Higher serum 25(OH)D concentrations are associated with improved FEV1 and FVC in adolescence

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ABSTRACT Vitamin D plays a role in the development of the immune system and the lung, as well as in airway remodelling. Therefore, this study investigated the association between serum 25-hydroxyvitamin D (25(OH)D) concentrations and spirometric lung function parameters at age 15 years.

In the German birth cohorts GINIplus and LISAplus, lung function testing by spirometry and 25(OH)D measurements were performed during the 15-year follow-up examinations. Valid lung function measurements pre- and/or post-bronchodilation and serum 25(OH)D concentrations, which were adjusted for the date of blood sampling to account for seasonal variability, were available for 2607 adolescents. Associations between 25(OH)D concentrations and spirometric parameters were analysed using generalised additive models adjusted for confounding factors.

Serum 25(OH)D concentrations were significantly associated with forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1) and FEV_1/FVC measured before bronchodilation after adjustment for potential confounders: FEV_1 increased by 10 mL (95% CI 2–17), FVC by 20 mL (95% CI 12–28) and FEV_1/FVC decreased by 0.177% (95% CI −0.286 to −0.067) per 10 nmol·L^{-1} increase in 25(OH)D concentrations. Flow rates (forced expiratory flow rates at 25, 50 and 75% of exhaled FVC (FEF_{25}, FEF_{50}, FEF_{75}) and mean flow rate between 25 and 75% of FVC ($FEF_{25–75}$)) were not associated with vitamin D. Similar associations were observed for lung function parameters measured after bronchodilation.

Vitamin D concentrations are positively associated with volume-related lung function parameters pre- and post-bronchodilation, suggesting structural changes in peripheral airways.

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Introduction

Humans obtain 10% of their vitamin D from food intake and 90% from endogenous synthesis after sunlight exposure [1]. Vitamin D_3 (cholecalciferol) is metabolised in the liver to 25-hydroxyvitamin D_3, which is then converted in the kidney to its active metabolite 1,25-dihydroxyvitamin D_3. 25-Hydroxyvitamin D (25(OH)D) is the main circulating vitamin D metabolite and can best represent vitamin D status in an individual [1–3]. The serum half-life of 25(OH)D is approximately 3 weeks [3]. As vitamin D production depends on the action of ultraviolet radiation, it shows a seasonal pattern [1, 3].

Besides its role in calcium and phosphate homeostasis, vitamin D is assumed to be related to allergic diseases [4]. Several studies have investigated the effect of vitamin D concentrations on the prevalence of asthma and other allergic diseases, but the findings are inconsistent [1, 4–6]. Some studies found a protective effect of vitamin D on the onset of allergic diseases and asthma, whereas other studies showed an adverse effect or no relationship.

The relationship between vitamin D and allergies might be explained by the immunomodulatory properties of vitamin D [1, 4]. For instance, the production of anti-inflammatory cytokines (such as IL-10) is increased, whereas the production of pro-inflammatory cytokines (such as IL-12) is reduced. Vitamin D can also inhibit the proliferation and growth of smooth muscle cells of the airways and thus play a role in airway remodelling in asthmatics [4, 7].

Although asthma is related to lower lung function, only a few studies have also investigated the association between 25(OH)D concentrations and lung function in children [8, 9], adolescents [10, 11] and adults [10, 12–16]. However, the results were inconclusive, as some studies found a positive relationship with lung function [8, 10], but others found no association [9, 11]. Furthermore, most studies only considered lung function measurements pre-bronchodilation. Consequently, the aim of this study was to analyse the association between serum 25(OH)D concentrations and spirometric lung function parameters, including forced expiratory flow rates, measured pre- and post-bronchodilation, at 15 years of age in two German birth cohorts.

Materials and methods

Study population

Data from two German birth cohorts were combined for this cross-sectional analysis. The GINIplus study (German Infant study on the influence of Nutrition Intervention PLUS Air pollution and Genetics on Allergy development) is an ongoing population-based birth cohort study. A total of 5991 Caucasian neonates were recruited between September 1995 and July 1998 in the German cities of Munich and Wesel. The GINIplus study comprised an intervention arm (n=2252) and an observation (n=3739) arm. Only children with at least one atopic parent or sibling were allocated to the intervention group, which prospectively investigated the effect of different types of hydrolysed formula on allergy development. In the prospective population-based birth cohort LISAplus (Influence of Life-style related factors on the development of the Immune System and Allergies in East and West Germany PLUS the influence of traffic emissions and genetics), 3097 healthy term born neonates were recruited in the German cities of Munich, Wesel, Leipzig and Bad Honnef between December 1997 and January 1999. Three of the originally recruited 3097 subjects withdrew their consent to participate. Details of the study design and recruitment have been described previously [17–20].

Support statement: The GINIplus study was mainly supported for the first 3 years by the Federal Ministry for Education, Science, Research and Technology (interventional arm) and Helmholtz Zentrum Munich (former GSF) (observational arm). The 4-year, 6-year and 10-year follow-up examinations of the GINIplus study were covered by the respective budgets of the five study centres (Helmholtz Zentrum Munich (former GSF), Research Institute at Marien-Hospital Wesel, LMU Munich, TU Munich, and from 6 years onwards also from IUF – Leibniz Research-Institute for Environmental Medicine at the University of Düsseldorf) and a grant from the Federal Ministry for Environment (IUF Düsseldorf, FKZ 20462296). The 15 year follow-up examination of the GINIplus study was partially supported by the Commission of the European Communities, the 7th Framework Programme, MeDALL project, as well as by the companies Mead Johnson and Nestlé. The LISAplus studies were mainly supported for the first 2 years by the Federal Ministry for Education, Science, Research and Technology, the Helmholtz Centre for Environmental Research – UFZ in Leipzig, and the Helmholtz Zentrum Munich (former GSF). The 4-year, 6-year, 10-year and 15-year follow-up examinations were covered from the respective budgets of the four study centres: Helmholtz Zentrum Munich, Research Institute at Marien-Hospital Wesel, and UFZ Leipzig, and also IUF – Leibniz Research Institute for Environmental Medicine at the University of Düsseldorf at age 6 and 10 years. The 15-year follow-up examination of the LISAplus study was also supported by the Commission of the European Communities, the 7th Framework Programme, and the MeDALL project. This work was supported by the Comprehensive Pneumology Center Munich (CPC-M) as a member of the German Center for Lung Research. Funding information for this article has been deposited with the Crossref Funder Registry.

