

Vitamin D and Influenza^{1,2}

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ABSTRACT

Vitamin D has become increasingly recognized in the literature for its extra-skeletal roles, including an effect on inflammation and the immune response to infection. Our goal was to describe the role of vitamin D in the immune response and implications for the risk of influenza infection in humans. In this review, we first consider literature that provides molecular and genetic support to the idea that vitamin D is related to the adaptive and innate immune responses to influenza infection in vitro and in animal models. We then discuss observational studies and randomized controlled trials of vitamin D supplementation in humans. Finally, we consider some of the knowledge gaps surrounding vitamin D and immune response that must be filled. *Adv. Nutr.* 3: 517–525, 2012.

Introduction

Vitamin D is a fat-soluble vitamin, unique in that it is primarily produced in the skin during sun exposure rather than absorbed from the diet (1). Vitamin D has long been known to play a role in the skeletal system and calcium homeostasis; vitamin D deficiency is known to be a cause of rickets and osteoporosis (2). Recently, cells of the immune system have been found to possess vitamin D receptors (VDR)³ and are capable of metabolizing the active form of vitamin D [also known as calcitriol, 1,25-dihydroxyvitamin D, or 1,25(OH)₂D] (1), suggesting that this nutrient may be an important factor in the immune response to infection (3). For example, activated T- and B-cells can convert the inactive form of vitamin D [also known as 25-hydroxyvitamin D, or 25(OH)D] to 1,25(OH)₂D in human cells in vitro (4); locally produced 1,25(OH)₂D then acts on immune cells in an autocrine or paracrine fashion. VDR have also been identified on peripheral blood mononuclear cells (PBMC) in human cells in vitro (5), lending support for a potential role of vitamin D in the regulation of the immune system and infectious diseases (6).

Further investigation has revealed that vitamin D plays important roles in signaling during both the adaptive and innate immune response to viral and bacterial infection

(3,7). 25(OH)D can be converted to 1,25(OH)₂D in human respiratory epithelium cells in vitro (8), and 1,25(OH)₂D and 25(OH)D have both been implicated in the immune response to several types of respiratory infections, including respiratory syncytial virus and tuberculosis, in vitro (8–10). Studies have also found associations between either 25(OH)D or 1,25(OH)₂D deficiency and clinical illnesses, including influenza (11–14), tuberculosis (15–19), respiratory syncytial virus (9,20), and other respiratory illnesses (21–24), in observational studies. An estimate provided by data from the NHANES states that more than one-half of U.S. adults have 25(OH)D <30 μg/L (23), a status defined as vitamin D insufficient (1). However, an Institute of Medicine report suggests that vitamin D deficiency be defined as 25(OH)D <10 μg/L (25), a condition shared by only 2% of Americans (23). No matter the cutoff, 25(OH)D levels have been found to be significantly lower among children with respiratory illnesses, older adults, women, and individuals with darker skin pigmentation (26–29).

In this review, we will describe evidence for the role of vitamin D in modulating the adaptive and innate immune response. We will then consider how those aspects of the immune system respond to influenza infection. We will also consider observational studies and randomized controlled trials of vitamin D supplementation in humans and the concurrent seasonality of poor vitamin D status and increased risk of influenza infection. Finally, we will note the problems in accurately assessing vitamin D and consider the next steps for assessing vitamin D in the context of respiratory illness.

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³ Abbreviations used: 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)₂D, 1,25-dihydroxyvitamin D; PBMC, peripheral blood mononuclear cell; Th, T helper cell; Treg, T regulatory cell; VDR, vitamin D receptor.

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Current status of knowledge

Vitamin D and adaptive immune response to infection

The adaptive immune response to infection is complex and multi-faceted and involves a diverse population of cell types and other factors such as cytokines, chemokines, enzymes, and hormones (3,30,31) (Fig. 1). Additionally, the immune response to an infectious insult is not static; characteristics of the response (measured by serum cytokines in human adults) change from the initial period of antigenic stimulation to the later stage of disease clearance (32). Vitamin D may therefore affect one component of an immune system response but not other components, meaning that the net effect of vitamin D on immune function and clinical illness is difficult to characterize. This also suggests that evidence of vitamin D's role in the immune system *in vitro* may not apply to vitamin D's role *in vivo*. Nevertheless, vitamin D [in particular, 1,25(OH)₂D] has been found to act on specific cell parameters of the adaptive immune response, most notably T- and B-cells (Fig. 1A).

