



## Early View

### Research Letter

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Please cite this article as: Stefanidis C, Bush A, Newby C, *et al.* Vitamin D replacement in children with acute wheeze: a dose-escalation study. *ERJ Open Res* 2022; in press (<https://doi.org/10.1183/23120541.00609-2021>).

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## **Vitamin D replacement in children with acute wheeze: a dose-escalation study**

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The authors whose names are listed above certify that they have no conflicts of interest to declare.

### **Contributorship statement**

Christos Stefanidis (CS), Andrew Bush (AB), Adrian R Martineau (ARM) and Christopher J Griffiths (CG) conceived the study and contributed to study design.

CS, AB, ARM, CG, Chinedu Nwokoro (CN), Susan Liebeschuetz (SL) and Imogen Phillipa Skene (IPS) participated in implementation of the study.

CS, AB, ARM, CG and Christopher Newby (CN) performed data analysis

CS, AB and ARM wrote the first draft of the article; all other authors critically reviewed it and approved the final version.

**Keywords:** pre-school wheeze, asthma attacks, vitamin D

**Acknowledgement:** The authors thank all the children and their parents / carers who participated in the research.

Word count: 1964 words

References: 12

## **Introduction**

Meta-analyses report protective effects of vitamin D supplementation against asthma exacerbations and acute respiratory infections in adults (1-3) but data relating to effects of vitamin D on risk of pre-school wheeze and asthma attacks in childhood are more limited (4). In preparation for a randomised controlled trial (RCT) of vitamin D in children with recurrent preschool wheeze or school-age asthma, we carried out a dose-escalation study to find the daily vitamin D<sub>3</sub> supplementation regimen that is most effective in elevating circulating 25-hydroxyvitamin (25[OH]D) concentrations in these children. We hypothesized that daily oral vitamin D<sub>3</sub> supplementation of 1,000 IU would be more effective than 400 IU (UK recommendation (5)) in elevating circulating 25(OH)D concentration  $\geq 75$  nmol/L at 3 months in vitamin D-insufficient children with recurrent pre-school wheeze or school-age asthma.

## **Methods**

Children were recruited from primary and secondary care if they were aged 1-4 years with  $\geq 2$  self-reported episodes of acute wheeze requiring unscheduled healthcare attendances, or aged 5-12 years with doctor-diagnosed asthma and  $\geq 1$  self-reported asthma attack requiring an unscheduled healthcare attendance in the preceding year. Exclusion criteria were baseline 25(OH)D level  $\geq 75$  nmol/L, concurrent vitamin D supplementation, or a history of other chronic or acute respiratory or systemic conditions. Approval was by Brent Ethics Committee (ref 16/LO/1218). Written informed consent was obtained from parents of all children, with assent additionally obtained from children aged  $\geq 7$  years.

Oral vitamin D<sub>3</sub> (Fultium-D<sub>3</sub> drops, Internis Pharmaceuticals Ltd) was initially administered at a dose of 400 IU/day for 3 months (phase 1). The dose was increased to 1,000 IU/day for a further 3 months (phase 2) if the 25(OH)D concentration at end-phase 1 was  $< 150$  nmol/L; otherwise, no dosing increment was applied during phase 2. Concentrations of 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> in capillary blood were measured from a fingerprick at baseline and at the end of phases 1 and 2 using liquid chromatography with tandem mass spectrometry and summed to give total circulating 25(OH)D concentration (6). Nasal epithelial lining fluid was sampled at baseline and at the end of phases 1 and 2 using nasosorption (7). Nasal

concentrations of cytokines and chemokines were determined using a multiplex assay (Meso Scale Discovery, #15050D). At baseline, body weight was measured with a validated scale. Children aged 5-12 years completed a baseline childhood asthma control test (c-ACT) under their parents / legal guardian's supervision. The volume of residual vitamin D<sub>3</sub> supplement was assessed at the end of phases 1 and 2.

### *Statistical analyses*

Co-primary outcomes were the proportions of participants attaining 25(OH)D  $\geq$ 75 nmol/L at the end of phases 1 and 2. Secondary outcomes were mean concentrations of 25(OH)D in capillary blood and concentrations of inflammatory mediators in nasal epithelial lining fluid. The proportions of children attaining serum 25(OH)D concentrations  $\geq$ 75 nmol/L or  $\geq$ 100 nmol/L at the end of phases 1 vs. 2 were compared using McNemar's test. Mean 25(OH)D levels and asthma control score by visits were compared using paired t-tests. Univariate linear regression was applied to determine independent predictors (body weight and age) of the response to vitamin D supplementation during phases 1 and 2. End-phase mean cytokine and chemokine concentrations were compared using paired t-tests with correction for multiple comparisons using the Benjamini & Hochberg method with a false discovery rate of 5% (8).