At the 15 year follow-up, 3198 adolescents from the GINIplus study and 1740 adolescents from the LISAplus study participated. Of these 2018 in GINIplus and 1104 in LISAplus underwent physical examinations at follow-up. The analyses in this study are based on a subsample of 2607 adolescents (1729 from GINIplus and 878 from LISAplus) who had valid lung function measurements pre- and/or post-bronchodilation and who provided vitamin D measurements.

Both studies were approved by the respective local ethics committees, and written informed consent was obtained from all participating families.

Lung function testing by spirometry

Spirometry before bronchodilation was performed in line with American Thoracic Society/European Respiratory Society (ATS/ERS) recommendations [21] during the physical examination at the 15 year follow-ups. A pneumotachograph-type spirometer (EasyOne Worldspirometer, ndd, Zurich, Switzerland) was used to obtain flow–volume curves. The participants performed at least three, but not more than eight, trials per test under the guidance of specifically trained examiners. All tests were visually inspected according to the ATS/ERS acceptability criteria [21], resulting in 1822 subjects from GINIplus and 935 subjects from LISAplus with valid lung function measurements pre-bronchodilation.

After completion of baseline spirometry, subjects inhaled salbutamol for the bronchodilator response according to ATS/ERS recommendations [21]. Spirometry was performed 15 min after salbutamol inhalation with 1732 and 915 subjects from GINIplus and LISAplus, respectively, meeting the ATS/ERS acceptability criteria [21].

Spirometric indices were taken from the manoeuvre with the largest sum of forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC). Further parameters evaluated were FEV_1/FVC, peak expiratory flow (PEF), forced expiratory flow rates at 25, 50 and 75% of exhaled FVC (FEF_{25}, FEF_{50} and FEF_{75}) and the mean flow rate between 25 and 75% of FVC ($FEF_{25–75}$). A positive bronchodilation response was defined as an increase of more than 12% and more than 200 mL in FEV_1 and/or FVC measured post-bronchodilation compared to pre-bronchodilation measurements, according to ATS/ERS standards [22]. Standardised *z*-scores were calculated based on the reference equations for spirometry from the Global Lung function Initiative (GLI, http://www.ers-education.org/guidelines/global-lung-function-initiative.aspx) to allow international comparisons [23].

Blood sampling and serum 25(OH)D concentrations

Blood samples were collected during the physical examination of the 15 year follow-ups from 2929 adolescents (1903 and 1026 from GINIplus and LISAplus, respectively). Blood samples were centrifuged after collection and stored frozen at −80°C until assayed for vitamin D.

Total 25(OH)D concentrations in serum were measured by Roche's vitamin D total test on the fully automated Modular system (E170, Roche Diagnostics, Mannheim, Germany). The specificity was reported by the manufacturer as $25(OH)D_2$=92%, $25(OH)D_3$=100%, $1,25(OH)_2D_2$=not detectable, $1,25(OH)_2D_3$=not detectable and $24,25(OH)_2D_3$=149%, and the lower limit of detection was 3 $ng{\cdot}mL^{-1}$. The intra-assay coefficient of variation was 2.2–6.8% for sera with concentrations between 8.35 and 69.6 $ng{\cdot}mL^{-1}$ and the inter-assay coefficient of variation as provided by the manufacturer was 3.4–13.1% for concentrations between 8.35 and 69.6 $ng{\cdot}mL^{-1}$.

Statistical analyses

All analyses were performed using the statistical software R, version 3.1.3 (http://www.R-project.org) [24]. To account for seasonal variability, serum 25(OH)D concentrations were adjusted for the date of blood sampling by fitting a generalised additive model (figure 1) which allows the modelling of a nonlinear association between the dependent and independent variable [25, 26]. Residuals were then added to the overall mean of the vitamin D concentrations. This resulted in season-adjusted vitamin D concentrations with an interpretable range of values [27]. Generalised additive models were also used to analyse the linear association between season-adjusted serum 25(OH)D concentrations and spirometric lung function parameters. By applying these models, potential nonlinear effects of continuous confounders on the outcome variable were taken into account [25].

Two models with different adjustment were fitted for all spirometric outcome variables (separately pre- and post-bronchodilation). Model 1 (minimal) was adjusted for sex, age, height and weight at lung function measurement and a combination of study (GINIplus and LISAplus) and study region (Munich, Wesel, Leipzig and Bad Honnef) and Model 2 (main) was adjusted for all variables included in Model 1 and additionally for parental education, birthweight, maternal smoking during pregnancy, breastfeeding,

