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REVIEW

Vitamin D modulation of innate immune responses to respiratory viral infections

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Summary

Vitamin D, in addition to its classical functions in bone homeostasis, has a modulatory and regulatory role in multiple processes, including host defense, inflammation, immunity, and epithelial repair. Patients with respiratory disease are frequently deficient in vitamin D, implying that supplementation might provide significant benefit to these patients. Respiratory viral infections are common and are the main trigger of acute exacerbations and hospitalization in children and adults with asthma and other airways diseases. Respiratory monocytes/macrophages and epithelial cells constitutively express the vitamin D receptor. Vitamin D, acting through this receptor, may be important in protection against respiratory infections. Whether the in vitro findings can be translated into a substantial in vivo benefit still remains uncertain. Here we review the in vitro data on the role of vitamin D in antiviral innate immunity, the data concerning the deficient levels of vitamin D in lung diseases, and the in vivo role of supplementation as protection against respiratory viral infections in healthy individuals and in patients with chronic respiratory diseases. Finally, we suggest ways of improving the effectiveness of vitamin D as an adjuvant in the prevention and treatment of acute respiratory infections.

KEYWORDS

innate immunity, respiratory viruses, vitamin D

1 | INTRODUCTION

Respiratory infections are frequently associated with severe colds in healthy people, especially in children and senior people, and with asthma exacerbations. 1α,25-dihydroxyvitamin D, the immunologically active form of vitamin D, through interaction with its own receptor, has the capacity to mediate epithelial cell proliferation, differentiation, and apoptosis. Furthermore, vitamin D has been shown to increase the antiviral potential of immune cells during respiratory viral infections in vitro, inducing increased expression of antimicrobial peptides, such as cathelicidin and defensin, and innate interferons, which are an important component of the airway responses against viruses.

In an era of debate on the rational use of expensive medicines, particularly in countries with limited resources, the use of vitamin D as a strategy to reduce the frequency and severity of respiratory infections needs to be seriously considered. However, the current evidence of efficacy of vitamin D in preventing viral respiratory infections is inconsistent. There is a lack of prospective cohort studies with detailed vitamin D and immune profiling of the patients studied.

In this review we attempt to unveil the pathogenic mechanisms underlying vitamin D treatment during respiratory viral infections, as introduced by several in vitro and in vivo/ex vivo models that have been published so far. More importantly, in the second part of the review, we focus on translating these findings into a clinical perspective and, through reviewing currently published randomized clinical trials and cohort studies, we aim to conclude whether vitamin D supplementation has been identified as a promising adjuvant during common cold episodes or exacerbations of asthma or whether current and future vitamin D trials are a waste of funding, as recently highlighted in *BMJ*.[1,2]

For our review we have taken into consideration the recently published reviews (systematic or not) on the topic.[3–5] Our review aims

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Abbreviations: 1,25(OH)$_2$D, 1α,25-dihydroxyvitamin D; 1α(OH)ase/CYP27B1, 1α-hydroxylase enzyme; LRTIs, lower respiratory tract infections; PBMCs, peripheral blood mononuclear cells; RTI, respiratory tract infection; TLRs, toll-like receptors; URTIs, upper respiratory tract infections; 24(OH)ase/CYP24A1, vitamin D-24-hydroxylase; VDR, vitamin D receptor

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to combine both the clinical evidence and the "in vitro" evidence regarding the functional role of vitamin D during "common cold" episodes and cold-induced exacerbations of asthma, a subject not covered in the current reviews.

We hope that presenting the available published studies of vitamin D and respiratory viral infections, also contradictory, will help to improve the future clinical studies of efficacy of vitamin D supplementation in preventing viral infections in healthy persons and in asthma exacerbations.

2 | SYNTHESIS AND REGULATION OF METABOLISM OF VITAMIN D

The immunologically active form of vitamin D, 1α,25-dihydroxyvitamin D (1,25[OH]$_2$D, calcitriol) is formed through sequential hydroxylation. Vitamin D$_3$ (cholecalciferol) or vitamin D$_2$ (ergocalciferol) is hydroxylated by the cytochrome P450 enzyme 25-hydroxylase (CYP27A1), generating 25-hydroxyvitamin D (25[OH]D: 25[OH]D$_3$, calcidiol, calcifediol, or 25[OH]D$_2$). This form is a biologically inactive circulating metabolite but is subsequently hydroxylated intracellularly in the kidney or extrarenally, by the mitochondrial 1α-hydroxylase enzyme (1α[OH]ase/CYP27B1) into 1,25(OH)$_2$D, the active form of calcitriol.[6]

25-hydroxyvitamin D levels are dependent on sun exposure and dietary intake of vitamin D, and serum levels are considered the most accurate marker for vitamin D status. Vitamin D levels over 20 ng/mL (50 nmol/L) are considered to be sufficient and ≤20 ng/mL (50 nmol/L) insufficient.[7,8] The Endocrine Society has set the lower threshold of adequate 25(OH)D levels at 30 ng/mL (75 nmol/L).[9] These levels are based on maintaining bone health and are suggested also for lung diseases, although the optimal levels for immune health have yet to be established.