### **Results**

Forty children had baseline 25(OH)D  $<$ 75 nmol/L and were offered vitamin D, of whom 8 withdrew from the study (withdrew consent / assent or consistently failed to attend scheduled study visits and did not reply to telephone calls) and 1 was excluded from statistical analyses as dosing was unchanged during phase 2. Mean baseline body weight for all children was 27.8 kg (standard deviation [SD] 17.4); 15.4 kg (SD 2.7) for pre-school children and 39.5 kg (SD 17.2) for schoolchildren. At baseline, twelve out of 16 (75%) schoolchildren had uncontrolled asthma (c-ACT score  $\leq$  19), and 4/16 (25%) had controlled asthma (score  $>$  19). Eleven of 31 (35.5%) children attained 25(OH)D  $\geq$ 75 nmol/L at the end of phase 1 vs. 16/31 (51.6%) at the end of phase 2 ( $p=0.06$ ). All children who attained 25(OH)D  $\geq$ 75 nmol/L at the end of phase 1 maintained 25(OH)D  $\geq$ 75 nmol/L at the end of phase 2. Four out of 31 (12.9%) children (all pre-school children) attained 25(OH)D  $\geq$  100 nmol/L at the end of phase 1 vs. 12/31 (38.7%) (10 pre-school

children and 2 schoolchildren) at the end of phase 2 ( $p=0.13$ ). Mean 25(OH)D concentration increased from baseline to end phase 1 (mean change 23.6 nmol/L; 95% CI 15.1 to 32.1,  $p<0.001$ ) and from end-phase 1 to end-phase 2 (mean change 12.7 nmol/L; 95% CI 1.4 to 23.9,  $p=0.03$ ) (Figure 1).

Ten of 15 (66.7%) and 11/15 (73.3%) pre-school children attained 25(OH)D  $\geq 75$  nmol/L at the end of phases 1 and 2, respectively ( $p>0.99$ ). One of 16 (6.3%) and 5/16 (31.3%) schoolchildren attained 25(OH)D  $\geq 75$  nmol/L at the end of phases 1 and 2, respectively ( $p=0.13$ ). We reasoned that a greater proportion of preschool children vs. schoolchildren attained 25(OH)D  $\geq 75$  nmol/L might arise as consequence of differences in body weight and age as reported elsewhere (9). We found that the 25(OH)D response to vitamin D supplementation during phases 1 or 2 was not associated with body weight (mean difference per additional kilogram of body weight during phase 1, -0.2 nmol/L, 95% CI; -0.9 to 0.4,  $p=0.47$ , mean difference per additional kilogram of body weight during phase 2, -0.1 nmol/L, 95% CI; -1.1 to 0.1,  $p=0.12$ ). The 25(OH)D response to vitamin D supplementation during phase 1 was not associated with age (mean difference per additional year of age 0.7 nmol/L, 95% CI; -2.5 to 3.9,  $p=0.66$ ). However, there was a trend for an inverse association between the 25(OH)D response to vitamin D supplementation during phase 2 with age (mean difference per additional year of age -3.3 nmol/L, 95% CI; -6.7 to 0.1,  $p=0.06$ ). In pre-school children, mean circulating 25(OH)D concentration increased from baseline to the end of phase 1 (mean change 28.7 nmol/L; 95% CI 11.9 to 45.4,  $p=0.002$ ) and from the end phase 1 to the end of phase 2 (mean change 24.3 nmol/L; 95% CI 4.0 to 44.6,  $p=0.02$ ). In schoolchildren, mean circulating 25(OH)D concentration increased significantly from baseline to the end of phase 1 (mean change 18.9 nmol/L; 95% CI 11.9 to 25.9,  $p<0.001$ ); no increase was seen between the end of phase 1 and the end of phase 2 (mean change 1.7 nmol/L; 95% CI -8.3 to 11.8,  $p=0.72$ ) (Figure 1). No participant experienced hypervitaminosis D (25[OH]D  $> 220$  nmol/L) or any adverse reaction. The highest 25(OH)D concentrations were 181 nmol/L in a schoolchild at the end of phase 1, and 175 nmol/L in a pre-school child at the end of phase 2. Mean asthma control score was not associated with baseline 25(OH)D concentration, or with the response to vitamin D supplementation during phases 1 or 2 ( $p\geq 0.19$ ). There were no statistically significant changes in nasal epithelial lining fluid cytokines or chemokines.