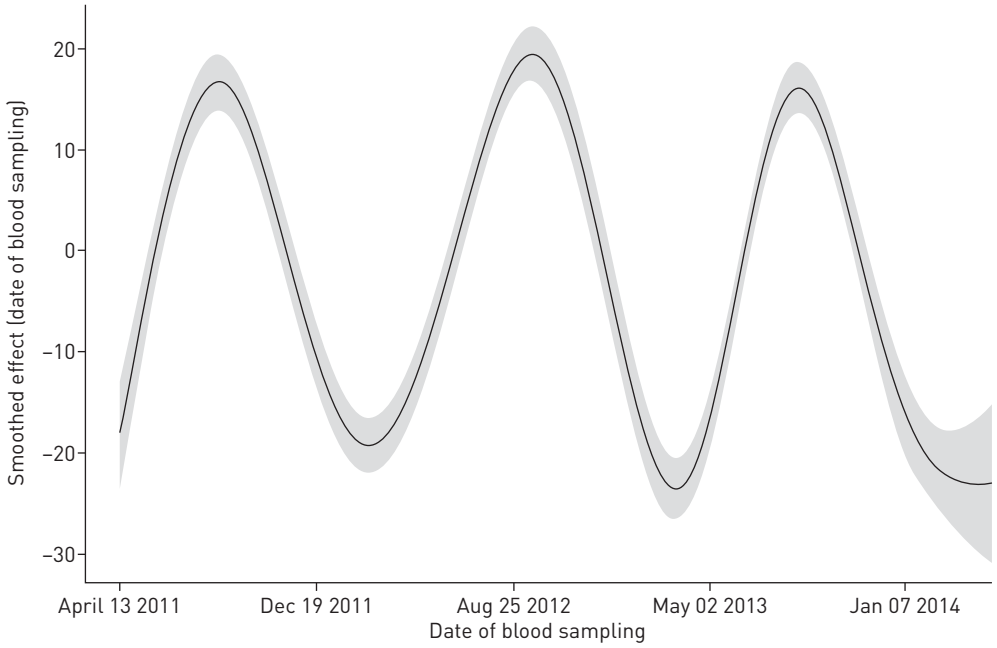

FIGURE 1 Plot showing the seasonal variability of serum 25(OH)D concentrations by fitting a generalised additive model for the smoothed association between the date of blood sampling and serum 25(OH)D concentrations.

parental atopy, exposure to tobacco smoke at home up to age 4 years, time spent in front of a television (TV)/personal computer (PC) and current asthma and/or a positive bronchodilation response.

Three categories, based on the highest number of years either mother or father attended school, were built to define parental education: low for less than 10 years, medium for 10 years, and high for more than 10 years. Parental atopy was defined as either mother or father reported having physician-diagnosed asthma, hay fever, allergic rhinitis, allergic conjunctivitis or atopic eczema. Time spent in front of a TV/PC, which was used as a surrogate for time spent inside, was defined as low if the adolescent spent not more than 1 h during summer and 2 h during winter in front of a TV or PC, high for more than 1 h during summer and more than 2 h during winter, and otherwise as medium. Current asthma was defined based on questions related to physician-diagnosed asthma ever, current wheezing at 15 years of age, and asthma medication at 15 years. An adolescent was defined as asthmatic if at least two of the three questions were answered yes.

Sensitivity analyses were conducted where asthmatics and adolescents with a positive bronchodilation response were excluded.

The results for the association between vitamin D and lung function indices are presented as regression coefficients (β) per 10 nmol·L^{-1} increase in season-adjusted 25(OH)D concentrations with corresponding 95% confidence interval (CI). Differences between males and females were tested using t-test or Pearson's chi-squared test.

Results

Table 1 shows the characteristics of the total study population as well as for males and females separately. The mean of the season-adjusted 25(OH)D concentrations in the total study population was 66.3 nmol·L^{-1}. Mean concentrations did not differ significantly between males (65.7 nmol·L^{-1}) and females (66.9 nmol·L^{-1}). Current asthma and/or a positive bronchodilation response were diagnosed in 10.6% of the study participants, and males tended to have a higher prevalence than females (12.0% *versus* 9.2%).

Mean and standard deviation of the spirometric lung function parameters measured pre- and post-bronchodilation as well as for the corresponding GLI *z*-scores are presented in table 2. Females had a higher mean FEV1/FVC ratio, pre- and post-bronchodilation, compared to males. Regarding the other lung function parameters, males had higher lung function values than females. For the sex-stratified mean GLI *z*-scores it was the other way round. Overall, the means of the GLI *z*-scores pre-bronchodilation were slightly negative.

The results for the association between season-adjusted 25(OH)D concentrations and spirometric lung function measurements pre-bronchodilation are summarised in table 3. In the minimal model, FEV1 and FVC increased by 13 mL (β=0.013, 95% CI 0.007–0.020) and 24 mL (β=0.024, 95% CI 0.017–0.031), respectively, per 10 nmol·L^{-1} increase in season-adjusted 25(OH)D concentrations. These associations also

TABLE 1 Study characteristics of participants at the 15 year follow-up, with valid lung function and vitamin D measurements