25-hydroxyvitamin D$_2$ binds to the carrier protein, vitamin D binding protein, with lower affinity than 25(OH)D$_3$, which may facilitate bioavailability to immune cells such as monocytes.[10] Recently, it has been shown that many innate immune cells, including epithelial cells, monocytes/macrophages, and dendritic cells, can synthesize 1,25(OH)$_2$D from 25(OH)D.[6,11,12] Extracellular 25(OH)D, bound to serum vitamin D binding protein, is endocytically internalized, and *intracellularly* synthesized 1,25(OH)$_2$D will act on the nuclear vitamin D receptor (VDR).[13] 1α,25-dihydroxyvitamin D and its precursor 25(OH)D are catabolized by vitamin D-24-hydroxylase (24[OH]ase/CYP24A1).[14]

Cellular and serum concentrations of 1,25(OH)$_2$D are regulated through a negative feedback loop.[15] Systemic levels of vitamin D are controlled via calcium and parathyroid hormone levels; however, local tissue production of 1,25(OH)$_2$D is regulated by calcium-independent mechanisms, and higher levels of 1,25(OH)$_2$D may be generated to activate innate immune responses.[16] There are data though supporting the view that intracellularly synthesized 1,25(OH)$_2$D does not leak into the circulation and is degraded locally.

The autocrine and paracrine effects of 1,25(OH)$_2$D are principally mediated through specific high-affinity VDR, referred to as "genomic" signaling and detected within hours of exposure to 1,25(OH)$_2$D. 1α,25-Dihydroxyvitamin D transactivates target gene expression by binding to the nuclear VDR, and target genes can be either upregulated or downregulated depending on the coregulatory proteins involved and the nature of the stimulus. 1α,25-dihydroxyvitamin D has the capacity to limit its own synthesis by exerting a negative feedback on the vitamin D signaling system,[17] decreasing CYP27B1 expression/1α(OH)ase transcription and also increasing CYP24A1 with an increase in 24(OH)ase catabolic function (autocrine regulation).

In addition, rapid, nongenomic actions were reported for 1,25(OH)$_2$D via a membrane receptor–associated, rapid response, steroid-binding protein and the nuclear VDR itself, and both membrane and nuclear receptor mediate rapid responses associated with cell proliferation, differentiation, and apoptosis.[18,19]

3 | VITAMIN D IMMUNOMODULATORY AND ANTIVIRAL ACTIVITY

In vitro studies show that vitamin D has immunomodulatory functions inhibiting cell proliferation, inducing cell differentiation and apoptosis in normal human cell types (normal human bronchial epithelial cells, monocyte-derived macrophages), and decreasing proinflammatory cytokine production. In addition vitamin D increases antiviral protein production, suggesting an important role in antiviral protection. These functions will be discussed in turn below.

3.1 | Vitamin D decreases proinflammatory cytokines in the lung

1α,25-Dihydroxyvitamin D directly modulates those cytokines that are dependent on nuclear factor κB (NF-κB) activity, in many cells including macrophages, by blocking NF-κB p65 activation via upregulation of the NF-κB inhibitory protein IκBα.[20] Toll-like receptors (TLRs) are transmembrane proteins that recognize conserved molecular motifs of viral and bacterial origin and initiate innate immune responses. TLR3 recognizes viral double-stranded RNA or synthetic double-stranded RNA (polyinosinic-polycytidylic acid) and is primarily involved in defense against viruses. Vitamin D treatment has been shown to attenuate double-stranded RNA-TLR3–induced expression of IL-8 in respiratory epithelial cells.[12]

25-hydroxyvitamin D and 1,25(OH)$_2$D also modulate T-cell adaptive immunity, decreasing the proinflammatory type 1 cytokines (IL-12, IFN-γ, IL-6, IL-8 and tumor necrosis factor-α) and IL-17 and increasing anti-inflammatory type 2 cytokines (IL-4, IL-5, and IL-10)[21,22] and regulatory T cells,[23] suggesting that a balance needs to be maintained to achieve a beneficial effect.

When primary CD4$^+$ T cells from normal donors were cultured in Th17-polarizing conditions, vitamin D reduced the expression of pathogenic Th17 markers and their secretion of proinflammatory cytokines (IL-17A and IFN-γ) and induced an expansion of CD25hi cells and upregulated their expression of CTLA-4 and Foxp3 regulatory markers.[24] Interestingly, the presence of the active form of vitamin D$_3$ during CD8$^+$ T-cell differentiation prevented IL-4–induced conversion to Tc2 IL-13 producers.[25]

In patients receiving oral vitamin D$_3$ (140 000 IU monthly) for 3 months, Tregs increased significantly.[26] Increased production of the suppressor cytokine IL-10 by non-CD4$^+$ non-CD8$^+$ T cells and decreased frequency of Th17 were reported in 4 healthy humans supplemented

with vitamin D_3 at a high dose (5000 to 10 000 IU/d).[27] In a randomized controlled trial in vitamin D–deficient patients, high-dose (4000 IU/d) vitamin D_3 for 2 months significantly reduced $CD4^+$ T cell–nonspecific activation as compared with the low dose (400 IU/d).[28]

3.2 | Antiviral activity

The local synthesis of vitamin D from its precursor 25(OH)D plays a crucial role in adequate local immune responses to respiratory virus infections.[29]

Human airway epithelial cells constitutively express VDR, high levels of activating 1α(OH)ase, and low levels of inactivating 24(OH)ase.[12,30] Vitamin D generated by lung epithelium could lead to increased expression of antimicrobial peptides (such as cathelicidin [LL-37] and defensin β4 [DEFB4, HBD2] in adjacent macrophages and other innate immune cells) (Figure 1).[6,12,31] In peripheral blood–derived cells, the highest levels of cathelicidin following treatment with $1,25(OH)_2D$ were in macrophages, while lower levels were found in granulocyte-macrophage colony-stimulating factor–derived dendritic cells.[32]