The T-cell profile shift

Vitamin D [especially 1,25(OH)₂D] is widely acknowledged to shift the T-cell response profile from a T helper cell (Th) 1- to a Th2-mediated response by inhibiting cells of the Th1 profile (3,31,33-43) *in vitro* in mouse fibroblasts,

pancreatic islets, cultured splenocytes, and host serum; and *in vivo* in mouse dendritic cells. Vitamin D also promotes cells of the Th2 profile (31,39,41,42,44,45) in mouse fibroblasts (Fig. 1A). This bias is thought to reduce inflammation and promote an immunosuppressive state (31). 1,25(OH)₂D inhibits the proliferation of Th1 helper cells in mouse lymphocytes and human T-cell clones *in vitro* in part by inhibiting IFN γ and IL-2, cytokines that promote Th1 production and recruitment and macrophage production (3,46-48). 1,25(OH)₂D also reduces IL-12 (another cytokine that promotes Th1 production and recruitment) *in vitro* in human PBMC (15); it is thought to accomplish this by downregulating molecules in human dendritic cells, which produce IL-12 (3,15,47,49), and by promoting IL-10, a cytokine thought to inhibit production of IL-12 (39,50,51). 1,25(OH)₂D suppresses an additional Th1-mediated cytokine, TNF α , *in vitro* in human monocytes, further pushing immune response to a Th2 profile (52,53). 1,25(OH)₂D has also been found to suppress the production and recruitment of Th17 cells in mice *in vivo* by downregulating Th17-mediated cytokines IL-23 and IL-6 (3,50) (Fig. 1B). In contrast, 1,25(OH)₂D has been shown to upregulate IL-4, IL-5, and IL-10 in mouse lymphocytes *in vitro*; these cytokines promote a Th2 response profile (3,46). IL-10 also promotes the proliferation of T regulatory (Treg) cells

