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VITAMIN D SERUM LEVELS AND EXERCISE-INDUCED BRONCHOCONSTRICTION IN CHILDREN WITH ASTHMA

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Summary (words 196)

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 one second (FEV_1) and the percentage fall in FEV_1 (ΔFEV_1) after a standardized exercise challenge in 45 children with intermittent asthma.

Only 11% of the children had desirable serum vitamin D levels (at least 30 to 40 ng/ml). 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 ($\Delta FEV_1 \geq 10\%$) presented lower serum levels of 25-hydroxyvitamin D than children with a negative challenge- 16.2 (5.2) vs 23.4 (7.0) ng/ml ($p=0.001$), respectively.

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 with increased reactivity to exercise.

Introduction

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 synthesize 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 recent data have suggested that vitamin D deficiency could be related to onset of asthma (6,7), only recently an association between low serum levels of vitamin D and markers of asthma severity has been described in children (8-10) and adults (11) with asthma.

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

Patients and Methods

Study Population

Forty-five Italian children with asthma, diagnosed according to the American Thoracic Society Guidelines (12) and consecutively seen in the out-patient clinic of the Department of Pediatrics of the University of Verona hospital, were enrolled in the study. Only patients with intermittent asthma were admitted to the study in order to avoid the interference of regular treatment on bronchial hyper-responsiveness and consequently on the results of the exercise challenge. Fifty nine 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 [measured as 25 hydroxyvitamin D, 25(OH)D] was obtained from all subjects using a radioimmunoassay method. Vitamin D level values were used as a continuous variable and were categorized in descriptive analyses as desirable (or sufficient) when scores were at least 30 to 40 ng/ml (75 to 100 nmol/l), insufficient between 20 and 30 ng/ml (50 and 75 nmol/l) and deficient when < 20 ng/ml, 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) maneuvers using an electronic spirometer (Jaeger, Master Screen IOS, Germany) calibrated before the arrival of each subject, using a 3 liter syringe (Cardinal Health Germany 234 GmbH). The FVC maneuvers were carried out with the child standing and wearing a nose clip. Subjects were instructed to avoid using short-acting bronchodilators at least six hours prior to undergoing testing.

Exercise challenge test

A standardized 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 (y)] 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, FEV_1 was measured at 1, 3, 5, 10, 15, 20 and 30 min after completing the exercise tests (single blows)**.** The severity of EIA was expressed as the maximum change after exercise from the baseline value of lung function ($\Delta FEV_1\%$). Children whose FEV_1 fell by 10% or more were classified as having EIB. No child on long-term treatment was admitted to the study.

Statistical Analysis

The statistical distributions of vitamin D, 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). On the contrary, Delta FEV_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 (gender, age, 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 percent-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 first identifying critical sample units by the leverage vs. squared-residual plot and then re-estimating correlations without these units.

P-values <0.05 were considered statistically significant. All calculations were done with Stata 11.0 statistical package (StataCorp. 2009. Stata Statistical Software: Release 11. College Station, TX, USA: StataCorp LP).

Results

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

As can be seen healthy subjects presented superior lung function values than patients but there was no difference in gender, age, body mass index, and vitamin D serum levels. Five (11.1%) patients and 7 (11.9%) controls had sufficient vitamin D levels (≥ 30 ng/ml). Insufficient levels (between 20 and 30 ng/ml) 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). There was no significant difference in median vitamin D serum levels between controls and patients.

The relationships between vitamin D levels and patients' baseline FVC, FEV_1 are shown in Figures 1a and 1b. A positive, moderate, statistically significant partial correlation was found between vitamin D serum and FVC (r = 0.34, 95% CI = 0.03 to 0.56, p = 0.037). A similar partial correlation was observed for FEV_1 (r = 0.32, 95%CI = -0.05 to 0.51, p = 0.037). The relationship between individual vitamin D serum 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 to 0.62, p = 0.007 and partial correlation r = 0.48, 95%CI = -0.05 to 0.51, 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 vitamin D range.

Twenty-one (46.7%) children had a positive result following the exercise challenge. In those children, mean (s.d.) vitamin D levels were significantly lower than those observed in subjects with a negative exercise challenge: 16.2 (5.2) vs. 23.4 (7.0) ng/ml, respectively, (p<0.001).

Mean percent-predicted baseline FEV_1 did not significantly differ between children with a positive response to exercise challenge and those with a negative test response: 99.6% (13.6) vs. 107.0% (16.6), respectively, p = 0.113.

Discussion

To our knowledge, to date 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 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 Rica asthmatic children, 28% of them showed insufficient levels of vitamin D (< 30 ng/ml) and an inverse relationship was observed between 25-hydroxyvitamin D levels and total IgE, eosinophil count, use of anti-inflammatory medication in the previous year, increased airway responsiveness to methacholine and any hospitalization 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) and this finding was not completely unexpected since 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-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 higher than 40ng/ml (100 nmol/l) may be essential for optimal 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 on animal models. First, an old study demonstrated that vitamin D deficiency is associated with an increase in mast cells in connective tissue (21). Second, 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 inhibits A23187-induced histamine release from mast cells (23). These events may be important since the primary source of inflammatory mediators involved in an asthma attack following exercise is likely to be mast cells (24). Third, vitamin D3 analogue significantly reduces the expression of

interleukin-13 (25), and IL-13 polymorphisms have been associated with EIB severity in asthmatic children (26). Fourth, 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 that these mechanisms may also be working in the airways since vitamin D receptors are present in respiratory epithelial cells (28) and human bronchial smooth muscle cells where 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).