There are data available supporting the antiviral activity of cathelicidin in infections with influenza A virus and respiratory syncytial virus (RSV)[12,33–35] by binding to virus, which leads to disruption of the virus envelope and modulation of cytokine and TLR3 responses.[33] Although defensin was induced by rhinovirus in primary epithelial cells, defensin showed no direct antiviral activity towards rhinovirus.[36] However, defensin inhibited RSV by destabilizing the viral envelope and consequently inhibiting cellular entry.[37]

Although to date there are no reports that vitamin D directly affects respiratory virus load, there are in vitro studies suggesting that vitamin D could contribute to reduced inflammation and less severe disease in RSV and influenza viral infections. In one study using epithelial cells isolated from tracheobronchial mucosa by enzymatic dissociation, $1,25(OH)_2D$ treatment induced the NF-κB inhibitor IκBα and, consequently, RSV induction of NF-κB–driven genes—such as the antiviral IFN-β and CXCL10—as well as antiviral IFN-stimulated genes (ISG) (myxovirus resistance A and ISG15) was downregulated.[38] However, the viral RNA or protein levels and viral replication were not increased.[38] Similarly, in vitro treatment of the human alveolar epithelial cell line A549 with $1,25(OH)_2D$ (100/30 nM) prior to/or after influenza A (H1N1) exposure decreased the levels of H1N1-induced proinflammatory cytokines and VDR in both experimental conditions, restored the increased apoptosis seen on H1N1 infection back to the constitutive level, but did not affect viral clearance.[39] Interestingly, antiviral IFN-β and ISG15 were decreased by $1,25(OH)_2D$ treatment prior to influenza A exposure but were increased by $1,25(OH)_2D$ treatment after exposure,[39] suggesting efficacy of $1,25(OH)_2D$ treatment during a viral infection. However, the antiviral role of vitamin D was questioned in another in vitro study, showing that vitamin D does not have a direct effect on the replication of rhinovirus.[40]

Overall, in vitro studies suggest that vitamin D plays a significant role in local "respiratory homeostasis" either by directly affecting the replication of respiratory viruses or by inducing the expression of antimicrobial peptides, by modulating the balance of Th1/Th2 or Tc1/Tc2 responses, and by inhibiting Th17 cytokine production.[25,41]

4 | VITAMIN D DEFICIENCY AND RESPIRATORY VIRAL INFECTIONS IN HEALTHY AND ASTHMATIC PEOPLE

Influenza A and B, parainfluenza 1 and 2, and RSV infections are all more common in the winter, and, as all are enveloped viruses, all of them may be sensitive to antimicrobial peptides, and therefore vitamin D may be important in host defense against them. Epidemiological data have linked vitamin D deficiency to increased susceptibility to acute viral respiratory infections.[42] Some of these studies suggested that the enhanced vitamin D status during summer may be linked to the reduced prevalence of viral infections as compared with the winter months when lower plasma levels of vitamin D have been reported.[43] A proposed mechanism has been that UV-B radiation could trigger the production of the active form of vitamin D, $1,25(OH)_2D$, leading to increased production of antiviral peptides such as cathelicidin and defensins.

5 | VITAMIN D DEFICIENCY AND RESPIRATORY VIRAL INFECTIONS IN HEALTHY PERSONS

5.1 | Vitamin D deficiency and respiratory infections in newborns and children

There was an inverse association between cord blood 25(OH)D levels and risk of respiratory infection and childhood wheezing[44]; healthy

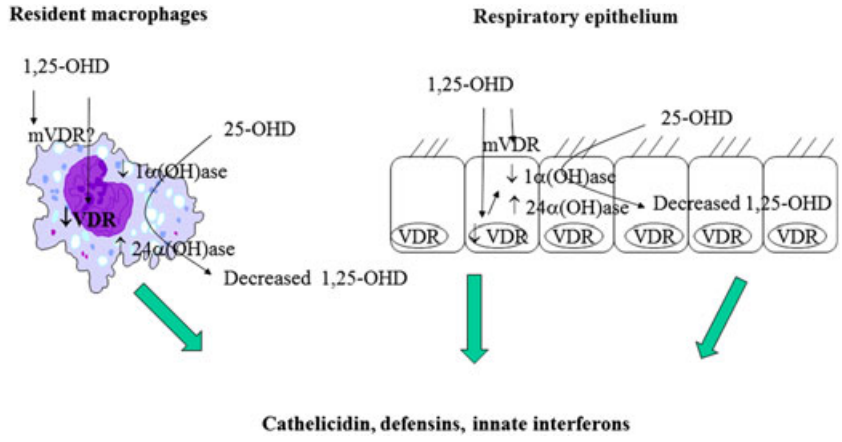

FIGURE 1 The antiviral pathway of vitamin D, 1α,25-dihydroxyvitamin D ($1,25[OH]_2D$) synthesized from 25-hydroxyvitamin D (25[OH]D), will act on vitamin D receptor (VDR) and induce the release of antiviral proteins (cathelicidin, defensins, and innate interferons) in the context of a respiratory viral infection

newborns with 25(OH)D <20 ng/mL in cord blood were 6 times more likely to develop RSV respiratory infection in the first year of life as compared with those with >30 ng/mL 25(OH)D.[45] Higher maternal circulating 25(OH)D concentrations in pregnancy were independently associated with reduced risk of lower respiratory tract infections (LRTIs) in offspring in the first year of life but not with wheezing or asthma in childhood,[46] and higher maternal midpregnancy 25(OH)D levels were associated with a modestly reduced risk of recurrent LRTI[47] by 36 months.