	Male	Female	Total
Subjects n	1301	1306	2607
Study and region			
Munich GINI	32.9 (428/1301)	34.3 (448/1306)	33.6 (876/2607)
Munich LISA	16.1 (209/1301)	14.2 (186/1306)	15.2 (395/2607)
Wesel GINI	32.1 (418/1301)	33.3 (435/1306)	32.7 (853/2607)
Wesel LISA	3.8 (49/1301)	3.7 (48/1306)	3.7 (97/2607)
Leipzig	10.9 (142/1301)	10.3 (134/1306)	10.6 (276/2607)
Bad Honnef	4.2 (55/1301)	4.2 (55/1306)	4.2 (110/2607)
Sex			
Male	100.0 (1301/1301)		49.9 (1301/2607)
Female		100.0 (1306/1306)	50.1 (1306/2607)
Age years	15.2±0.3	15.2±0.3	15.2±0.3
Weight at 15 years kg[#]	64.9±12.9	58.5±9.7	61.7±11.8
Height at 15 years cm[#]	176.2±7.6	166.9±6.2	171.5±8.4
Season adjusted vitamin D concentration nmol·L^{-1}	65.7±24.0	66.9±25.1	66.3±24.6
Birthweight g[#]	3537.1±461.2	3412.9±439.7	3474.8±454.7
Parental education			
Low	5.8 (76/1301)	5.5 (72/1304)	5.7 (148/2605)
Medium	27.9 (363/1301)	27.0 (352/1304)	27.4 (715/2605)
High	66.3 (862/1301)	67.5 (880/1304)	66.9 (1742/2605)
Exclusive breastfeeding			
No	21.8 (270/1239)	21.0 (263/1250)	21.4 (533/2489)
1–4 months	29.9 (371/1239)	28.8 (360/1250)	29.4 (731/2489)
>4 months	48.3 (598/1239)	50.2 (627/1250)	49.2 (1225/2489)
Smoking during pregnancy			
No	88.5 (1068/1207)	86.8 (1029/1186)	87.6 (2097/2393)
Yes	11.5 (139/1207)	13.2 (157/1186)	12.4 (296/2393)
Parental atopy			
No	41.6 (526/1263)	42.6 (540/1269)	42.1 (1066/2532)
Yes	58.4 (737/1263)	57.4 (729/1269)	57.9 (1466/2532)
Exposure to tobacco smoke at home up to age 4 years			
No	61.9 (767/1240)	62.0 (764/1233)	61.9 (1531/2473)
Yes	38.1 (473/1240)	38.0 (469/1233)	38.1 (942/2473)
Time spent in front of TV/PC[#]			
Low	12.0 (153/1271)	19.5 (249/1279)	15.8 (402/2550)
Medium	27.6 (351/1271)	32.4 (415/1279)	30.0 (766/2550)
High	60.3 (767/1271)	48.1 (615/1279)	54.2 (1382/2550)
Current asthma			
No	93.1 (1201/1290)	94.2 (1211/1286)	93.6 (2412/2576)
Yes	6.9 (89/1290)	5.8 (75/1286)	6.4 (164/2576)
Positive bronchodilation at age 15 years[#]			
No	94.9 (1143/1205)	96.7 (1183/1224)	95.8 (2326/2429)
Yes	5.1 (62/1205)	3.3 (41/1224)	4.2 (103/2429)
Current asthma and/or positive bronchodilation[#]			
No	88.0 (1054/1198)	90.8 (1101/1212)	89.4 (2155/2410)
Yes	12.0 (144/1198)	9.2 (111/1212)	10.6 (255/2410)

Data are presented as mean±SD or % (n/N), unless otherwise stated. [#]: significant difference between males and females (tested using chi-squared test or t-test).

remained statistically significant in the main model after adjustment for further confounding factors (FEV_1: β=0.010, 95% CI 0.002–0.017; FVC: β=0.020, 95% CI 0.012–0.028). The ratio of FEV_1 to FVC was negatively associated with 25(OH)D; *i.e.* it decreased by 0.182% (β=−0.182, 95% CI −0.281 to −0.084) in the minimal model and 0.177% (β=−0.177, 95% CI −0.286 to −0.067) in the main model. Similar associations were observed when considering the *z*-scores calculated according to GLI (FEV_1: β=0.021, 95% CI 0.005–0.037; FVC: β=0.039, 95% CI 0.023–0.055; FEV_1/FVC: β=−0.029, 95% CI −0.046 to −0.011 in the main models). Flow rates (FEF_{25}, FEF_{50}, FEF_{75}), mean flow rate ($FEF_{25–75}$) and peak expiratory flow (PEF) were not significantly associated with 25(OH)D concentrations, neither in the minimal nor in the main model.

Table 4 presents the results for the associations between vitamin D and lung function measured post-bronchodilation. As for the measurements pre-bronchodilation, FEV_1 and FVC were positively significantly

TABLE 2 Characteristics of spirometric lung function parameters measured pre- and post-bronchodilation, for study participants with valid vitamin D measurements

	Male	Female	Total
Subjects n	1301	1306	2607
Before bronchodilation			
FEV_1 L#	3.82±0.64	3.20±0.42	3.50±0.62
FVC L#	4.51±0.76	3.64±0.49	4.07±0.77
FEV_1/FVC %#	84.95±6.32	88.12±6.10	86.55±6.41
PEF $L{\cdot}s^{-1}$#	7.64±1.30	6.48±0.97	7.06±1.28
FEF_{25} $L{\cdot}s^{-1}$#	6.52±1.26	5.85±0.95	6.18±1.16
FEF_{50} $L{\cdot}s^{-1}$#	4.64±1.11	4.20±0.90	4.42±1.03
FEF_{75} $L{\cdot}s^{-1}$#	2.28±0.77	2.12±0.64	2.20±0.71
FEF_{25-75} $L{\cdot}s^{-1}$#	4.07±0.99	3.71±0.80	3.89±0.91
z-score FEV_1 GLI	−0.59±0.95	−0.52±0.88	−0.56±0.92
z-score FVC GLI#	−0.56±0.96	−0.45±0.88	−0.50±0.92
z-score FEV_1/FVC GLI	−0.11±1.00	−0.12±1.00	−0.11±1.00
z-score FEF_{75} GLI	−0.14±0.94	−0.11±0.92	−0.13±0.93
z-score FEF_{25-75} GLI#	−0.49±0.95	−0.37±0.93	−0.43±0.94
After bronchodilation			
FEV_1 L#	3.94±0.65	3.28±0.42	3.61±0.64
FVC L#	4.50±0.75	3.63±0.49	4.06±0.77
FEV_1/FVC %#	87.71±5.58	90.68±5.17	89.20±5.58
PEF $L{\cdot}s^{-1}$#	7.78±1.31	6.66±0.98	7.22±1.28
FEF_{25} $L{\cdot}s^{-1}$#	6.78±1.24	6.08±0.96	6.43±1.16
FEF_{50} $L{\cdot}s^{-1}$#	5.09±1.10	4.60±0.87	4.84±1.02
FEF_{75} $L{\cdot}s^{-1}$#	2.64±0.85	2.47±0.70	2.55±0.78
FEF_{25-75} $L{\cdot}s^{-1}$#	4.50±1.00	4.11±0.78	4.30±0.92
z-score FEV_1 GLI	−0.34±0.95	−0.30±0.88	−0.32±0.92
z-score FVC GLI#	−0.56±0.96	−0.46±0.90	−0.51±0.93
z-score FEV_1/FVC GLI	0.33±0.92	0.31±0.91	0.32±0.92
z-score FEF_{75} GLI	0.31±0.91	0.38±0.91	0.34±0.91
z-score FEF_{25-75} GLI#	−0.05±0.89	0.11±0.89	0.03±0.89

