Results
(Revuelta-Iniesta, Causer et al., 2021) UK	Strong	Low	Multi-centre retrospective study July 2017 to October 2019	90 patients with CF. included both adults and children >9 years of age. 54 children included Median (IQR)16.60 (13.0–25.4) years PI n = 41 (79.9%)	Plasma 25OHD concentration M: Liquid chromatography-tandem mass spectrometry technique; CV% 8.9 25OHD status definition: deficient <50 nmol/L; insufficient ≥50 ≤ 75 nmol/L and sufficient >75 nmol/L (Endocrine Society, Holick et al., 2011)	Comparison between 25OHD sufficient vs. insufficient vs. deficient BMI *Z* score and height for age (H/A) *Z* scores. Associations between 25OHD and aerobic fitness measured as maximal (peak) aerobic fitness (VO_{2peak}) M: Stature measured using a stadiometer; weight on calibrated scales recorded at each CPET visit. UK-WHO BMI *Z*-scores were calculated for children using the LMSgrowth® (2012); aerobic fitness (Hebestreit et al., 2015; Radtke et al., 2019)	Median (IQR) BMI *Z* score of 25OHD status; sufficient −0.15 (−1.02, 0.60) vs. insufficient 0.05 (−0.90, 095) vs. deficient −0.40 (−1.07–0.87); $p = 0.74$ 25OHD significantly predicted VO_{2peak} $R^2 = 0.13$; $\beta = -0.36$; $p = 0.05$; 95% CI (−0.26 to −0.005) Differences in BMI *Z* score between PI and PS N/R
(Simoneau, Bazzaz et al., 2014) USA	Weak	Moderate	Retrospective chart review January 2009–December 2011	148 children under 12 years of age with CF (10 months–12 years) Mean ± SD age 82.7 ± 40.8 months PI n = 120 (81.1%) PS n = 28 (18.9%)	25OHD status defined as: deficiency <25OHD 20 ng/mL; insufficiency ≥20 ≤ 29.9 ng/mL; sufficiency ≥30 ng/mL M: BCH Clinical Laboratory using liquid chromatography–tandem mass spectrometry (AB Sciex, Foster City, CA); CV% N/R	Associations between 25OHD insufficiency and BMI percentiles Weight for height percentiles <2 years BMI percentiles >2 years M: N/R Confounding variables accounted for (age, sex, BMI percentile, genotype, pancreatic insufficiency, FEV_1% predicted, CRP, IgG, IgE, and history of *Pseudomonas*)	The mean ± SD BMI percentiles did not differ between the 25OHD sufficient (52.1 ± 2.7); insufficient (50 ± 28.3) and deficient groups (62.4 ± 32) percentile; $p = 0.496$. No correlation between 25OHD and BMI; r = N/R; P = N/R Differences in BMI *Z* score between PI and PS N/R
(Simoneau, Sawicki et al., 2016) USA	Moderate	Moderate	Randomised control trial April 2012–June 2013	47 children age 6–21 years with CF Mean ± SD 14.3 ± 4.3 years PI 100%	25OHD outcome >30 ng/mL M: 25OHD concentration was determined by liquid chromatography–tandem mass spectrometry (AB Sciex, FosterCity, CA) and the laboratory external quality control through DEQA Vitamin D supplementation: Compare 50,000 IU of Vitamin D2 twice weekly for 8 weeks vs. 50,000IU of D2 weekly	Comparison of BMI percentile before and after vitamin D supplementation M: N/R	No statistical significant differences in BMI kg/m^2 before and after vitamin D supplementation Mean ± SD Baseline 18.92 ± 2.36 to follow up 19.13 ± 2.34; $p = 0.05$ D2: Baseline 18.72 ± 2.87 to follow-up 18.96 ± 2.78; $p = 0.03$ Equal BMI kg/m^2 mean change between the two arms mean ± SE; D2 (0.20 ± 0.10) and D3 (0.24 ± 0.10); $p = 0.81$ 95% CI N/R Differences in BMI kg/m^2 between PI and PS NR