Serum 25(OH)D levels were associated with viral respiratory tract infection (RTI) in 743 children (3-15 y old)[48] followed between December 2008 and June 2009. A third of participants developed at least 1 viral RTI (confirmed by polymerase chain reaction in nasopharyngeal specimens). Serum 25(OH)D levels[48] under 75 nmol/L augmented the risk of viral RTIs by 50%, and levels <50 nmol/L by 70%.

5.2 | Vitamin D deficiency and respiratory infections in healthy adults

Data from a prospective cohort study over the autumn and winter of 2009-2010 in 198 healthy adults linked the levels of 25(OH)D to the recovery time from acute RTI (infection confirmed in nasopharyngeal swabs), suggesting that concentrations of ≥38 ng/mL of 25(OH)D may be associated with a markedly quicker recovery.[49]

In healthy adults 45 to 47 years of age, participants in the 1958 British nationwide birth cohort, the prevalence of respiratory infections had a strong seasonal pattern, opposing the direction of the 25 (OH)D concentration pattern. Vitamin D status had a linear relationship with respiratory infections: each 10 nmol/L increase in 25(OH)D was associated with a 7% lower risk of self-reported acute respiratory infection (ARI).[50] These associations were confirmed in another large, nationally representative sample in the United States.[42] After season was adjusted for, demographic factors, and clinical data, in 14 108 participants in National Health and Nutrition Examination Survey 2001-2006, 25(OH)D levels <30 ng/mL were associated with 58% higher odds of ARI compared with ≥30 ng/mL levels.[42]

In another report, athletes with high vitamin D levels, 25(OH) D > 120 nmol/L, had fewer upper RTIs (URTI), less total number of URTI symptom days, and lower median symptom severity scores during a 4-month winter period than a vitamin D–deficient group with <30 nmol/L 25(OH)D.[51] The plasma cathelicidin concentration positively correlated with the plasma 25(OH)D concentration.

6 | VITAMIN D DEFICIENCY AND VIRAL-INDUCED EXACERBATIONS OF ASTHMA

Viral infections, mostly due to rhinoviruses, constitute the major cause of exacerbations of asthma.[52]

6.1 | Mothers and babies

A protective role of high maternal serum levels of vitamin D against viral infection was supported by several human and animal studies,[53–56] suggesting that high serum levels of vitamin D in pregnancy might protect the infant from developing ARIs or viral-induced wheezing episodes within the first years of life. However, other studies were unable to support these associations[57] or highlight any protective effect of high maternal 25(OH)D concentrations on outcomes of allergic airway disease and lung function at early adulthood.[58]

6.2 | Asthmatic children

Several studies, principally in children, suggest that vitamin D insufficiency is associated with both asthma severity and poorer control.[59–61] Asthmatic children with low vitamin D levels suffer more severe exacerbations of the disease and have an increased risk of emergency department visits or hospitalizations.[59,60,62] There is also evidence linking vitamin D insufficiency and deficiency to an increased incidence of acute bronchiolitis. A recent systematic review and meta-analysis concluded that polymorphisms at the VDR level—which might be linked to functional vitamin D deficiency—are usually present in patients hospitalized because of severe RSV bronchiolitis, which is strongly associated with the development of wheezing and asthma later on in life.[63]

6.3 | Adult asthmatics

Ginde et al reported that the association between low 25(OH)D level and the incidence of URTI was stronger in individuals with asthma and chronic obstructive pulmonary disease: people with deficient circulating 25(OH)D (<25 nmol/L or <10 ng/mL) always exhibited a higher risk than people with adequate circulating 25(OH)D (>75 nmol/L or >30 ng/mL).[64]

In adult asthma, 25(OH)D levels below 30 ng/mL are common especially in patients with severe and/or uncontrolled asthma, in particular in patients with a sputum eosinophil count ≥3% despite treatment with inhaled and oral corticosteroids,[65,66] and levels correlate significantly with impaired lung function and poor response to treatment.[65]

In patients with mild to severe persistent asthma, vitamin D sufficiency was significantly associated with a decreased total number of asthma exacerbations, decreased total severe asthma exacerbations, and decreased emergency room visits, suggesting that vitamin D deficiency may be linked to the risk of severe asthma exacerbations in adults.[67,68]

Finally, elderly asthmatics commonly exhibit a profound vitamin D deficiency or insufficiency (also mirrored in their bone health status) and have poor control of the disease.[69] In a recent study in the United States, the group of older adults with asthma who presented with vitamin D deficiency had an increased number of hospitalizations and increased morbidity.[70] However, old people also probably spent less time outside and consequently were less exposed to UV-B radiation.