Data are presented as mean±SD. FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; PEF: peak expiratory flow; FEF_{25}, FEF_{50}, FEF_{75}: forced expiratory flow rates at 25, 50 and 75% of exhaled FVC; FEF_{25-75}: mean flow rate between 25 and 75% of FVC; GLI: *z*-scores according to the Global Lung function Initiative [23]. #: significant difference between males and females (tested using t-test).

related to 25(OH)D concentrations (main model: FEV_1: β=0.011, 95% CI 0.004–0.018; FVC: β=0.019, 95% CI 0.011–0.027), whereas FEV_1/FVC showed a negative relationship (main model: β=−0.139, 95% CI −0.236 to −0.042). Comparable results were observed for the GLI *z*-scores.

Excluding asthmatics and those showing a positive bronchodilation response did not substantially affect the associations (figures 2 and 3).

Discussion

Summary

This cross-sectional study investigated the association between serum 25(OH)D concentrations and spirometric lung function indices measured before and after bronchodilation at 15 years of age. Serum 25(OH)D concentrations were positively associated with the volume-related lung function parameters FEV_1 and FVC, and stronger effects were observed for FVC than FEV_1. As the results were similar for lung function parameters measured pre- and post-bronchodilation, structural changes in peripheral airways are suggested. The findings did not substantially change if analyses were restricted to apparently lung healthy subjects, excluding those with positive bronchodilation response or asthma.

Results from other studies

Several previous cross-sectional studies have analysed the association between vitamin D and lung function, but only a few studies have focused on adolescents [8, 10, 11, 28]. For instance, the study by YAO *et al.* [8], conducted in 1282 Taiwanese children and adolescents aged 5 to 18 years, observed positive and significant

TABLE 3 Results for the association between season-adjusted 25-hydroxyvitamin D concentrations per 10 nmol·L^{-1} increase and lung function measurements pre-bronchodilation

	Model 1[#]			Model 2[¶]		
	β	95% CI	p-value	*β*	95% CI	p-value
FEV_1	**0.013**	0.007–0.020	**<0.001**	**0.010**	0.002–0.017	**0.010**
FVC	**0.024**	0.017–0.031	**<0.001**	**0.020**	0.012–0.028	**<0.001**
FEV_1/FVC	**−0.182**	−0.281–−0.084	**<0.001**	**−0.177**	−0.286–−0.067	**0.002**
PEF	0.013	−0.004–0.029	0.125	0.014	−0.004–0.033	0.135
FEF_{25}	−0.001	−0.018–0.016	0.896	0.004	−0.015–0.023	0.690
FEF_{50}	0.000	−0.015–0.016	0.975	−0.003	−0.020–0.015	0.773
FEF_{75}	−0.005	−0.015–0.006	0.419	−0.008	−0.021–0.004	0.182
$FEF_{25–75}$	0.000	−0.014–0.014	0.993	−0.004	−0.019–0.011	0.619
z-score FEV_1 GLI	**0.028**	0.014–0.042	**<0.001**	**0.021**	0.005–0.037	**0.010**
z-score FVC GLI	**0.046**	0.032–0.060	**<0.001**	**0.039**	0.023–0.055	**<0.001**
z-score FEV_1/FVC GLI	**−0.029**	−0.045–−0.014	**<0.001**	**−0.029**	−0.046–−0.011	**0.002**
z-score FEF_{75} GLI	−0.006	−0.021–0.009	0.465	−0.010	−0.027–0.007	0.250
z-score $FEF_{25–75}$ GLI	−0.001	−0.016–0.014	0.914	−0.004	−0.021–0.013	0.619

Values presented in bold are statistically significant at p<0.05. FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; PEF: peak expiratory flow; FEF_{25}, FEF_{50}, FEF_{75}: forced expiratory flow rates at 25, 50 and 75% of exhaled FVC; $FEF_{25–75}$: mean flow rate between 25 and 75% of FVC; GLI: *z*-scores according to the Global Lung function Initiative [23]. [#]: adjusted for sex, study and study region, age, height and weight at lung function testing. [¶]: adjusted for all variables in Model 1 plus birthweight, time spent in front of a TV/PC, current asthma and/or positive bronchodilation response, exposure to tobacco smoke at home up to age 4 years, parental education, parental atopy, maternal smoking during pregnancy and breastfeeding.

associations between serum 25(OH)D concentrations and FEV_1 and FVC: per ng·mL^{-1} increase in serum 25(OH)D, FEV_1 increased by 2.4 mL and FVC by 3.6 mL. As these effect sizes correspond to increases by 9.6 mL and 14.4 mL for FEV_1 and FVC, respectively, per 10 nmol·L^{-1} increase in serum 25(OH)D, these

TABLE 4 Results for the association between season-adjusted 25-hydroxyvitamin D concentrations per 10 nmol·L^{-1} increase and lung function measurements post-bronchodilation