(Timmers, Stellato et al., 2019) The Netherlands	Strong	Low	Retrospective study January 2012 June 2016	190 CF patients above the age of 6 PI 100%	Vitamin D supplementation 10–50 μg (400 –2000 IU) for all ages (Sinaasappel et al., 2002) 25OHD status deficient (<50 nmol/L); sufficient (≥50 ≤ 75 nmol/L) & high sufficient (>75 nmol/L) as per European Union guidelines. Serum 25OHD concentration M: Electrochemiluminescence sandwich immunoassay, CV% 8.7%	Associations between BMI *Z* scores and 25OHD concentration	A strong negative correlation found between weight kg and 25OHD concentration r = -0.79; 95% CI -1.20 – (–0.29); $p = 0.002$ Each kg increase in body weight resulted in a 0.79 nmol/L decrease in 25OHD concentration BMI data N/R
(Wani, Nazir et al., 2019) India	Weak	High	Retrospective cohort January–December 2016	62 children with CF < 15years Median (IQR) 11.4 (6.01 –14.47) PI n = 53 (85.4%) PS n = 9 (14.6%)	Vitamin D supplementation 400–800 IU/day 25OHD status defined as deficiency (<12 ng/mL); insufficiency (≥12 < 20 ng/mL) and sufficiency in D sufficient (≥20 ng/mL) as per Global Consensus recommendations (2016) 25OHD concentration M: Liquid chromatography	Weight and height comparisons between 25OHD criteria at baseline	Median (IQR) Weight kg Deficient; 15 [12 –17], sufficient; 16 [11–17]; $p = 0.38$ Height cm deficient 97.5 (87.4 –102.2); sufficient; 101 (88.2 –104.5); $p = 0.22$ 95% CI N/R

BMI: Body mass index; CF: Cystic fibrosis; CI: confidence interval; CRP: C-Reactive Protein; CV%: percentage coefficient of variation; FEV1% predicted: forced expiratory volume in 1 s expressed as percentage; FVC: forced vital capacity; H/A: Height for age; IgE: Immunoglobulin E; IgG: Immunoglobulin G; IQR: interquartile range; M: Method; N/R = Not reported in the study; OR: odd ratio; SD: standard deviation; VO2peak; aerobic fitness measured as maximal (peak) aerobic fitness; 1.25OHD; 1,25 dihydroxyvitamin D; 25OHD: 25-hydroxyvitamin D; *- included both adult and children, but only paediatric data was used for this review.

statistical analysis [54] or included pancreatic insufficiency cypCF only [47,48]. These studies showed no statistical significant differences in BMI (Kg/m^2) before and after vitamin D supplementation [48], no statistically significant correlation between BMI *Z*-score and 25OHD concentration [54] and a strong negative significant correlation between weight (Kg) and 25OHD ($r = -0.79$; $p = 0.002$) [47].

Only one study (4.8%) [43] explored the relationship between 25OHD and aerobic fitness expressed as maximal aerobic fitness (VO_{2peak}) in cypCF and reported a statistically significant association between 25OHD and VO_{2peak} (Table 4).

4. Discussion

This is the first systematic review appraising evidence of the association of 25OHD concentration on pulmonary function as a primary outcome with secondary outcomes analysed including frequency of pulmonary exacerbations and 25OHD status and the relationships between growth measurements and aerobic fitness and 25OHD status in cypCF. Indeed, a recent systematic review of RCT evaluating the effects of vitamin D supplementation on health outcomes including lung function on children and adults with CF was recently published; however, no study including pulmonary function in cypCF met their criteria [21]. Henceforth our results add to the existing evidence based literature. As our review identified 21 studies that were highly variable in quality, at present there is insufficient robust evidence to accurately determine the role of 25OHD concentration on pulmonary function, exacerbations and its relationship with growth in cypCF. Moreover, owing to the considerable diversity of the variables investigated and the lack of results reported (mainly correlation coefficients) in many studies, this review performed a meta-analysis of five studies only investigating differences in pulmonary function, assessed using FEV_1% predicted, between 25OHD status categories. Nonetheless, this systematic review importantly shows that FEV_1% predicted is statistically significantly higher in those who are 25OHD sufficient compared to their 25OHD deficient counterparts [FEV_1% predicted mean difference (95% CI) 7.71 (1.69–13.74) %; $p = 0.01$] and clinically meaningful. Furthermore, the mean FEV_1% predicted of the deficient group (86.9 ± 13.2%) was healthy but borderline with mild lung disease (70–84%), which highlights a higher risk of comorbidities and mortality in cypCF who are 25OHD deficient [56].