7 | SUMMARY

The majority of published evidence suggests that vitamin D deficiency is associated with negative outcomes in respiratory disease. This implies that supplementation with vitamin D might have a beneficial role on both the course and the outcome of these diseases and might provide significant benefit in terms of the quality of life of these patients. From a

recently published systematic review of randomized controlled trials, it can be concluded that, in the majority of people, daily intake of 1000 IU should result in >50 nmol/L circulating 25(OH)D levels[71] and that it is reasonable and safe to take approximately 1000 IU of vitamin D daily to optimize nonspecific immunity and prevent infection.[72]

8 | FORMS OF VITAMIN D SUPPLEMENTATION

The most common forms of inactive vitamin D used for supplementation are cholecalciferol/vitamin D_3 and ergocalciferol/vitamin D_2. Given its longer serum half-life, greater potency, and lower cost, in theory, cholecalciferol/vitamin D_3 should be the preferred treatment for the correction of vitamin D deficiency.[73,74] Vitamin D_3 has been shown to be 87% more potent in raising and maintaining serum 25(OH)D concentrations and to produce up to 3-fold greater storage of vitamin D as compared with vitamin D_2 in equimolar amounts.[73,75]

Whether cholecalciferol/vitamin D_3 or ergocalciferol/vitamin D_2 is equally effective in increasing and maintaining serum 25(OH)D concentration is controversial.[10] Studies favoring ergocalciferol reported that the ingestion of 1000 IU ergocalciferol in vitamin D–insufficient adults is effective in raising total serum concentrations of 25(OH)D and in sustaining serum concentrations of total $1,25(OH)_2D$.[76] By contrast, in another study, in only 7 healthy participants, circulating 25(OH)D was increased from 27 to 43 ng/mL with a single oral dose of ergocalciferol (100 000 IU).[77]

The indications for vitamin D supplementation should be based on scientific evidence. It has been shown that only 25(OH)D levels <32 ng/mL positively correlate with both baseline antimicrobial cathelicidin levels and changes in cathelicidin levels following high-dose ergocalciferol treatment (50 000 IU every other day for 5 d) in healthy patients.[78] This implies that there is evidence that vitamin D supplementation could be recommended to prevent viral infections, but the baseline serum 25(OH)D levels will be a significant indication as to whether the supplementation will be effective. The treatment of persons with high 25(OH)D levels is not beneficial and is even dangerous. The supplementation trials should include only people with low baseline levels of 25(OH)D.[5,7,79–81]

In addition, intermittent high-dose vitamin D supplementation seems not to work as well as daily supplementation.[82–85] Bergman reported in a systematic review and a meta-analysis of published randomized controlled trials that vitamin D has a protective effect against RTIs; specifically, when using daily doses instead of intermittent large bolus doses at long intervals (1-3 mo) of vitamin D, a significantly better therapeutic effect is achieved.[82,86] When bolus vitamin D_3 (250 000 IU) was given once in November to young healthy adults, there was a robust increase in plasma 25(OH)D after 5 days, but after 90 days this effect was lost.[87] Intermittent dosing with vitamin D seems also to be less effective in reducing the incidence of exacerbations of asthma despite its favorable role in preventing bone fractures. However, an intermittent dosing scheme might improve adherence and could be directly observed.

The appropriate form of vitamin D supplementation might vary between the different respiratory conditions, and indeed between supplementation for respiratory and bone disease and an evidence-based consensus on both the dose and the frequency needs to be reached.

9 | VITAMIN D SUPPLEMENTATION IN PREVENTING ARIs IN HEALTHY PEOPLE

The results from clinical trials of vitamin D supplementation in preventing and/or treating common colds in healthy subjects are inconsistent (Table 1). Values established as normal, insufficient, and deficient for circulating 25(OH)D were chosen to optimize bone health, and optimal levels of vitamin D for immune function were not considered in these studies. The majority of the studies show that maintaining serum 25(OH)D levels above 30 ng/mL (30 μg/L or 75 nmol/L) is associated with better respiratory outcomes. In some clinical trials vitamin D supplementation was not given to subjects with baseline serum 25(OH)D deficiency, and in other studies, the baseline levels of 25(OH)D were not measured. In addition, in some clinical trials the dose of vitamin D given was very low. Conversely, in some studies, intermittent, high-dose vitamin D was given, which may be suboptimal in time and is also possibly potentially dangerous immediately after administration: a high dose of vitamin D could be immunosuppresive.[28,88]

9.1 | Vitamin D_3 supplementation during pregnancy and infancy

Camargo et al reported that cord blood levels of 25(OH)D >75 nmol/L have inverse associations with risk of respiratory infection and childhood wheezing.[44]

Vitamin D_3 supplementation during pregnancy and infancy reduces primary care visits for ARIs during early childhood.[89] In a randomized, double-blind, placebo-controlled trial conducted in New Zealand, healthy pregnant women, from 27 weeks' gestation to birth, and their infants, from birth to age 6 months, were assigned to placebo or 1 of 2 doses of daily oral vitamin D_3 (1000 IU/400 IU or 2000 IU/800 IU).[89] The primary care records of enrolled children were inspected to age 18 months. In comparison with the placebo group, the proportion of children making any ARI visits was significantly smaller in the higher dose, but not the lower-dose vitamin D_3 group. The median number of visits per child for ARI was less in the higher-dose vitamin D_3 group from age 6 to 18 months (placebo 4, lower dose 3, higher dose 2.5; $P = .048$ for higher-dose vitamin D_3 versus placebo).[89]

A recent systematic review of correlation between in utero vitamin D exposure and childhood infection found that higher in utero vitamin D exposure lowers the risk of LRTI (pneumonia, bronchiolitis, croup, bronchitis, and/or otherwise unspecified chest infections) in young children.[90]

9.2 | Vitamin D_3 supplementation in preventing respiratory viral infections in healthy children

There are 2 studies in healthy children where preventive administration of vitamin D lowered the risk of respiratory infection.[91,92]

TABLE 1 Selective clinical trials investigating the protective role of vitamin D during an acute respiratory infection in healthy individuals