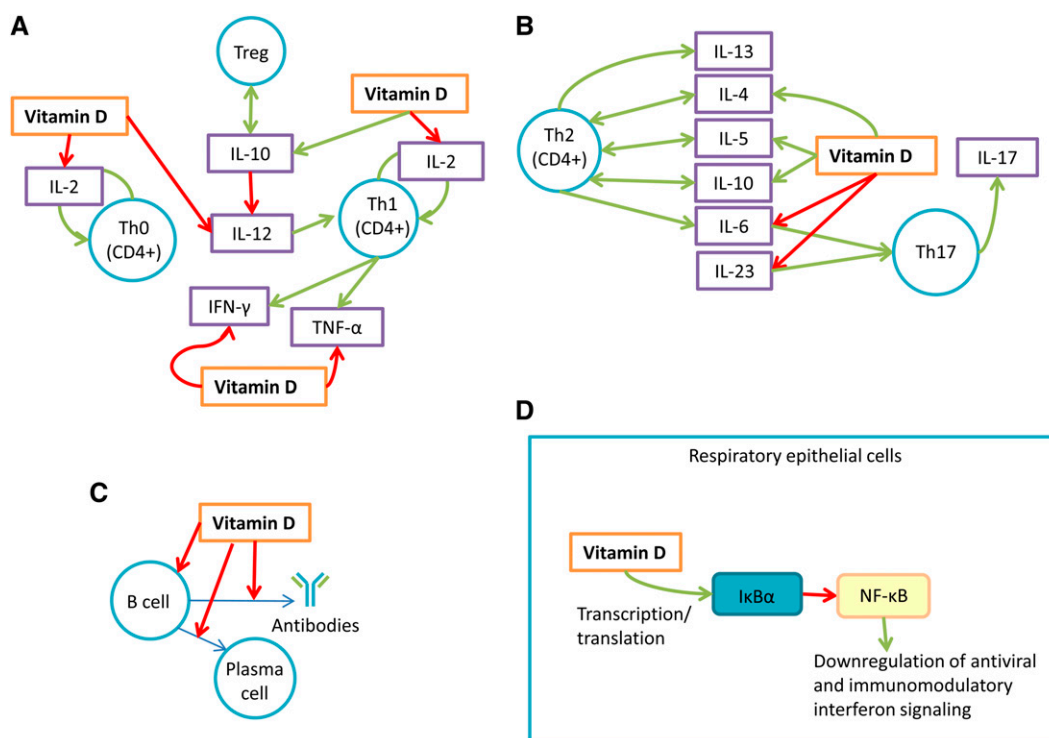


Figure 1 Vitamin D and its various actions in the immune system. (A) Vitamin D inhibits the production and proliferation of Th1 and Th0 cells by inhibiting IL-2, IFN γ , and TNF α ; vitamin D promotes the production of Treg cells by facilitating production of IL-10. (B) Vitamin D promotes a Th2-mediated immune response profile by promoting IL-4, IL-5, and IL-10. Vitamin D inhibits a Th17-mediated immune response profile (and thus inhibits IL-17) by inhibiting IL-6 and IL-23. (C) Vitamin D inhibits the production of B-cells, the differentiation of B-cells into plasma cells, and the production of antibodies by B-cells. (D) Vitamin D promotes nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, α in respiratory epithelial cells, which inhibits NF- κ B, in turn promoting antiviral and immunomodulatory interferon signaling. Th, T helper cell; Treg, T regulatory cell.

in mouse colon cells *in vivo*; these Treg cells inhibit the Th1 response profile (3,50) (Fig. 1A).

Despite overwhelming *in vitro* evidence that 1,25(OH)₂D biases the immune system response toward a Th2-dominated profile, it has been shown to repress both Th1 and Th2 response profiles in human cord blood cells *in vitro* (54). This suggests that the effects of 1,25(OH)₂D on Th helper cell selection are more complex *in vivo* and in differing molecular and cellular environments (44). A study on the immune response to allergy stimulus in mice *in vivo* suggested that vitamin D supplementation [100 ng 1,25(OH)₂D injection] given after the initial period of sensitization prevented high levels of eosinophils associated with reduced local inflammatory response in bronchoalveolar lavage fluid and lung tissue. However, constant vitamin D supplementation [100 ng 1,25(OH)₂D injection every other day during the study] did not protect against a high eosinophil count in mice respiratory epithelia (38). The proposal that the effect of vitamin D is time-sensitive is further bolstered by a study that showed that low vitamin D [measured by serum 25-hydroxy-(ergocalciferol + cholecalciferol)] was common among patients with tuberculosis (35.6 μg/L compared with 37.2 μg/L in patients without tuberculosis; *P* < 0.05) but was not associated with the initial, acute-phase response to infection (measured by levels of α₁-antichymotrypsin, an acute-phase protein) (19). A 3-mo prospective, randomized controlled trial investigating whether vitamin D has an effect on cytokine levels in humans showed that supplementation of 2000 IU cholecalciferol in ambulatory adults does not have a significant effect on the association between levels of serum 25(OH)D and IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IFNγ, and TNFα, although this study did not take into account infection status and there was no difference between 25(OH)D levels pre- and postsupplementation in the group randomized to receive cholecalciferol (55).

In contrast, another study found that many of the cytokines inhibited by 1,25(OH)₂D in humans (IL-2, IL-12, IFNγ, IL-6, TNFα, IL-17, and IL-23) are produced in significantly greater quantities in individuals with pandemic H1N1 influenza compared with healthy controls; some cytokines that are promoted by 1,25(OH)₂D (IL-5, IL-10) are also produced in significantly greater quantities in those individuals (3,56) (measured by serum cytokine levels) (Fig. 1A,B). Early secretion of Th1- and Th17-mediated cytokines was also found to be significantly increased in individuals infected with severe novel H1N1 influenza (37), suggesting that serum 25(OH)D may play a role in inflammatory response during influenza infection, even if it does not affect levels of inflammatory cytokines in a noninfectious state.