4.1. Associations of 25OHD concentration with lung function, pulmonary exacerbations and aerobic fitness.

Over half of the studies included here found a positive relationship between 25OHD concentration and pulmonary function assessed by FEV_1% predicted and FVC % predicted. As many of the studies, particularly those that did not find correlation between 25OHD concentration and pulmonary function (FEV_1% predicted and FVC % predicted) did not report the data, a meta-analysis could not be performed. Therefore, the associations between 25OHD and pulmonary function may be clinically significant.

Consensus exist in regard to 25OHD concentration and frequency of pulmonary exacerbations across the included studies [24,39,42,46,53]. These data suggest either an inverse relationship between 25OHD concentration and frequency of pulmonary exacerbations or a higher rate of exacerbations reported in those who were 25OHD deficient. Of note, these studies were retrospective but only analysed data at one time point. Therefore the associations between basal 25OHD concentration and pulmonary exacerbations (before vitamin D supplementation intake) is unknown. A single study performed by members of our group [43] reported a positive association between 25OHD and aerobic fitness measured using

Fig. 2. Risk of bias of all eligible studies.

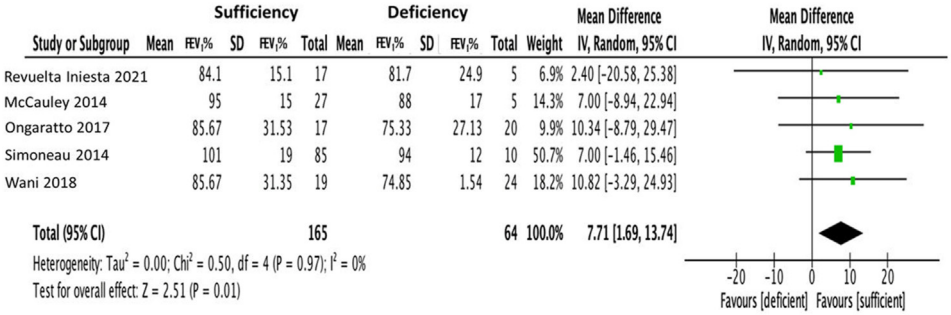

Fig. 3. Meta-analysis to estimate the associations of 25OHD status with pulmonary function (FEV1% predicted). The vertical line represents no effect. The horizontal line represents the 95% confidence intervals. The black diamond around the mean difference point shows the proportion of the study weight. The pooled diamond is centred on the grouped estimate.

Question: Vitamin D deficient compared to Vitamin D sufficient for the prediction of lung function (FEV1% predicted) in paediatric cystic fibrosis patients
Setting:
Bibliography:

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Vitamin D deficient	Vitamin D sufficient	Relative (95% CI)	Absolute (95% CI)		
FEV1%												
5	observational studies	serious [a]	serious [b]	not serious	serious [c]	none	64	165	-	MD **7.71 % higher** (1.69 higher to 13.74 higher)	⨁◯◯◯ VERY LOW	NOT IMPORTANT abc

CI: Confidence interval; **MD:** Mean difference

Explanations

a. Risk of bias for all of the studies were 75% moderate and 25% weak. Lack of randomisation.
b. There was a difference between the dose of vitamin D supplied to the patients across the studies
c. Number of participants was lower than 400. The width of confidence intervals is too high

Fig. 4. Certainty assessment of the Meta-analysis (Fig. 3), with explanations of how the certainty and importance ratings were obtained.