Interventional Clinical Trial Number	Type of Study	Participants	Description of the Study: Dose (IU) and Trial Duration	Posted/Published Results	Comments/Limits
UMIN000001373, PMID: 20219962, Urashima et al[91]	randomized placebo-controlled double-blinded multicenter study	schoolchildren aged 6-15 vitamin D_3 (n = 167) or placebo (n = 167)	1200 IU/d vitamin D_3 or placebo for 4 winter months—incidence of influenza A and B and flu-like illness determined by antigen detection in nasopharyngeal swabs	42% lower risk of laboratory-confirmed influenza A infection in children in the vitamin D_3 group	Positive study: low doses of vitamin D; baseline 25(OH)D levels not available
NCT00886379, PMID: 22908115, Camargo et al[92]	randomized placebo-controlled double-blinded multicenter study	Mongolian schoolchildren daily ingestion of milk fortified with 300 IU of vitamin D_3 (n = 143) or unfortified regular milk (control; n = 104)	milk fortified with 300 IU/d vitamin D_3 for 7 wk versus placebo; baseline serum 25(OH)D in intervention was 17.5 nmol/L and placebo 17.0 nmol/L	higher 25(OH)D levels at the end of the study (17.5 vs 47.3 nmol/L) in the fortified milk treated; 50% risk reduction in parent-reported ARI among children, ages 9-11; the decrease in ARI was seen in children both above and below the median baseline 25(OH)D levels	Positive study: post hoc analysis; very low dose of vitamin D; short time; study started late in winter (January-March)
NCT00656929, PMID: 19296870, Li-Ng et al[96]	randomized placebo-controlled double-blinded single-center study	adults (age 18-80) receiving vitamin D_3 (n = 78) versus placebo (n = 70)	vitamin D_3 2000 IU/d or placebo for 12 wk; biweekly questionnaire to record the incidence and severity of RTI symptoms over a 3-month period; baseline serum 25(OH)D measurement	25(OH)D levels increased significantly from 64.3 to 88.5 nmol/L in the vitamin D group, not in placebo group, $P < .0001$; vitamin D_3 did not decrease the incidence or severity of symptomatic RTIs	Negative study: single-center self-reported RTIs; short time of administration (12 wk); high levels at baseline of 25(OH)D and no difference between groups; study over spring period only, April-June
NCT01131858, PMID: 23242238, Bergman et al[99]	randomized, placebo-controlled, double-blinded, single-center study	patients with immunodeficiency with increased susceptibility to RTIs (>4 bacterial RTIs/year)	4000 IU/d vitamin D_3 for 1 y versus placebo; baseline serum 25(OH)D in intervention was 51.5 nmol/L (n = 62) and placebo 46.9 nmol/L (n = 62)	25(OH)D levels increased from 51.5 to 133.4 nmol/L after 3 mo and remain increased, $P < .001$; vitamin D_3 reduced the symptoms and antibiotic utilization among patients with immunodeficiency or frequent RTI, decreased number of days on antibiotics, increased vitamin D levels, $P < .001$	Positive study: patient-reported information; single center, small sample size; a selected group of patients; not only healthy persons, the patient population was very heterogeneous with regard to immune deficiency and concomitant diseases (only few participants healthy, mostly ill with asthma; bronchiectasis; chronic obstructive pulmonary disease etc); incidence of viral infections was not addressed
NCT01182870, PMID: 22026455, Jorde et al[98]	randomized, placebo-controlled, double-blinded, multicenter interventional study	adults received vitamin D (n = 289) versus placebo (n = 280)	different doses of vitamin D: 2000 IU/d; 2800 IU/d; 20 000 or 40 000 IU/wk; 100 000 IU vitamin D_3, taken at 0, 2, and 4 mo; 100 000 IU taken every 4 wk during 1 y (n = 289) or placebo (n = 280)	supplementation with vitamin D in subjects randomized to vitamin D did not protect against "influenza"	Negative study: post hoc analysis; retrospective study that relied on self-reported symptoms; baseline 25(OH)D levels not available; some patients with very high bolus dose of vitamin D
ACTRN12609000486224, PMID: 23032549, Murdoch et al[83]	randomized, placebo-controlled, double-blinded, multicenter study	adults with near-normal vitamin D levels (over 29 ng/mL) received vitamin D (n = 161)	participants received an initial dose of 200 000 IU oral vitamin D_3, then 200 000 IU 1 mo later, then 100 000 IU/mo for a total of 18 mo;	25(OH)D levels increased from 72.5 to 122.5 nmol/L, $P < .001$; no significant difference in	Negative study: levels at baseline of 25(OH)D, nearly sufficient in both treated and control groups; high bolus doses administered

(*Continues*)

		versus placebo (n = 161)	baseline serum 25(OH)D in intervention was 72.5 nmol/L and placebo 70.0 nmol/L; questionnaire to record the number, duration, severity of RTIs; PCR of nasopharyngeal swabs for viruses	reported or laboratory-confirmed number of upper RTIs	
NCT01158560, PMID: 24885201, Goodall et al[100]	randomized, placebo-controlled trial	students 17 y of age or older received vitamin D (n = 70) versus placebo (n = 80)	a weekly dose of 10 000 IU of vitamin D_3 (oral capsule) for around 2 mo	supplementation with vitamin D was associated with 46% risk reduction of laboratory-confirmed viral RTIs and may reduce the risk of clinical infections	Positive study: short time of administration; 10% of subject with asthma
NCT01069874 PMID: 26063508 Martineau et al[84]	randomized, placebo-controlled, double-blinded study	active treatments: 137; participants: 115 sheltered accommodation and 22 carers, versus control participants: 79 sheltered and 24 carers	residents: daily dose of 400 IU + 6 times (X) bolus of 96 000 IU every 2 mo vitamin D_3; carers: 6 X bolus of 120 000 IU every 2 mo vitamin D_3 versus placebo over a period of 1 y	25(OH)D levels increased, 42.4 to 85.3 nmol/L; supplementation with vitamin D was not associated with a decrease in RTI, but with increased risk and duration of URI	Negative study: mixture of sheltered accommodation residents and carers subjects; mixed ethnicity; high bolus doses of vitamin D_3