	Model 1[#]			Model 2[¶]		
	β	95% CI	p-value	*β*	95% CI	p-value
FEV_1	**0.014**	0.007–0.020	**<0.001**	**0.011**	0.004–0.018	**0.004**
FVC	**0.023**	0.015–0.030	**<0.001**	**0.019**	0.011–0.027	**<0.001**
FEV_1/FVC	**−0.148**	−0.235–−0.061	**0.001**	**−0.139**	−0.236–−0.042	**0.005**
PEF	0.016	−0.001–0.033	0.060	0.016	−0.003–0.035	0.091
FEF_{25}	0.004	−0.013–0.021	0.617	0.011	−0.008–0.030	0.259
FEF_{50}	0.007	−0.008–0.022	0.380	0.005	−0.012–0.022	0.578
FEF_{75}	−0.005	−0.017–0.007	0.449	−0.010	−0.023–0.004	0.168
$FEF_{25–75}$	0.002	−0.011–0.016	0.723	−0.001	−0.016–0.015	0.934
z-score FEV_1 GLI	**0.030**	0.016–0.045	**<0.001**	**0.025**	0.008–0.041	**0.003**
z-score FVC GLI	**0.044**	0.030–0.058	**<0.001**	**0.038**	0.022–0.053	**<0.001**
z-score FEV_1/FVC GLI	**−0.025**	−0.040–−0.010	**0.001**	**−0.024**	−0.041–−0.007	**0.005**
z-score FEF_{75} GLI	−0.006	−0.021–0.009	0.462	−0.011	−0.028–0.006	0.199
z-score $FEF_{25–75}$ GLI	0.002	−0.012–0.017	0.756	0.000	−0.017–0.016	0.956

Values presented in bold are statistically significant at p<0.05. FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; PEF: peak expiratory flow; FEF_{25}, FEF_{50}, FEF_{75}: forced expiratory flow rates at 25, 50 and 75% of exhaled FVC; $FEF_{25–75}$: mean flow rate between 25 and 75% of FVC; GLI: *z*-scores according to the Global Lung function Initiative [23]. [#]: adjusted for sex, study and study region, age, height and weight at lung function testing. [¶]: adjusted for all variables in Model 1 plus birthweight, time spent in front of a TV/PC, current asthma and/or positive bronchodilation response, exposure to tobacco smoke at home up to age 4 years, parental education, parental atopy, maternal smoking during pregnancy and breastfeeding.

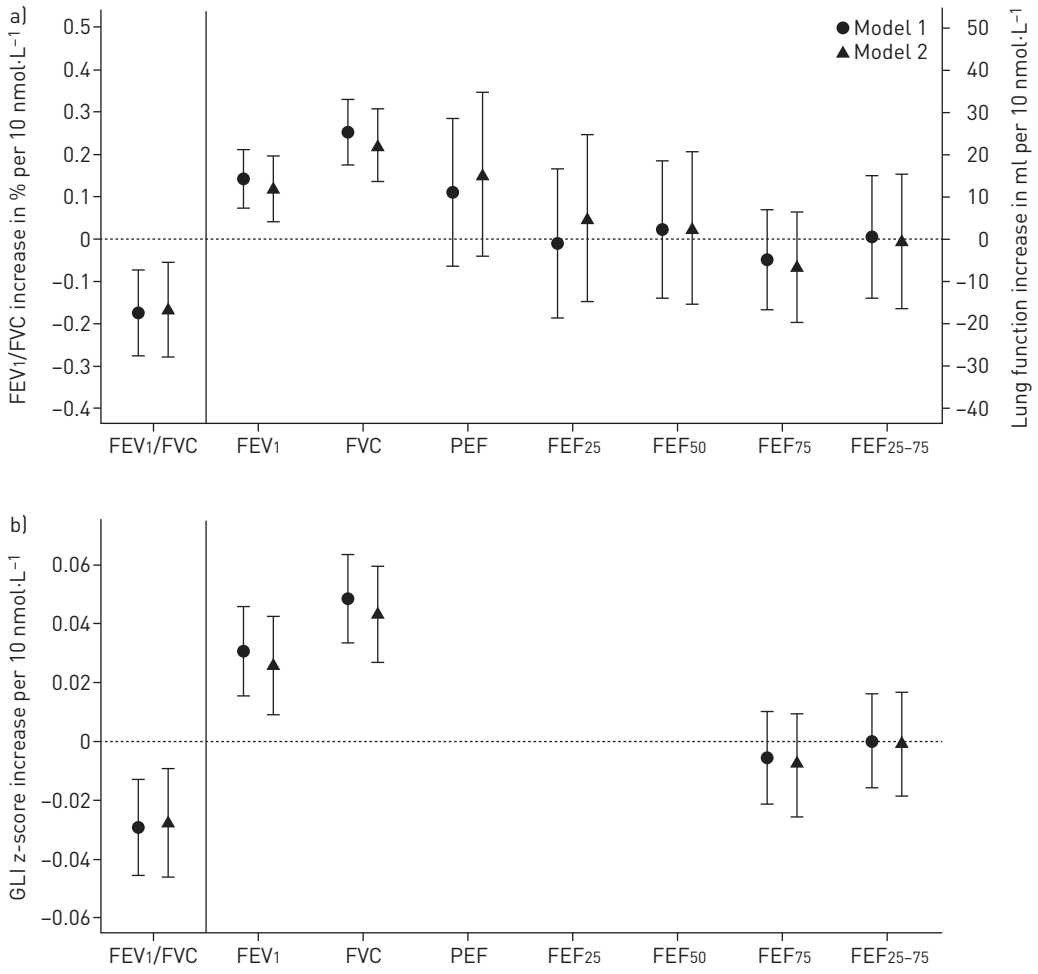

FIGURE 2 Results for the association between season-adjusted 25(OH)D concentrations per 10 nmol·L^{-1} increase with a) spirometric lung function measurements and b) GLI *z*-scores pre-bronchodilation for the study population restricted to non-asthmatics and subjects with no positive bronchodilation response. Model 1: adjusted for sex, study and study region, age, height and weight at lung function testing. Model 2: adjusted for all variables in Model 1 plus birthweight, time spent in front of a TV/PC, exposure to tobacco smoke at home up to age 4 years, parental education, parental atopy, maternal smoking during pregnancy and breastfeeding. FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; PEF: peak expiratory flow; FEF_{25}, FEF_{50}, FEF_{75}: forced expiratory flow rates at 25, 50 and 75% of exhaled FVC; FEF_{25-75}: mean flow rate between 25 and 75% of FVC; GLI: *z*-scores according to the Global Lung function Initiative.

effects are comparable to the results of the current study. Because, in the study by Yao *et al.* [8] as well as in the current study, stronger effects were observed for FVC than for FEV_1, the associations with vitamin D might be indicative of changes in the lung volume rather than airway alterations with airflow limitations.

