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Serum vitamin D levels and exercise-induced bronchoconstriction in children with asthma

I. Chinellato, M. Piazza, M. Sandri, D.G. Peroni, F. Cardinale, G.L. Piacentini and A.L. Boner

ABSTRACT: Epidemiological studies have established a relationship between low levels of serum vitamin D and reduced lung function in healthy adults, and asthma onset and severity in children. However, no study has examined the relationship between vitamin D levels and exercise-induced bronchoconstriction in asthmatic children.

We evaluated the relationship between 25-hydroxyvitamin D concentrations and baseline forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1) and change in FEV_1 (ΔFEV_1) after a standardised exercise challenge in 45 children with intermittent asthma.

Only 11% of the children had desirable serum vitamin D levels (at least 30–40 $ng \cdot mL^{-1}$). A positive correlation was found between serum 25-hydroxyvitamin D and both FVC ($r=0.34$; $p=0.037$) and FEV_1 ($r=0.32$; $p=0.037$). Subjects with a positive response to the exercise challenge ($\Delta FEV_1 \geqslant 10\%$) presented lower serum levels of 25-hydroxyvitamin D than children with a negative challenge (mean $\pm$ SD 16.2 ± 5.2 *versus* 23.4 ± 7.0 $ng \cdot mL^{-1}$, respectively; $p=0.001$).

Our results indicate that hypovitaminosis D is frequent in asthmatic children who live in a Mediterranean country. In those children, lower levels of vitamin D are associated with reduced lung function and increased reactivity to exercise.

KEYWORDS: Childhood asthma, exercise-induced asthma, lung functions, serum vitamin D levels

AFFILIATIONS
Dept of Paediatrics, University of Verona, Verona, Italy.

CORRESPONDENCE
A.L. Boner
Dept of Paediatrics
Policlinico GB Rossi
Piazzale L. Scuro
37134 Verona
Italy
E-mail: attilio.boner@univr.it

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Vitamin D exerts many of its effects through contact with vitamin D receptors [1], which have been found in a variety of cells, including lung cells [2] and many cells of the immune system [3]. The finding that most tissues and cells in the body have vitamin receptors and that several possess the enzymatic apparatus to synthesise the active form 1,25-dihydroxyvitamin D from the primary vitamin D, 25-hydroxyvitamin D, has provided new insight into the role of this vitamin deficiency in several diseases [4], including asthma [5].

Although previous data have suggested that vitamin D deficiency could be related to onset of asthma [6, 7], only recently has an association between low serum levels of vitamin D and markers of asthma severity been described in children [8–10] and adults [11] with asthma.

The aim of our study was to correlate serum vitamin D levels with severity of exercise-induced bronchoconstriction (EIB) in children with intermittent asthma.

PATIENTS AND METHODS

Study population

45 Italian children with asthma, diagnosed according to the American Thoracic Society (ATS) guidelines [12] and consecutively seen in the outpatient clinic of the Dept of Paediatrics of the University of Verona Hospital (Verona, Italy), were enrolled in the study. Only patients with intermittent asthma were admitted to the study in order to avoid the interference of regular treatment in bronchial hyperresponsiveness and, consequently, on the results of the exercise challenge. 59 children with no asthma served as healthy controls.

The study was approved by the hospital ethical committee, and the parents and children gave their informed consent to be enrolled in the study.

Vitamin D status definitions

A single measurement of vitamin D concentration (measured as 25-hydroxyvitamin D) was obtained from all subjects using a radioimmunoassay method. Vitamin D level values were used

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as a continuous variable, and were categorised in descriptive analyses as desirable (or sufficient) when scores were at least 30–40 ng·mL^{-1} (75–100 nmol·L^{-1}), insufficient between 20 and 30 ng·mL^{-1} (50 and 75 nmol·L^{-1}), and deficient when <20 ng·mL^{-1}, as previously recommended [4, 13].

Spirometry

Measurements of lung function, the best of three blows according to ATS criteria [14], were taken from full forced vital capacity (FVC) manoeuvres using an electronic spirometer (Master Screen IOS; Jaeger, Hoechberg, Germany) calibrated before the arrival of each subject, using a 3-L syringe (Cardinal Health Germany 234 GmbH, Hoechberg). The FVC manoeuvres were carried out with the child standing and wearing a nose clip. Subjects were instructed to avoid using short-acting bronchodilators for ⩾6 h prior to undergoing testing.