B-cell differentiation

1,25(OH)₂D inhibits proliferation and promotes apoptosis of activated human B-cells *in vitro*, although the initial production of B-cells remains unaffected (4). 1,25(OH)₂D is thought to thereby inhibit the differentiation of B-cells into plasma cells (4) (Fig. 1C). B-cell deficiency in mice and ferrets has been associated with decreased heterologous

immunity between seasonal and pandemic H1N1 influenzas (57) and memory B-cells against influenza infection have persisted for >5 mo after infection in lung epithelial cells *in vitro* (58). Although there is relatively little evidence linking influenza infection to B-cell levels in humans, a small study involving 15 children aged 2–14 y with pandemic or seasonal H1N1 influenza showed that a significantly higher percentage of B-cells were found in children infected with any strain of influenza, compared with controls (59).

Vitamin D and innate immune response

Inflammation and cell signaling. Both the active form of vitamin D [1,25(OH)₂D] and a fluorescent vitamin D analogue have been found to decrease proinflammatory chemokine production during infection *in vitro* in human respiratory epithelial cells (for the active form of vitamin D) and mouse pancreatic islets (for the vitamin D analogue) (9,35). Additionally, 1,25(OH)₂D has been found to downregulate proinflammatory cytokines such as IL-1, IL-6, IL-8, and TNFα in many different cell types *in vitro* (60). Significantly higher serum levels of IL-6, IL-8, IL-17, and TNFα were found in patients hospitalized with pandemic H1N1 influenza in a case control study (37) and have elsewhere been implicated in influenza infection (32,56).

In human lymphocytes *in vitro*, the antiinflammatory effect of vitamin D is carried out in part through inhibition of NF-κB (61) (Fig. 1C). NF-κB is a protein complex that is associated with the transcription of inflammatory proteins during infection (62), including cytokines and chemokines, acute phase proteins, adhesion molecules, and inducible effector enzymes (63). 1,25(OH)₂D induces nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor, α, the inhibitor of NF-κB, in human airway epithelial cells *in vitro* during infection with respiratory syncytial virus (9). NF-κB itself has been found to modulate T-cell response profiles in various types of mouse cells (64) and to downregulate antiviral and immunomodulatory interferon signaling involved against influenza infection in mouse embryonic fibroblasts (65,66). 1,25(OH)₂D inhibits NF-κB *in vitro* in human promyelotic leukemia HL-60 cells during the early infection period (67) (Fig. 1D) but promotes it later on (67).

Monocytes and differentiation. 1,25(OH)₂D has been shown to promote the differentiation of monocytes into macrophages in both mouse and human cells *in vitro* (47) and suppress the differentiation of human monocytes into dendritic cells (49) (Fig. 2). 1,25(OH)₂D (10 nmol/L) has also been found to induce a tolerogenic state in human myeloid (but not plasmacytoid) dendritic cells *in vitro* (68) and has an effect on the trafficking and translocation of differentiated but immature dendritic cells in mice (69). Mouse dendritic cells exposed to 1,25(OH)₂D *in vivo* for 24 h were able to retain antigen-presenting abilities and avoid sequestration in lymph nodes. However, mouse dendritic cells that were differentiated in the constant presence of 1,25(OH)₂D *in vitro* did not retain these capabilities (69), a result that supports