This may have obvious consequences since early respiratory infections may predispose to onset of asthma (35-38) and the development of bronchial hyper-reactivity (39). However, no evaluation of the prevalence of respiratory infections was done on our cohort.

We confirm that serum 25-OH vitamin D is positively associated with FEV_1 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 FEV_1/FVC and vitamin D serum 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 hyper-responsiveness and lung function. Such studies are urgently needed since 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 on

mild asthmatics, and in a period of time when, at our latitude, no vitamin D is produced as an effect of poor exposure to the sun, argues in favor of a direct cause-effect relationship between low vitamin D and lung function and exercise-induced lability. Furthermore, vitamin D serum 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 to precisely disentangle the cause-effect relationships. Obviously, randomized 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.

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Table 1: Demographic data, vitamin d serum levels and lung function of controls and patients (medians and IQR).

	Controls	Patients	p-value*	$\Delta FEV_1<10\%$	$\Delta FEV_1\geq10\%$	p-value**
Number of subjects	59	45	-	24	21	-
Female	23 (39%)	18 (40%)	0.916	9 (37.5%)	9 (42.9%)	0.767
Age (y)	10 (9 – 12)	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/cm^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 FEV_1 (%)	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)	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

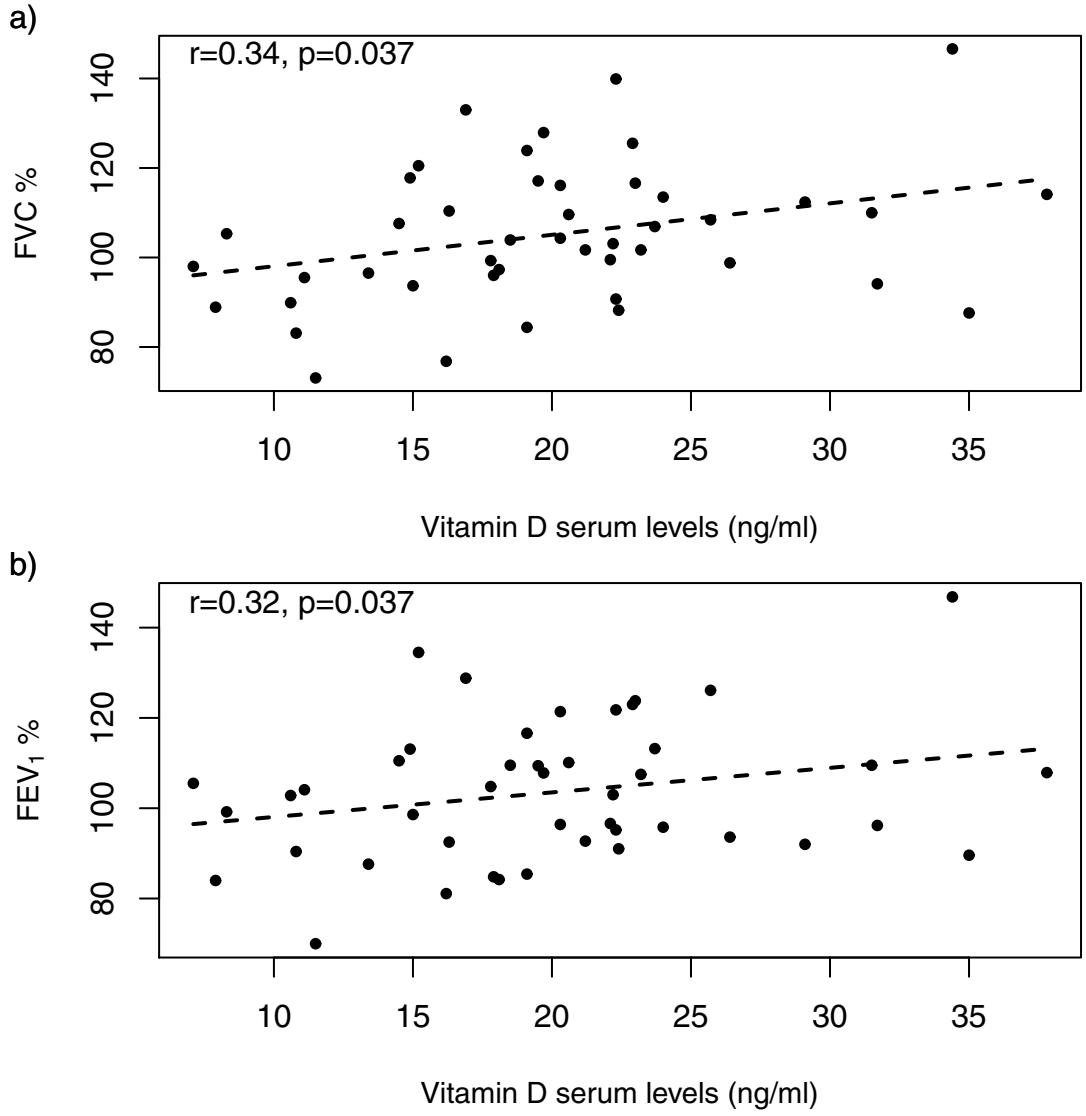
* p-values of the Wilcoxon rank-sum test between controls and patients