Exercise challenge test

A standardised exercise test protocol was used [15, 16]. Briefly, the children performed baseline spirometry and then ran for 6 min on a treadmill at a speed able to achieve a heart rate that was 85% of the maximum predicted value (220 - age in yrs) by the end of the exercise period. The laboratory was air-conditioned (19–21°C; relative humidity <50%) during all exercise tests. Nose clips were applied to ensure breathing through the mouth. After the exercise challenge, forced expiratory volume in 1 s (FEV_1) was measured at 1, 3, 5, 10, 15, 20 and 30 min after completing the exercise tests (single blows). The severity of exercise-induced asthma was expressed as the maximum change in FEV_1 (ΔFEV_1) from the baseline value of lung function after exercise. Children whose FEV_1 decreased by ⩾10% were classified as having EIB. No child undergoing long-term treatment was admitted to the study.

Statistical analysis

The statistical distributions of vitamin D level, FEV_1 and FVC were approximately Gaussian (p-values of the Shapiro–Wilk test for normal data 0.257, 0.599 and 0.837, respectively). However, ΔFEV_1 was markedly skewed and normality was rejected ($p<0.001$).

The degree of association between vitamin D levels, and baseline FVC and FEV_1 after removing the effect of potential confounders (sex, age and sensitivity to different allergens) was quantified using partial linear correlations [17]. The association between vitamin D and ΔFEV_1 was also measured by Spearman's rank correlation. Differences between mean levels of vitamin D and % predicted baseline FEV_1 in subjects with negative exercise challenge were tested using the unpaired t-test with equal variances. The effect of outliers and influential observations on estimated correlations was evaluated by first identifying critical sample units by the leverage *versus* r^2-plot, and then re-estimating correlations without these units.

p-values <0.05 were considered statistically significant. All calculations were performed using the Stata 11.0 statistical package (StataCorp, College Station, TX, USA).

RESULTS

Demographic data of patients and healthy controls are reported in table 1.

As can be seen, healthy subjects presented superior lung function values to patients, but there was no difference in sex, age, body mass index or serum vitamin D levels. Five (11.1%) patients and seven (11.9%) controls had sufficient vitamin D levels (⩾30 ng·mL^{-1}). Insufficient levels (between 20 and 30 ng·mL^{-1}) were found in 17 (37.8%) patients and in 27 (45.8%) controls. The remaining 23 (51.1%) patients and 25 (42.4%) healthy subjects showed deficient levels (<20 ng·mL^{-1}). There was no significant difference in median serum vitamin D levels between controls and patients.

The relationships between vitamin D levels and patients' baseline FVC and FEV_1 are shown in figure 1. A positive, moderate, statistically significant partial correlation was found between serum vitamin D level and FVC (r=0.34, 95% CI 0.06–0.61; p=0.037). A similar partial correlation was observed for FEV_1 (r=0.32, 95% CI 0.04–0.60; p=0.037). The relationship between individual serum vitamin D values and ΔFEV_1 is shown in figure 2. Again, a positive, statistically significant association was found (Spearman's correlation 0.40 (95% CI 0.12–0.62; p=0.007) and partial correlation r=0.48 (95% CI 0.28–0.68 (p=0.001)). No child with sufficient vitamin D levels showed a positive response to exercise challenge. The above correlations were not significantly influenced by outliers and values at the extremes of the vitamin D range.

21 (46.7%) children showed a positive result following the exercise challenge. In those children, mean±SD vitamin D levels were significantly lower than those observed in subjects with a negative exercise challenge: 16.2±5.2 *versus* 23.4±7.0 ng·mL^{-1}, respectively (p<0.001).

Mean±SD baseline FEV_1 did not differ significantly between children with a positive response to exercise challenge and those with a negative test response: 99.6±13.6 *versus* 107.0±16.6% pred, respectively (p=0.113).

DISCUSSION

To our knowledge, there have been no studies on the relationship between vitamin D status and the prevalence of EIB. The results of our study are in agreement with and further extend the findings of recent studies on the relationship between vitamin D deficiency and asthma severity [8–10]. In a study on Costa Rican asthmatic children, 28% showed insufficient levels of vitamin D (<30 ng·mL^{-1}), and an inverse relationship was observed between 25-hydroxyvitamin D levels and total immunoglobulin E, eosinophil count, use of anti-inflammatory medication in the previous year, increased airway responsiveness to methacholine and any hospitalisation in the previous year. In our study, only 11% of the patients and 12% of healthy controls had desirable 25-hydroxyvitamin D levels (⩾30 ng·mL^{-1}), and this finding was not completely unexpected, as the study was conducted during the winter/early-spring time and our town is located at a latitude of 45°27′ N. People living above the 35th latitude cannot produce adequate pre-vitamin D3 during the winter [18], and in our country (Italy) foods are not fortified with vitamin D [19]. The fact that almost all our children had insufficient or deficient levels of 25-hydroxyvitamin D is worrying; although those levels have been set for optimal calcium, phosphorus and bone metabolism, there are proposals that concentrations >40 ng·mL^{-1} (100 nmol·L^{-1}) may be essential for optimal