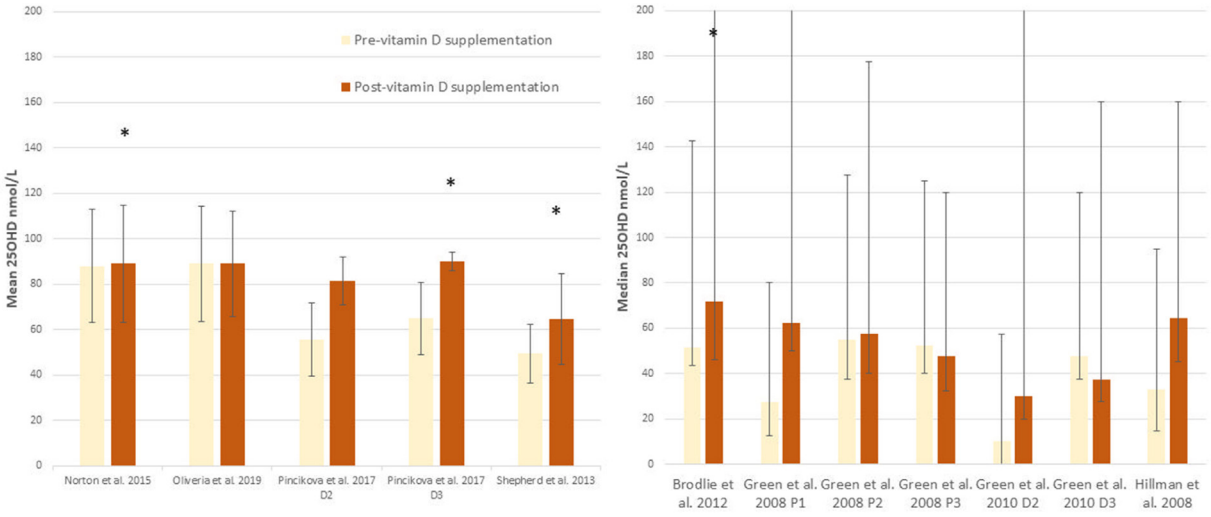

Fig. 5. Studies reporting 25OHD nmol/L pre- and post-vitamin D supplementation. Left figure: studies presenting their data in mean ± SD; Right figure: studies presenting their data using median and range; *$p < 0.05$; Vitamin D supplementation dosages (from left to right): Norton et al., 2015: Vitamin D3 400 IU/day if 25OHD between 60 and 75 nmol/L and 1000 IU for 25OHHD concentration <60 nmol/L); Oliveria et al., 2019: 400IU < 1 year and 400IU–800IU > 1 year; Pincikova et al., 2017: D2/D3 < 16 years old 35,000 IU/week (5000 IU/day) and ≥16 years old 50,000 IU/week (7143 IU/day); Shepherd et al., 2013: intramuscular single dose 100,000–600,000 IU; Brodlie et al., 2012: 800–3800 IU/day; Green et al., 2008: Protocol 1 (P1): 50,000 IU of ergocalciferol (D2) for 8 weeks; P2: 50,000 IU twice a week for 8 weeks; P 3: 50,000IU three times a week for 8 weeks; Green et al., 2010: Deficiencies treated with 50,000 IU D2 or D3 once per day for 28 days; Hillman et al., 2008: 400 IU or 2000 IU per day.

CPET (VO_{2peak}). Noteworthy, as all studies looking at 25OHD and lung health outcomes in cypCF are of epidemiological nature, these findings are not causal. Therefore, these associations may be influenced by disease state, such as more severe pulmonary diseases, infections associated to malnutrition, dietary intake and malabsorption [1] and a more catabolic state induced by a pro-inflammatory state [57].

Our systematic review findings contrast with those of Juhasz et al. [21] in which two interventional studies of adults with CF showed no effect of vitamin D supplementation (and 25OHD concentration) on FEV_1% predicted. The data was described narratively [21]. Our results align with a large cross-sectional study (n = 896) performed in Scandinavian patients (children and adults) with CF [58], which did not meet the eligibility criteria [58]. Pincikova et al. [58] showed a positive association between 25OHD and FEV_1% predicted ($r^2 = 0.308$; $p = 0.02$). Unfortunately, the relationship between 25OHD concentration and pulmonary exacerbations was only analysed as a confounding factor [58]. Nonetheless, pulmonary exacerbations significantly influenced the relationship of 25OHD and FEV_1% predicted [58]. Such findings that have been replicated in other populations diagnosed with Chronic Obstructive Pulmonary Disease [59] and Asthma [60].