** p-values of the Wilcoxon rank-sum test between patients with $\Delta FEV1<10\%$ versus those with $\Delta FEV1\geq10\%$

Figure legends

Figure 1: Relationship between vitamin D serum levels (ng/ml) and a) FVC% predicted, b) FEV_1% predicted.

Figure 2: Relationship between vitamin D serum levels (ng/ml) and percent fall in FEV_1 post-exercise challenge.


a)
r=0.34, p=0.037
FVC %
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Vitamin D serum levels (ng/ml)
b)
r=0.32, p=0.037
FEV1 %
80
100
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140
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15
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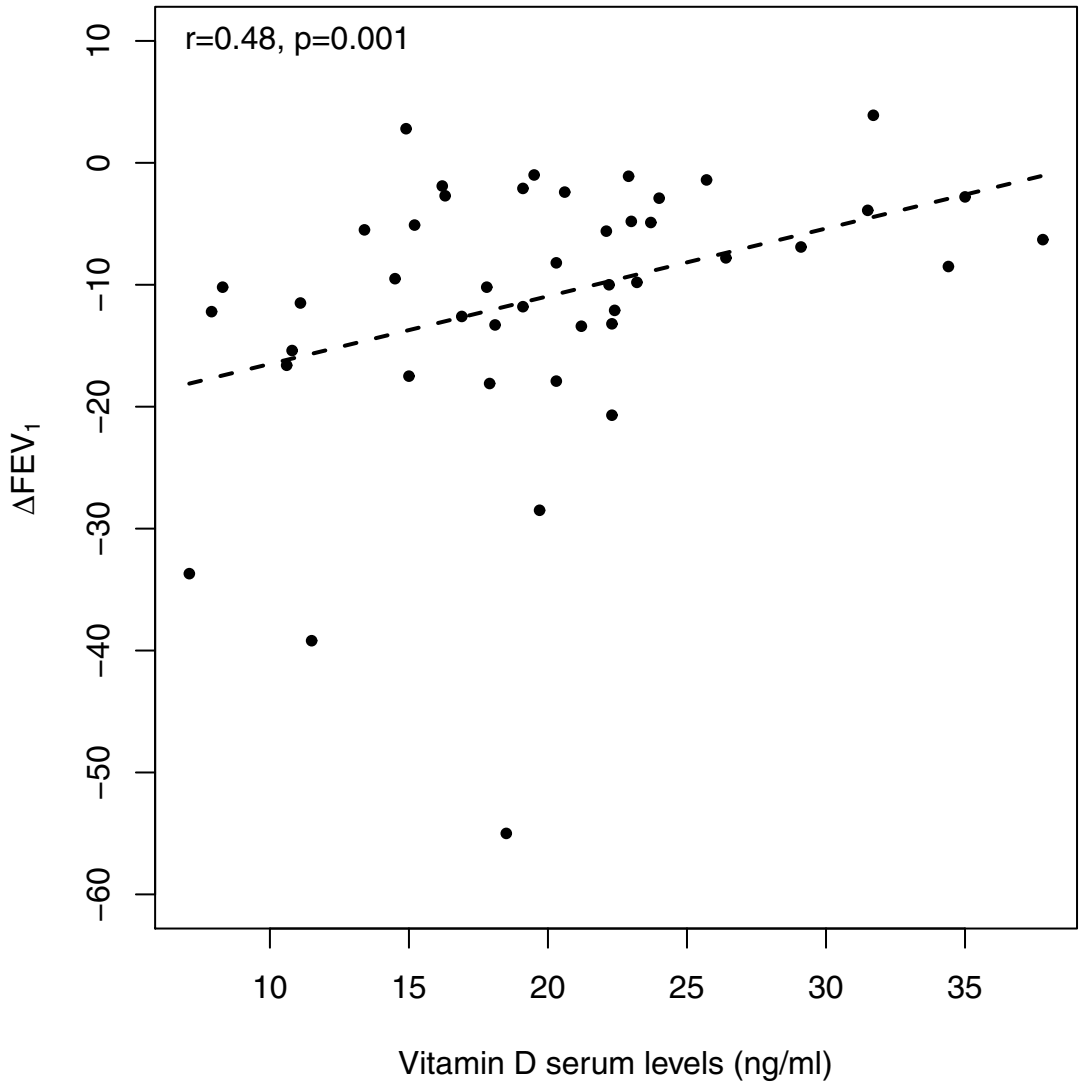
Vitamin D serum levels (ng/ml)

Figure 1


r=0.48, p=0.001
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Vitamin D serum levels (ng/ml)

Figure 2