TABLE 1 Demographic data, serum vitamin D levels and lung function of controls and patients

	Controls	Patients	p-value#	ΔFEV1 <10%	ΔFEV1 ⩾10%	p-value¶
Subjects n	59	45		24	21	
Females	23 (39)	18 (40)	0.916	9 (37.5)	9 (42.9)	0.767
Age yrs	10 (9–2)	10 (9–11)	0.717	10 (9–11)	9 (9–11)	0.620
Weight kg	37.5 (30.0–50.0)	39.0 (31.0–49.0)	0.738	41.5 (30.0–46.5)	38 (35–51)	0.554
Height cm	142 (133–154)	143 (136–151)	0.875	143 (134–151)	143 (140–151)	0.873
BMI kg·m^{-2}	18.6 (16.8–20.8)	19.1 (17.2–21.4)	0.544	19.5 (17.0–20.8)	18.9 (17.3–24.0)	0.585
Baseline FVC %	109.9 (102.0–116.3)	103.9 (95.5–114.1)	0.035	109.8 (99.2–116.4)	98.0 (90.7–104.3)	0.064
Baseline FEV1 %	110.8 (105.2–117.5)	103.0 (92.5–110.5)	0.002	108.7 (93.1–117.3)	99.2 (91.0–105.5)	0.136
Vitamin D ng·mL^{-1}	21.6 (16.3–25.7)	19.7 (15.2–23.0)	0.268	23.0 (17.7–27.8)	17.9 (11.1–20.3)	0.001

Data are presented as n (%) or median (interquartile range), unless otherwise stated. FEV1: forced expiratory volume in 1 s; BMI: body mass index; FVC: forced vital capacity. #: Wilcoxon rank-sum test between controls and patients; ¶: Wilcoxon rank-sum test between patients with ΔFEV1 <10 *versus* ⩾10%.

immune functioning and overall health [20], but no child in our study population reached such levels.

Even if a precise role of vitamin D in the pathogenesis of EIB has never been determined, vitamin D may be connected to the severity of bronchoconstriction through several mechanisms that can be speculated from *in vitro* studies or those performed in animal models. First, a previous study demonstrated that vitamin D deficiency is associated with an increase in mast cells in connective tissue [21]. Secondly, it has been shown that 1,25-dihydroxyvitamin D3 promotes apoptosis and inhibits maturation of mouse bone marrow-derived mast cell precursors [22] as well as inhibiting A23187-induced histamine release from mast cells [23]. These events may be important, as the primary source of inflammatory mediators involved in an asthma attack following exercise is likely to be mast cells [24]. Thirdly, vitamin D3 analogue significantly reduces the expression of interleukin (IL)-13 [25], and IL-13 polymorphisms have been associated with EIB severity in asthmatic children [26]. Fourthly, it has recently been observed that vitamin D3 diminishes endothelium-dependent contractions in the aorta by reducing calcium influx into the endothelial cells, hence decreasing the production of endothelium-derived contracting factors [27]. There are no reasons to exclude the possibility that these mechanisms may also be working in the airways, as vitamin D receptors are present in respiratory epithelial cells [28] and human bronchial smooth muscle cells, in which vitamin D regulates the expression of genes implicated in asthma pathogenesis [29]. Finally, several recent studies have established that vitamin D is a principal controller of innate immunity and adaptive immune responses [30]. In fact, vitamin D deficiency has been shown to predispose children to respiratory infections [31–33], and vitamin D supplements have been shown to decrease the incidence of respiratory infections [34].