It is increasingly recognised that both systemic and localised 1,25OHD are responsible for many pulmonary immune effects [61]. In particular, locally formed 1,25OHD by the action of 1α−hydroxylase, which is present in alveolar macrophages, dendritic cells and lymphocytes as well as in airway epithelia, acts in an autocrine and paracrine fashion to modulate cell proliferation, cell differentiation and immune function [61]. Alveolar macrophages are the first line of immune defence and are activated by pathogens,

which in turn stimulate the conversation of localised 25OHD into 1,25OHD [61]. This leads to an increase of 25OHD regulated antimicrobial peptide cathelicidin, which facilitates bacterial phagocytosis and killing [62]. Common pathogens inducing pulmonary exacerbations in cystic fibrosis are *Pseudomonas auruginosa*, *Methicillin staphylococcus aureus* (including MRSA) [46]. Indeed, it is plausible that higher 25OHD concentration may activate alveolar macrophages and induce an immune response that protects particularly against *Pseudomonas aeruginosa* [46]. Furthermore, systemic and localised dendritic cells' 1,25OHD might induce an immunosuppressive response by decreasing the expression of major histocompatibility complex (MHC) molecules, via vitamin D binding protein regulated genes, leading to a decrease of IL-12 and an increase of IL-10 synthesis [22,61]. More studies investigating the relationship between 25OHD and lung function in cypCF are now warranted. Ideally, they should be interventional and aim to elucidate 25OHD's immune physiological and cellular mechanistic response.

4.2. Effects/impact of vitamin D supplementation on 25OHD status in ctyCF

Our systematic review showed that vitamin D supplementation is necessary to treat 25OHD deficiency (<50 nmol/L) [15] in cypCF, whilst sunlight exposure in the summer months and vitamin D rich foods do not provide enough to avoid 25OHD deficiency. Unfortunately, this systematic review was unable to show the optimal vitamin D dosage to achieve sufficiency (≥75 nmol/L) [15] and the frequency in which this should be taken in cypCF. These findings mirror other studies [21,63]. The inability of studies to demonstrate a vitamin D optimal dosage has traditionally been attributed to malabsorption and maldigestion; however, a review suggested other potential pathophysiology [63]. This include (i) a reduction in photobiogenesis through epidermis; (ii) low DBP concentration due to systemic inflammation, which impairs 25OHD transport to target cells and tissues; (iii) a reduction in the synthesis of 25OHD from its precursors due to liver dysfunction; (iv) altered 25OHD storage capacity in the adipose tissue; (v) an increased in the catabolism of 1,25OHD and its excretion due to renal dysfunction and (vi) 25OHD peripheral metabolism dysfunction where vitamin D receptors are expressed, which include, but are not limited to, immune cells such as macrophages and B-cells [63].

Nevertheless, the highest 25OHD concentration increase was seen from studies that used 50,000 IU of vitamin D taken orally once or twice per week for 28–42 days, which raised mean 25OHD concentration by 20–50 nmol/L, and a single dose of IM vitamin D of 100,000–600,000 ("*stoss therapy*"), which was effective at achieving sufficiency (>75 nmol/L) for 6 months and avoid insufficiency (<50 nmol/L) for 12 months [45].