By contrast, in 3735 adolescents and adults aged 13 to 69 years participating in the Canadian Health Measures Survey [28], 25(OH)D concentrations were significantly negatively associated with FEV_1/FVC, but no association was found with FEV_1 and FVC. Tolppanen and co-workers [10, 11] investigated the same research question in two studies. In the first study, which was a cross-sectional study in adolescents aged 12–19 years and adults aged 20–59 years from the third National Health and Nutrition Examination Survey, a positive association between serum 25(OH)D concentrations and FVC was found in adolescents as well as in adults [10]. The relationship with FEV_1 was significant among adults, but not among adolescents. However, the effect of serum 25(OH)D concentrations was stronger for FVC than for FEV_1, irrespective of the significance of the results. In the second study, which was based on data from the population-based Avon Longitudinal Study of Parents and Children, spirometric lung function parameters measured after bronchodilation were taken into account [11]. In this prospective study, 25(OH)D concentrations were measured at the age of 10 years, whereas lung function testing was performed at the age of 15 years. Furthermore, a differentiation between $25(OH)D_2$ and $25(OH)D_3$ was included: $25(OH)D_2$ concentrations were weakly associated with FEV_1 and FVC, whereas $25(OH)D_3$ concentrations were not

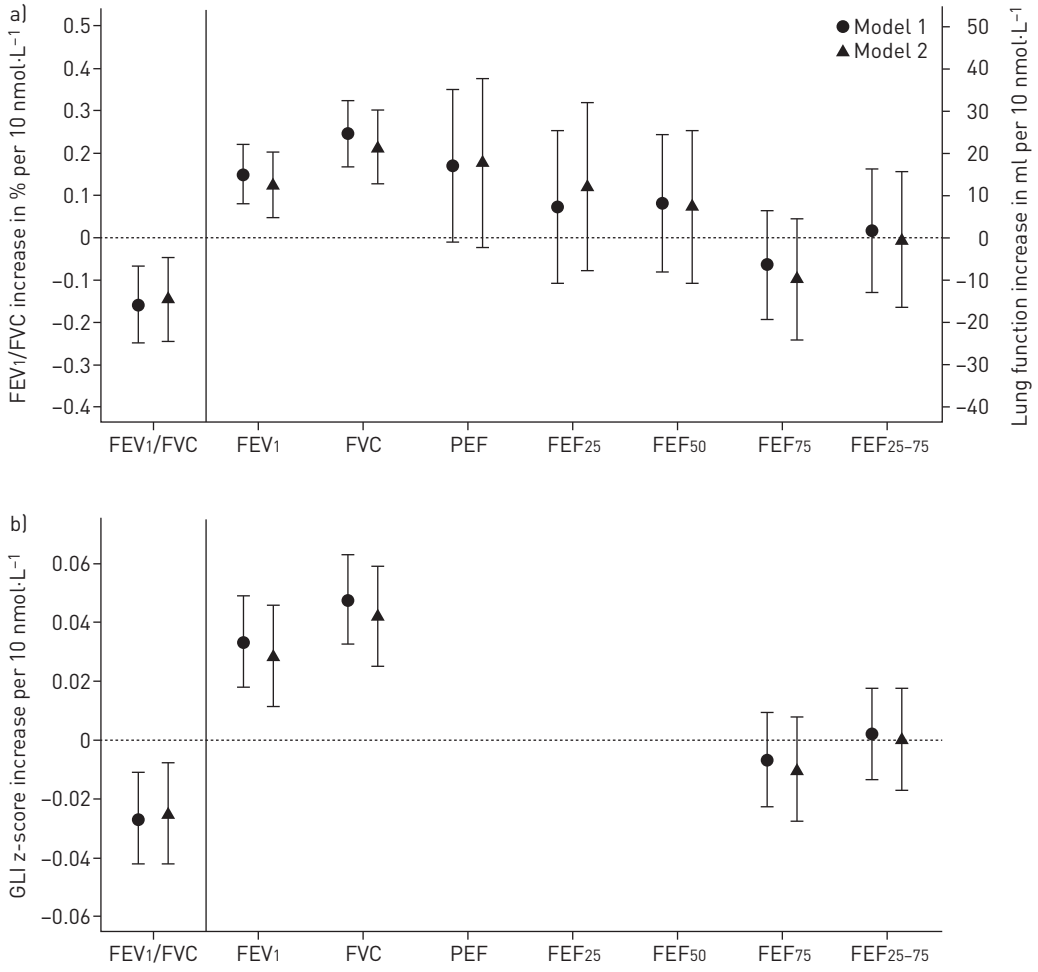

FIGURE 3 Results for the association between season-adjusted 25(OH)D concentrations per 10 nmol·L^{-1} increase with a) spirometric lung function measurements and b) GLI *z*-scores post-bronchodilation for the study population restricted to non-asthmatics and subjects with no positive bronchodilation response. Model 1: adjusted for sex, study and study region, age, height and weight at lung function testing. Model 2: adjusted for all variables in Model 1 plus birthweight, time spent in front of a TV/PC, exposure to tobacco smoke at home up to age 4 years, parental education, parental atopy, maternal smoking during pregnancy and breastfeeding. FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; PEF: peak expiratory flow; FEF_{25}, FEF_{50}, FEF_{75}: forced expiratory flow rates at 25, 50 and 75% of exhaled FVC; FEF_{25-75}: mean flow rate between 25 and 75% of FVC; GLI: *z*-scores according to the Global Lung function Initiative.

associated with lung function. However, none of these studies reported results for lung function parameters measured pre- as well as post-bronchodilation.