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; ARI, acute respiratory infection; PCR, polymerase chain reaction; PMID, PubMed identifier; RTI, respiratory tract infection.

Influenza A occurred in 10.8% of a group of 167 schoolchildren receiving 1200 IU/d vitamin D_3. This was significantly lower than the placebo group where over 18.6% of children were infected.[91]

In a recent systematic review of vitamin D supplementation to prevent ARI (influenza and pneumonia) in healthy kids, Xiao et al[93] found that from 4 clinical trials[91,92,94,95] only in Urashima's study, where the risk of viral infection was lowered, was the vitamin D supplementation[91] 1200 IU/d. In 2 studies, where vitamin D supplementation was at a low dose or administered as a 3-monthly bolus of 100 000 IU, the ARIs were not reduced in healthy children.[94,95]

9.3 | Vitamin D_3 supplementation in preventing respiratory viral infections in healthy adults

In contrast, the majority of vitamin D supplementation studies in adults reported negative results.

Vitamin D supplementation had no effect in healthy persons with self-reported upper respiratory infection (URI).[83,96] In a 3-month prospective placebo-controlled trial of vitamin D_3 supplementation, a dose of 2000 IU/d of cholecalciferol in ambulatory adults decreased neither the incidence nor the severity of symptomatic URTI during winter.[96,97] A lack of effect was also seen in a study in New Zealand with over 29 ng/mL mean baseline 25(OH)D level of participants.[83] Vitamin D supplementation (vitamin D_3, 200000 IU, then 200000 IU 1 mo later, then 100 000 IU monthly thereafter for a total of 18 mo) resulted in an increase in serum 25(OH)D levels that were maintained at greater than 48 ng/mL throughout the study, but there was no difference in the incidence of URTI of sufficient severity to bring them to medical attention. However, both vitamin D–treated and placebo groups had serum levels of 25(OH)D above deficiency and similar at baseline: 64.3 nmol/L in the vitamin D group versus 63.0 nmol/L in the placebo group[96] and 72 nmol/L in the vitamin D group versus 70 nmol/L in the placebo group.[83]

In another report, in 569 subjects from 10 different placebo-controlled clinical trials of vitamin D supplementation, of whom 289 received vitamin D (1111-6800 IU/d) and 280 received placebo,[98] influenza-like disease did not differ, although the duration of illness was significantly longer among those in the vitamin D group. The results do not support the hypothesis that high doses of vitamin D supplementation will have a pronounced effect on influenza-like disease in populations not having a high influenza risk.[98]

Bergman, in a study in adult patients with antibody deficiency or frequent RTI, reported that 4000 IU/d cholecalciferol reduced symptoms and antibiotic utilization.[99]

In university students who completed weekly electronic surveys and submitted self-collected midturbinate nasal swabs, vitamin D_3 supplementation at doses of 10 000 IU/wk significantly reduced the risk of URTI, with a significantly lower mean viral load.[100] Clinical URTI were reported in 23.3% of a group of 70 participants receiving vitamin D_3 as compared with 26.7% of 80 participants receiving placebo.

In a clinical trial of intermittent high-dose vitamin D_3 versus low-dose vitamin D_3 supplementation for ARI prevention in residents of sheltered-accommodation housing blocks and their carers in London, United Kingdom, the addition of intermittent bolus dose vitamin D_3 supplementation to a daily low-dose regimen did not influence risk of

ARI in older adults and their carers but on the contrary was associated with increased risk and duration of URIs.[84] Inadequate vitamin D status was common at baseline: 220 of 240 (92%) participants had serum 25(OH)D concentration <75 nmol/L. The active intervention did not influence time to first ARI. In fact, when URI and lower respiratory infections were analyzed separately, allocation to the active intervention was associated with increased risk of URI and increased duration of URI symptoms, but not with altered risk or duration of lower respiratory infection.

Simpson et al reported that a protective vitamin D effect (20 000 IU/wk cholecalciferol 17 wk during winter) towards acute infections (respiratory, gastrointestinal, urinary tract, eye, ear, skin, and cold sore infections) could only be observed in the subgroup of healthy adults with initial 25(OH)D levels <40 nmol/L (n = 4 of 34 healthy adults).[101] No microbiological verification of symptoms was available.