There is a paucity of evidence reporting the optimal 25OHD concentration required to improve pulmonary function (or reduce lung function decline), rate of pulmonary exacerbations and aerobic fitness in cypCF. The meta-analysis performed here suggests that sufficiency (≥75 nmol/L) [15] is associated with optimal FEV_1 (94.7 ± 31.9) % as compared to deficiency (86.9 ± 13.2%). Furthermore, Green et al. [40] found that an increase of 2.5 nmol/L (1.0 ng/mL) of 25OHD was associated with a 10% improvement in FEV_1% predicted; whilst McCauley [24] reported that 25 nmol/L (10 μg/L) increase in 25OHD was associated with a 5.5% improvement in FEV_1% predicted. Similarly, 25OHD sufficiency was associated with a reduction in respiratory infections, which ranged between 0 and 4.3 rate of pulmonary exacerbations per year vs. 3–13 per year in the deficiency group, and improve aerobic fitness (VO_{2peak}). These findings are not surprising as the main aetiology of lung function decline is pulmonary exacerbations and 25OHD appears to be associated with a reduced rate of pulmonary exacerbations. Current vitamin D guidelines for CF aim at preventing rickets, fractures and low bone mineral density and differ in both the vitamin D dosages recommended and the target 25OHD concentration [64,65]. For instance, in the USA the target 25OHD is ≥ 75 nmol/L and dosages range between 400 and 4000 IU/day (12 months < 10 years old) and prescriptions are determined by serum 25OHD concentration and age; whilst in the UK [64] and Europe [66] the target is ≥ 50 nmol/L and supplementation for children >1 year old ranges between 400 and 5000 IU/day. Neither are based on strong evidence and the role of vitamin D on non-skeletal muscle health, such as pulmonary immune function is yet to be considered. The findings from this review suggest that 25OHD concentration of ≥75 nmol/L may help slow down lung function decline probably by reducing the rate of pulmonary exacerbation. Further studies to prospectively evaluate rates of pulmonary exacerbation and change in lung function are warranted, with the use of registry data being ideal for the design of such studies.

It is worth noting that the present systematic review only contains studies performed before the introduction of the triple-combination CFTR-modulator therapy, Elexacaftor in combination with Tezacaftor and Ivacaftor (ETI), which is licensed for patients with CF and at least one copy of the F508del mutation aged over 6 years in the UK. Eligible cypCF are expected to have improvements in their lung function, longevity, nutritional status and wellbeing [67] and real world studies are underway, including PROMISE [68] and RECOVER [69]; however, neither are investigating the role of vitamin D. The National Institute for Health Research [70] has highlighted the importance of evaluating the consequences of the implementation of this therapy and establishing any change to clinical management needs. Together, these findings, in addition to the finding that plasma/serum 25OHD is positively associated with lung function possibly due to a reduction in rate of exacerbations, provide an exciting rationale for investigating the efficacy of vitamin D supplementation in both cypCF treated with ETI and the 10%ers (ineligible for ETI who remain on supportive treatments). International collaborations using registry data may facilitate large scale studies in this area.

4.3. Relationship between 25OHD concentration and markers of growth in cypCF

Contrary to studies performed in healthy children [71] and adults [72] whereby the BMI extremes are associated to lower 25OHD concentration, this systematic review suggests that 25OHD concentration is not associated with growth measurements and there is no difference in BMI *Z*-score between 25OHD deficient and sufficient in cypCF. This finding also contrast with Mangas Sánchez et al. [73] whereby cypCF who have lower 25OHD concentration had significantly lower BMI *Z*-scores than those with higher 25OHD concentration (−0.29 ± 0.82 vs. 0.1 ± 1.02; $p = 0.004$). Although vitamin D is essential for bone health and growth in all children [15], the aetiology of growth in cypCF is complex and multifactorial [74]. The most important contributing factors are maldigestion and malabsorption [75]. These derive from inadequate pancreatic enzyme supplementation in pancreatic insufficient people with CF, a reduced intestinal absorptive area and permeability to nutrients, which results from the loss of chloride secretion and viscous intestinal mucus, and dysmotility in patients with intestinal resection [76]. Furthermore, specific genetic mutations of the CFTR gene, inadequate food intake and an increase in total energy and micronutrient requirements associated to systemic chronic inflammation and malabsorption all may play a bigger role on growth than 25OHD status in cypCF. Nonetheless, body composition, impaired hydroxylation of 25OHD, corticosteroids use [73,76]

and the time taken between blood 25OHD sampling and assessment of growth measurements may have affected the findings from this systematic review. Of note, no study reported nutritional intake and only three controlled for the time taken between 25OHD sampling and outcome measures. The half-life of 25OHD ranges between 15 and 45 days [77] and therefore any associations performed outwit this range may be inaccurate [43].