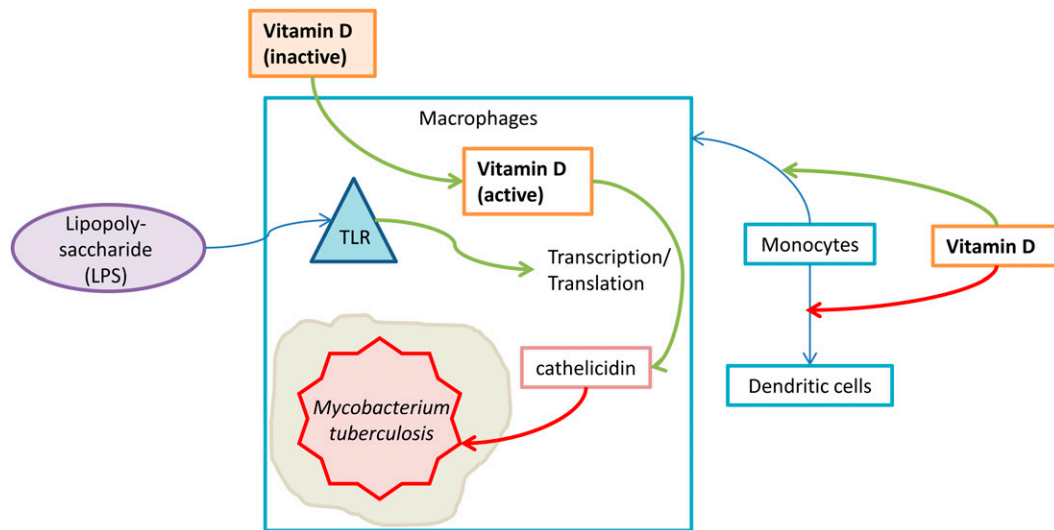


Figure 2 Vitamin D activation and mycobacterial immune response. Vitamin D inhibits the differentiation of monocytes into dendritic cells and promotes the differentiation of monocytes into macrophages. When toll-like receptors are activated by circulating LPS, they promote vitamin D-mediated transcription of cathelicidin, an antimicrobial peptide that kills *Mycobacterium tuberculosis*.

the idea that immune system involvement of active vitamin D is modulated by infection time frame as well as the type of immune cell involved. In older adults, loss of dendritic cell function (including reduction in dendritic cell-mediated cytokines, such as $\text{TNF}\alpha$) is associated with poor influenza vaccine response (70) and impaired response to influenza infection as a result of decreased induction of dendritic cell-stimulated CD8^+ T-cells (71). Notably, infection with influenza A virus induced human blood monocytes to rapidly differentiate into mature dendritic cells in vitro (72). However, it has been found that plasmacytoid dendritic cells, which are unaffected by $1,25(\text{OH})_2\text{D}$, may be responsible for dendritic cell-mediated protection to influenza infection in mice (73).

$1,25(\text{OH})_2\text{D}$ is also thought to suppress $\text{IFN}\gamma$ -mediated activation of macrophages through inhibition of the Th1 response profile (3); the deactivation of $\text{IFN}\gamma$ -activated macrophages is contingent upon a functional VDR in vitro in mouse macrophages (74). However, macrophages are capable of responding to and producing $1,25(\text{OH})_2\text{D}$ in human alveolar macrophages in vitro (75), and $1,25(\text{OH})_2\text{D}$ increases the production of cathelicidin in human macrophages in vitro by increasing expression of the VDR (76).

Antimicrobial properties. $1,25(\text{OH})_2\text{D}$ is associated with increased bactericidal activity in human PBMC (15) and the innate antibacterial response in human trophoblasts in vitro (77). VDR expression is upregulated by the activation of toll-like receptors in human macrophages and trophoblasts; this upregulation leads to the transcription of cathelicidin, which kills intracellular *Mycobacterium tuberculosis* (42,77) (Fig. 2). This finding has been corroborated by in vivo evidence: vitamin D supplementation (a single oral mega-dose of 100,000 IU ergocalciferol) has been found to improve antimycobacterial immunity in humans (78).

Because cathelicidin's main mechanism of action is the destruction of envelope proteins of foreign agents, it may also be implicated in the destruction of influenza virus, which possesses an envelope protein (2,79).

Additionally, $1,25(\text{OH})_2\text{D}$ has been found to upregulate human β -defensin 2 in a variety of human cells (80); human β -defensin 2 is thought to act as a chemoattractant for monocytes during viral infection (7). $1,25(\text{OH})_2\text{D}$ also induces hydrogen peroxide production in human monocytes in vitro (81). However, serum $25(\text{OH})\text{D}$ was not associated with levels of serum cathelicidin or β -defensin-2 in patients with community-acquired pneumonia (82).

In total, the literature describing vitamin D's role in the adaptive and innate immune systems suggests that vitamin D is involved in reducing inflammation during infection. Although $1,25(\text{OH})_2\text{D}$ suppresses the response of Th1 cells and proinflammatory cytokines, it promotes antimycobacterial factors such as cathelicidin and human β -defensin 2. Additionally, the literature suggests that vitamin D's role in modulating immune system response to infection is not constant over time and changes according to the host and state of infection; $25(\text{OH})\text{D}$ deficiency has been shown to be related to tuberculosis but not the acute phase of infection in humans (19); $1,25(\text{OH})_2\text{D}$ has been shown to have a positive effect on infection only when given after the initial phase of infection in mice (38); $1,25(\text{OH})_2\text{D}$ has been shown to affect $\text{NF-}\kappa\text{B}$ in different ways at different time points of infection in human leukemia cells (67) and prevents mouse dendritic cells from presenting antigens and differentiating only at certain time points (69).