The volume of vitamin D<sub>3</sub> supplement returned at the end of phase 1 was higher than the expected volume based on 100% adherence in 10/13 (76.9%) schoolchildren and in 7/14 (50.0%) pre-school children. At the end of phase 2, 2/7 (28.6%) pre-school children returned their vitamin D<sub>3</sub> supplement with a higher residual volume than was compatible with 100% adherence. Only a limited number of vials were handed back at the end of phases 1 (27 vials) and 2 (12 vials).

## **Discussion**

Neither vitamin D replacement regimen investigated was particularly effective in elevating 25(OH)D concentrations  $\geq 75$  nmol/L in vitamin D-insufficient children with pre-school wheeze or asthma: nearly half the children failed to attain 25(OH)D concentration  $\geq 75$  nmol/L after 3 months of supplementation with 1,000 IU vitamin D<sub>3</sub>. A greater proportion of pre-school children attained 25(OH)D concentrations  $\geq 75$  nmol/L at end of phases 1 (10/15 vs. 1/16) and 2 (11/15 vs. 5/16) compared to school children ( $p \geq 0.001$ ). A daily oral dose of 400 IU vitamin D safely elevated circulating 25(OH)D levels by a mean of 23.6 nmol/L at 3 months. Escalation of this dose to 1,000 IU/day resulted in a further mean increase of 12.7 nmol/L. Stratification of the analysis by age-group revealed that increases in 25(OH)D were higher among pre-school vs. schoolchildren.

The main strength of the study is the prospective, individual tailoring of supplementation and the use of a fingerprick to measure 25(OH)D levels, which was well-tolerated. We acknowledge we did not record information on prescribed asthma treatments. Additionally, we highlight the fact that we excluded children who received vitamin D supplementation at the time of enrolment as those are more likely to have circulating 25(OH)D concentration  $\geq 75$  nmol/L; this may have limited the generalizability of our findings. The main limitation related to monitoring adherence: weighing returned bottles only reveals what medication has not been taken, and we cannot know if the missing drops were taken regularly, all at once before return (dose-dumping) or discarded. Better adherence in pre-school vs. schoolchildren may therefore explain their higher attained 25(OH)D levels. Alternatively, children with asthma may have dysregulated vitamin D metabolism associating with a blunted 25(OH)D response to vitamin D<sub>3</sub> supplementation, as recently reported in adults (10). Finally, reference ranges for 25(OH)D levels are based on requirements to support optimal bone health; the levels needed to support optimal immune function are unknown.

Our data have implications for the design of RCTs of vitamin D supplementation in pre-school wheeze and school-age asthma. It cannot be assumed that conventional supplementation will achieve adequate levels in all participants. Given the safety data here, it would seem reasonable initially to supplement children who are vitamin D deficient with 1,000 IU rather than 400 IU/day, since this regimen resulted in higher mean attained 25(OH)D levels without increasing risk of hypervitaminosis D; it may be that doses of more than 1,000 IU/day are needed to prevent exacerbations. The use of smart phone applications for remote direct observation of daily vitamin D<sub>3</sub> supplement intake may be an inexpensive method to address adherence accurately and allow researchers to prompt participants with poor adherence (11). Financial incentives or rewards for those whose adherence can be documented remotely can optimize adherence and encourage patients to complete the study as per protocol (12).



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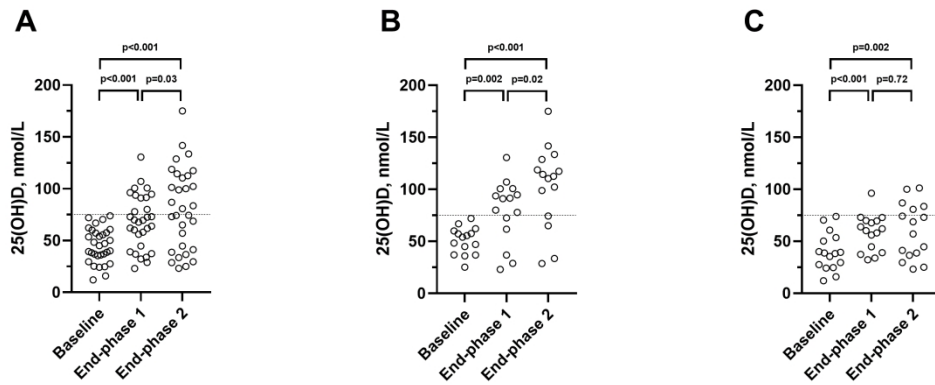


Figure 1: 25-hydroxyvitamin D (25[OH]D) concentrations by study time point in A) all participants (n=31), B) the sub-set of participants with pre-school wheeze (n=15) and C) the sub-set of participants with asthma (n=16). Dotted line represents threshold denoting optimal 25(OH)D concentration (>75 nmol/L). P values from paired Student's t-tests.