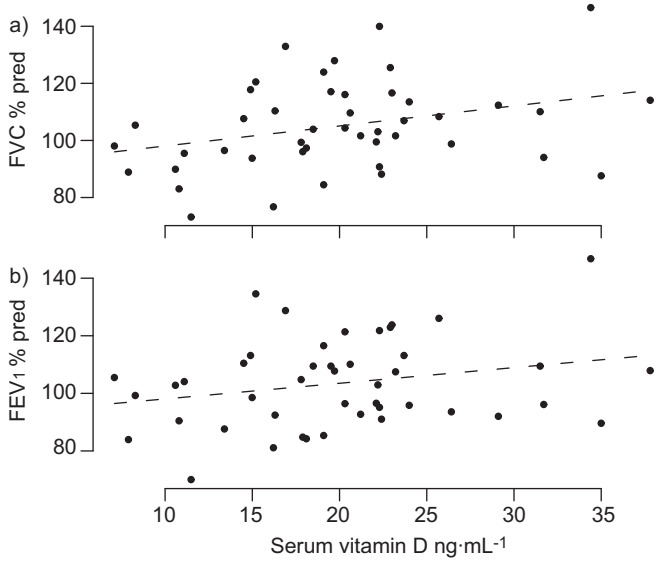

FIGURE 1. Relationship between serum vitamin D serum level and a) forced vital capacity (FVC) (r=0.34; p=0.037) and b) forced expiratory volume in 1 s (FEV1) (r=0.32; p=0.037). % pred: % predicted.

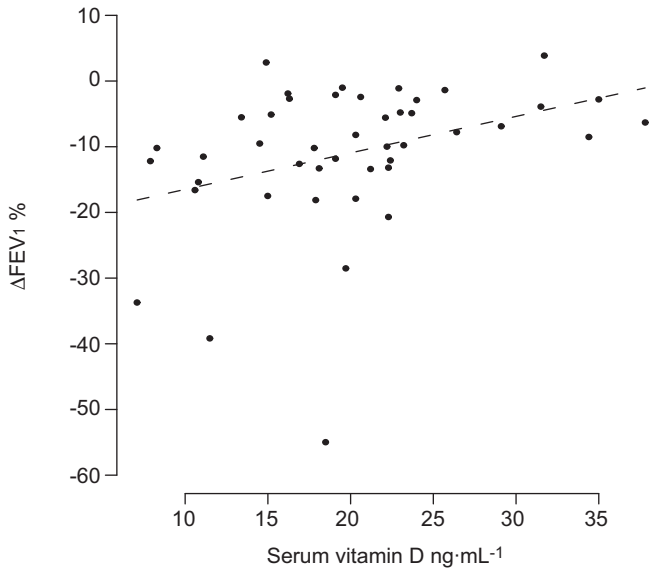

FIGURE 2. Relationship between serum vitamin D levels and change in forced expiratory volume in 1 s (ΔFEV1) after exercise challenge (r=0.48; p=0.001).

This may have obvious consequences, as early respiratory infections may predispose to the onset of asthma [35–38] and the development of bronchial hyperreactivity [39]. However, no evaluation of the prevalence of respiratory infections was performed on our cohort.

We confirm that serum 25-hydroxyvitamin D levels are positively associated with FEV1 and FVC, as has been previously reported in large cross-sectional studies in randomly selected adolescents and adults [40], and in agreement with these studies, no correlation was found in our patients between FEV1/FVC and serum vitamin D levels (data not shown).

However, epidemiological observational studies suggest an association but do not prove causality, and no interventional trials on individuals with low serum concentrations of vitamin D have evaluated the effect of supplementation on asthma exacerbations, bronchial hyperresponsiveness or lung function. Such studies are urgently needed, as it remains difficult to ascertain from cross-sectional investigations whether vitamin D deficiency is responsible for reduced lung function and asthma, or whether asthma-associated lifestyles, such as less outdoor exercise and, thus, decreased exposure to sunlight, or less milk consumption and, thus, decreased dietary intake [6], are responsible for lower serum levels of vitamin D. However, the fact that our study was conducted in mild asthmatics and in a period of time when, at our latitude, no vitamin D is produced as a result of low exposure to the sun, argues in favour of a direct cause–effect relationship between low vitamin D and lung function and exercise-induced lability. Furthermore, serum vitamin D levels were similar in healthy controls and patients. The study has some weaknesses, such as the limited number of patients and the cross-sectional design, which does not allow us to precisely disentangle the cause–effect relationships. Obviously, randomised interventional trials on vitamin D supplementation will be needed to confirm the ability of this intervention to reverse the suboptimal outcomes associated with vitamin D insufficiency in asthmatic subjects.

In conclusion, hypovitaminosis D is re-emerging as an important public health problem in different parts of the world, not only in relation to bone metabolism, but also in connection with a variety of other common chronic conditions, including respiratory diseases.

STATEMENT OF INTEREST

None declared.

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