Vitamin D and respiratory diseases in humans. Due to the complexity of adaptive and innate immune responses to antigenic stimulation, it is difficult to pinpoint the overall effect of vitamin D during infection. This complexity

Table 1. Randomized controlled trials investigating the association between vitamin D supplementation and increased risk of respiratory illness as an outcome

Author(s)	Year	Sample size and participants	Location	Dose and duration of supplementation	Outcome measure	Strength of association
Jorde et al. (93)	2011	n = 569 adults 32–84 y old	Norway, Austria, USA, Scotland, Denmark, and Belgium	1111–6800 IU/d cholecalciferol for at least 12 wk during influenza season	Risk of influenza-like illness, measured by self-report	RR of influenza-like illness ¹ : 0.88 (95%CI: 0.58–1.32) Median increased duration of ILI ² : 3 d (P < 0.01)
Urashima et al. (12)	2010	n = 334 school-children	Japan	One 200-IU tablet cholecalciferol 3 times/d for 120 d	PCR-confirmed influenza A	RR of infection ¹ : 0.58 (95%CI: 0.34–0.99)
Li-Ng et al. (22)	2009	n = 162 adults 18–80 y old	USA	2000 IU cholecalciferol/ d for 12 wk	Risk of URTI	RR of URTI ¹ : 0.96 (95%CI: 0.75–1.23)
Wejse et al. (100)	2009	n = 281 adults with TB	Guinea-Bissau	100,000 IU of cholecalciferol at inclusion and again at 5 and 8 mo after TB treatment	Clinical severity and mortality of tuberculosis	RR of mortality ¹ : 1.19 (95%CI: 0.58–1.95)
Aloia and Li-Ng (14)	2007	n = 208 postmenopausal African American women	USA	800 IU cholecalciferol/d for 2 y, then increased to 2000 IU/d for an additional year (3 y total)	Risk of self-reported cold or influenza	RR of cold or influenza ¹ : 0.31 (95%CI: 0.15–0.65)
Avenell et al. (92)	2007	n = 3444 adults ≥70 y old	England and Scotland	800 IU/d cholecalciferol, 1000 mg calcium, both, or placebo, for 24–62 mo	Risk of any infection, measured by self-report	Relative odds of any infection ¹ : 0.90 (95%CI: 0.76–1.07)
Martineau et al. (78)	2007	n = 131 adults >17 y old	England	Single dose of 100,000 IU ergocalciferol at beginning of study	Immunity to TB mycobacteria measured by BCG-Iux luminescence ratio at 24 h postinfection	Relative increase in immunity ³ : 20.4% (95%CI: 1–25%)
Nursyam et al. (17)	2006	n = 67 TB patients 15–59 y old with moderately advanced TB lesions	Indonesia	10,000 IU vitamin D (unspecified)/d	Rate of sputum conversion	Relative odds of conversion ⁴ : 1.32 (95%CI: 1.09–1.60)
Morcos et al. (18)	1998	n = 24 children with TB, 1–13 y old	Egypt	1000 IU cholecalciferol/d for the length of tuberculosis treatment	Concentration of serum vitamin D in supplemented vs. unsupplemented groups	Difference in vitamin D (pg/mL) between supplemented and unsupplemented groups was not significant (data not shown)
Rehman (102)	1994	n = 47 children 3–12 y old	India	60,000 IU vitamin D (unspecified)/wk for 6 wk, plus 650 mg calcium/d	Frequency of any infection in children	No difference in frequency seen between supplemented and control groups (data not shown)

¹ RR refers to the risk of illness while being supplemented with vitamin D compared with being supplemented with placebo. TB, tuberculosis; URTI, upper respiratory tract infection.