10 | VITAMIN D SUPPLEMENTATION IN PREVENTING RESPIRATORY INFECTION IN PATIENTS WITH ASTHMA

Although epidemiological studies have revealed a strong association between lower vitamin D levels and increased asthma exacerbations, suggesting that these patients would benefit from vitamin D supplementation, there are few studies that show efficacy of vitamin D supplementation. Several studies used a high dose of vitamin D and intermittent administration as bolus dose, whose efficacy is questioned.[102–104]

A cause of inefficacy of vitamin D in preventing viral infection in allergic asthma could be the characteristic high levels of type 2 cytokine IL-4 and IL-13, which could initiate the catabolism of vitamin D, affect VDR levels, induce type 1 to type 2 conversion of T cells.[25,105–108]

10.1 | Vitamin D supplementation in pregnant women in preventing wheezing in offspring

Vitamin D supplementation of pregnant women did not protect the offspring from developing recurrent wheezing episodes or physician diagnosed asthma later in life.[109,110]

In a double-blind, placebo-controlled trial conducted in India, 230 mother-newborn pairs were randomized to receive, monthly for 9 months, a high dose of cholecalciferol (3000 μg = 120 000 IU) for the mother (group A) or 10 μg = 400 IU/d for the infant (group B) or double placebo (group C).[104] At 3.5 months, infants' median 25(OH)D was lower in placebo group C (45.3 nmol/L) than in group A (60.8 nmol/L) or B (61.3 nmol/L). There were no significant differences between groups in respiratory infections (we do not know how respiratory infections were diagnosed) over 9 months. There are no results concerning the differences between baseline and 3.5 months.

In 2 recent well-designed clinical trials, vitamin D supplementation did not decrease significantly the incidence of RTIs.[111,112] In a multiracial cohort of pregnant women at high risk of having a child with asthma, the supplementation with vitamin D at doses of 4400 IU/d, although significantly increased vitamin D levels, did not significantly decrease the incidence of LRTI and recurrent wheezing in their children at age 3 years as compared with a group taking only 400 IU/d vitamin D.[111] However, the authors recognized that the studies may have been underpowered and short in time. The high risk of having a child with asthma means that the group was not homogenous: the mother or the father has a range of very different diseases such as asthma (not specified if allergic or nonallergic), eczema, and allergic rhinitis. Other causes for a negative study results could be that the group was multiracial and the dose of vitamin D was too small.

Another study found that supplementation with 2800 vs 400 IU/d of vitamin D during the third trimester of pregnancy did not result in a statistically significant reduced risk of persistent wheezing in the offspring through 3 years of age.[112] Vitamin D supplementation did not influence the number of URTI (episodes of common cold, acute tonsillitis, croup, and acute otitis media) or LRTI (pneumonia and bronchiolitis). The authors admitted a reduced statistical power of the study and that the vitamin D supplementation doses may have been too low. In addition, the authors commented that they may have begun supplementation too late (only 81% of the women had serum vitamin D above 30 ng/mL after the intervention). Finally, the study did not include postnatal supplementation of the children, which could have induced an additive effect if the effects of maternal supplementation declined postnatally.

10.2 | Vitamin D supplementation in preventing asthma exacerbations in children

Episodes of airway obstruction with wheezing, which are mostly triggered by viral infection in the first 3 years of life, although transient, are a major cause of morbidity, and hospitalization rates are high.[113,114]

In 2 randomized, placebo-controlled double-blind multicenter studies in children, vitamin D supplementation reduced ARI and asthma attacks in children with vitamin D deficiency.[91,93,94] In school-aged children, vitamin D_3 supplementation at 1200 IU/d reduced the incidence of symptomatic influenza A infections during the influenza season and reduced the number of asthma attacks.[91]

10.3 | Vitamin D supplementation in preventing asthma exacerbations in adults

In a recent trial (Vitamin D Add-on Therapy Enhances Corticosteroid Responsiveness in Asthma) that assessed the effect of vitamin D supplementation to adults with persistent asthma with vitamin D deficiency (serum 25[OH]D < 30 ng/mL), vitamin D_3 (100 000 IU once, then 4000 IU/d for 28 wk) did not reduce asthma exacerbations.[102] However, the trial was underpowered, and when analyses were limited to those that achieved a sufficient level of vitamin D (30 ng/mL or greater), an effect was seen for decreasing the overall number of asthma exacerbations.[115]

In another study, a bolus dose of vitamin D_3 supplementation did not influence time to exacerbation or URI in a population of adults with asthma with a high prevalence of baseline vitamin D insufficiency.[103]

A current ongoing study VITAL will evaluate the effect of vitamin D supplementation, 2000 IU, administered once per day, rather than intermittent large boluses, on acute exacerbations of asthma.[115]

11 | CONCLUSIONS

Vitamin D has been shown to increase the antiviral and anti-inflammatory responses of airway epithelial cells during respiratory viral infections in vitro. There is definite evidence for an association between vitamin D deficiency and an incidence of pulmonary exacerbations in chronic airway diseases such as asthma. However, the results from clinical trials of vitamin D supplementation in preventing or ameliorating treating RTIs are inconsistent. The negative results could be due to underpowered studies, a lower dose of vitamin D, a high dose of vitamin D given at a large interval of time, dosing schedule, short follow-up time, use of supplementation in subjects with normal 25(OH)D levels, self-reporting of the cold/infection, and, very frequent, the absence of a laboratory diagnostic of the viral infection.

Better-designed clinical studies, using (i) cholecalciferol/vitamin D_3, (ii) given vitamin D in a daily rather than as intermittent high dose, (iii) for longer than 3 months, (iv) in placebo-controlled homogenous large groups with deficient 25(OH)D levels, could confirm its efficiency in preventing viral infection and virus-induced exacerbations of asthma. In addition, vitamin D could increase the efficacy of medications such as corticosteroids in preventing respiratory exacerbations of these diseases. More studies investigating the role of vitamin D deficiency in respiratory disease are eagerly anticipated.

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CONFLICT OF INTEREST

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