The different results of the mentioned studies might also be due to different confounding variables and different measurement methods [29] for the 25(OH)D concentrations. Only the study by Yao *et al.* [8] used the same assay to measure vitamin D concentrations as was used in the GINIplus and LISAplus studies. As the vitamin D concentrations were categorised in some studies, 25(OH)D measurements are not directly comparable between studies.

In summary, although there are differences in the studies regarding the association between vitamin D concentrations and lung function in adolescence, the findings are related to FVC, suggesting an involvement of peripheral lung structures.

Biological mechanisms

The exact mechanisms underlying the association between vitamin D and lung function are not completely understood. However, there are some hypothesised mechanisms that might explain this relationship. One possible explanation is the role vitamin D plays in airway remodelling [30]. Airway remodelling occurs early in asthmatics and is characterised by increased airway smooth muscle mass, fibrosis with alterations in extracellular matrix composition as well as subepithelial membrane thickening, all indicating structural

changes [30, 31]. As the results of the current study have shown similar associations for lung function measurements pre- and post-bronchodilation, structural, and not functional, changes in the lung are suggested, which would support the hypothesis of vitamin D influencing airway remodelling. Another hypothesis might be related to the influence of vitamin D on the development and modulation of the immune system [1, 32, 33]. For instance, the active vitamin D metabolite can lead to an inhibition of the activation and proliferation of T-lymphocytes [32, 34]. It has been shown that Th1-associated cytokine production is inhibited, whereas Th2 responses could be inhibited as well as enhanced [32]. Both Th1 and Th2 cells are involved in asthma by producing pro- and anti-inflammatory cytokines. Furthermore, vitamin D might support regulatory T-cells, which inhibit airway hyperresponsiveness and airway inflammation [32]. Lung development, growth and maturation as well as surfactant secretion might also be affected by vitamin D *via* its receptors present in fetal type II alveolar cells [30, 32, 35]. The study by Zosky *et al.* [36, 37] has shown that vitamin D-deficient mice have a significantly reduced lung volume, in terms of thoracic gas volume, compared to vitamin D-replete mice. Lower vitamin D concentrations also resulted in reduced numbers of alveoli in female mice. Furthermore, an influence of vitamin D on the alveolar epithelial–mesenchymal interactions was observed in the study by Sakurai *et al.* [37, 38]. The active vitamin D metabolite inhibits apoptosis and thus increases alveolar type II cell proliferation. Besides surfactant synthesis, the production of interstitial fibres, *i.e.* collagen type I alpha 1, is affected by vitamin D deficiency [39]. These experimental studies support the hypothesis that vitamin D affects the alveolar region of the lung, causing changes in lung volume rather than airflow limitations, which is in line with our and other findings that FVC is mainly affected by serum 25(OH)D concentrations.

Besides these mechanisms, genetic variation and epigenetic mechanisms might also be involved in regulating the relationship between vitamin D concentrations and respiratory health [40, 41].

Strengths and limitations

One of the strengths of this study is the lung function measurement pre- and post-bronchodilation, as well as the inclusion of forced flow rates by spirometry. As lung function testing was also performed after inhalation of salbutamol, which has an airway-widening effect, this study allowed to investigate whether the association between vitamin D concentrations and lung function parameters is only based on reversible lung function impairment or whether it is rather based on fixed, structural changes of the lung. Furthermore, parameters of small airways and airway narrowing were also measured as well as the commonly used parameters indicating lung volume and size and airway obstruction. Another strength of this study is that the serum 25(OH)D concentrations were adjusted for the exact date of blood sampling to carefully correct for the seasonal variation of vitamin D. Most other studies only adjusted for season of sampling.

Besides these strengths, this study also has some limitations. As with most other studies, our findings are based on a cross-sectional study design. Thus, reverse causation cannot be ruled out, even though the biological mechanisms probably do not support the notion. Theoretically, it is possible that lung function affects vitamin D concentrations, *i.e.* adolescents having asthma and therefore a reduced lung function are less active and spend less time outdoors. However, sensitivity analyses restricted to non-asthmatics and those without a positive bronchodilation response were conducted and showed similar results. We therefore consider a reverse causation unlikely. Moreover, the standardised *z*-scores according to GLI did not fit very well to the current study population [42]. Thus, the regression models calculated for the association between serum 25(OH)D concentrations and GLI *z*-scores were also adjusted for sex as well as age and height at lung function measurement. It is thus unlikely that measurement error or insufficient standardisation errors for lung function data would introduce major bias. Another limitation of the current study might be the restricted generalisability of these findings to the general German population of adolescents, because only four regions with two metropolitan areas were used to recruit study subjects. In addition, participants of parents with a high socioeconomic status are overrepresented in the GINIplus and LISAplus studies. However, whether the participants were assigned to the intervention (with hydrolysed *versus* cow's milk formulae) or the observation arm of the GINIplus study did not affect the observed associations (data not shown). Another common problem in prospective birth cohorts is loss to follow-up.

Conclusion

The results of this study suggest that higher vitamin D concentrations are associated with higher lung volume-related spirometric parameters in adolescence. Similar associations were observed for lung function parameters measured pre- and post-bronchodilation, suggesting structural changes in peripheral airways.

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