4.4. Future directions, strengths and limitations

Taking into consideration the results of this systematic review and the limited number of studies available to date, future research is proposed (Box 1). Several limitations have been identified in this review. Firstly there was a paucity of evidence investigating most outcome measures, particularly rate of pulmonary exacerbations, aerobic fitness and growth. Following the risk of bias assessment and data extraction, it became clear that the quality of the studies varied considerably, there was a lack of control for confounding factors, unreported data and heterogeneity in the reported outcomes, which precluded the performance of further Meta-analysis. There was only two studies reporting the effects of vitamin D supplementation on pulmonary function using two different markers (FEV_1% and FVC % predicted) with data reporting pre and post intervention. Therefore, we were unable to report pulmonary function changes resulting from vitamin D supplementation.

Potential bias might have occurred as this systematic review excluded studies in which individuals younger and older than 21 years of age were included in the same study and where data were analysed altogether. Furthermore, the heterogeneity (I_2) obtained in the Meta-analysis was 0%. This should be interpreted with caution as I_2 is often biased and imprecise in Meta-analysis of small sample size ($n < 7$) and large 95% CI [78]. In spite of these limitations, the strength of this systematic review lies in its robust methodology. A comprehensive search of five electronic databases of Spanish and English language was conducted. Experts in the field were contacted and the reference list of the identified studies were searched. Still, potential eligible studies, for example those published in different languages, might have been missed.

Box 1
Recommendations for future studies investigating 25OHD concentration and pulmonary health, aerobic fitness and growth in cypCF.

- Results from studies should be presented appropriately. For instance, correlation coefficients for statistically significant and non-statistically significant correlations should be presented.
- Studies investigating the impact of 25OHD on pulmonary health, aerobic fitness and growth should be of prospective nature and the sampling time between 25OHD and the aforementioned outcomes within 15 days.
- Studies exploring the relationship between 25OHD concentration and growth should take into consideration the confounding factors highlighted below.
- Research investigating the relationship between 25OHD, body composition and lung function in cypCF is warranted.
- Adherence to vitamin D supplementation, medication, severity of disease, sun exposure, dietary intake, bone mineral density, and body composition (fat mass and fat free mass) and physical activity should be all measured.
- Data should also be stratified by treatment (ETI therapy) and age groups.
- Control trials and mechanistic studies in which the role of 25OHD on pulmonary health in cypCF that include 25OHD concentration (and its metabolites) and the following; macrophages DBP expression, localised 1,25OHD present in alveolar macrophages, dendritic cells and lymphocytes as well as in airway epithelia, are now warranted.

5. Conclusion

This systematic review is the first to date to review the existing literature on the associations of vitamin D with pulmonary function, aerobic fitness, 25OHD status and growth in cypCF. Unfortunately, there was a small number of eligible studies signified by the failure of researchers to report results (correlation coefficients) and the poor quality of the studies. This systematic review did highlight that 25OHD sufficiency is associated with a better lung function by reducing pulmonary infections. Vitamin D supplementation dosages of 50,000 IU taken orally once or twice per week for 28–42 days or a single dose of IM vitamin D of 100,000–600,000 may be most effective at achieving 25OHD sufficiency (>75 nmol/L) and that 25OHD concentration does not appear to be associated to BMI in cypCF. Future clinical trials and mechanistic studies are warranted.

Authorship

All authors have made substantial contributions to all of the following: [1] the conception and design of the study (RRI, GO, DU, ML, PK) or literature searching and screening (RRI, SC, GO), data extraction and analysis (RRI, GO, SC) and interpretation of extracted data (all authors); [2] drafting the article (RRI, SC) and revising it critically for important intellectual content (all authors), [3] final approval of the version to be submitted (all authors).

Declaration of competing interest

There are no conflict of interest to declare.

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