² “Relative increase in duration” refers to the extra number of days that participants in the placebo group reported experiencing ILI symptoms, compared with the number of days that supplemented participants reported; P-value is a chi-square value comparing the 2 proportions.

³ “Relative increase” refers to the increase in immunity in vitamin D-supplemented compared with placebo-supplemented participants.

⁴ “Relative conversion” refers to the conversion proportion of vitamin D-supplemented compared with conversion proportion of placebo-supplemented participants.

sometimes results in different results in *in vitro* compared with *in vivo* studies. In several observational studies, lower 25(OH)D serum levels have been associated with increased risk of respiratory infection in adults (21,23,83,84), children (26,85,86), and infants (20). A robust, dose-response association was found between lower levels of 25(OH)D and increased risk of upper respiratory infection in large, population-based studies in the United States (23) and Great Britain (21). 25(OH)D deficiency has also been connected to increased severity of acute lower respiratory infection in children (27,86) and mortality from pneumonia in adults (82). Furthermore, children with VDR gene polymorphisms (specifically, the *FokI ff* or *TaqI* polymorphisms) are more likely to have acute lower respiratory infections (87). The *FokI ff* polymorphism results in a downregulation of the vitamin D target gene, *CYP24A1*, which codes for an enzyme that degrades 1,25(OH)₂D (88); the *TaqI* polymorphism is associated with lower VDR protein levels (89).

However, randomized controlled trials supplementing vitamin D have yielded mixed results; a summary of these results can be found in **Table 1**. No significant effect was found for 1,25(OH)₂D supplementation as an adjuvant to increase the efficacy of an influenza vaccine in the general adult population (90) or in HIV-positive (91) adults, and supplementation with oral cholecalciferol did not significantly reduce the risk of infection in an elderly population (92). General vitamin D supplementation (median daily dose of 2000 IU/d, or 50 µg/d) was not associated with improved serologic response to an influenza vaccine (measured by a ≥1:40 hemagglutinin antibody inhibition titer ratio or 4-fold increase in hemagglutinin antibody inhibition titer at 3-mo postvaccination) in prostate cancer patients (13). However, the baseline 25(OH)D serum concentration in the same population was associated with an improved response to influenza vaccine ($P = 0.045$) (13). Supplementation with cholecalciferol (1200 IU/d for 4 mo) was also found to be associated with a reduced risk of seasonal influenza A in Japanese schoolchildren ($P = 0.04$) (12), but the study did not measure serum concentrations of 25(OH)D or serum antibody concentrations to influenza. An additional study found a lower rate of upper respiratory infection and influenza in participants taking a cholecalciferol supplement (2000 IU or 50 µg/d), but this analysis was based on self-reported illness (14) and the results were not reproduced (93). A systematic review of randomized controlled trials supplementing vitamin D for the prevention or treatment of infectious disease found that the strongest evidence for the effectiveness of vitamin D in prevention or treatment of infectious diseases is for the reduction of risk of acute respiratory illness and influenza (45).

Influenza and vitamin D: seasonality. Edgar Hope-Simpson (94) was the first to make the argument that the seasonality of influenza hinges upon times of less sunlight; since then, several researchers have corroborated the finding that influenza is more prevalent in the winter during times of less sunlight and therefore less available vitamin D (40,95,96). Healthy

volunteers inoculated with live attenuated influenza virus in northern latitudes of Russia during different seasons of the year were 8 times more likely to have influenza infection during the winter than summer (97). However, another study recently found that model simulations of vitamin D seasonal fluctuation are not able to consistently reproduce observed seasonal patterns in influenza; the authors concluded that seasonal variation in vitamin D is unlikely to be the main factor affecting the seasonality of influenza (98). Because there are other important environmental factors that could affect virulence of influenza that are synchronous with periods of less sunlight (e.g., colder temperatures and lower humidity), it may be difficult to separate these factors in an analysis of influenza and vitamin D seasonality.

Problems in assessing vitamin D. Lee (99) has pointed out several problems in assessing vitamin D. First, a consensus has not yet been reached on what level of vitamin D is considered “deficient” (1), and studies with different definitions of deficiency may not have comparable results. Additionally, there is no consensus on biologically relevant doses of vitamin D for use in randomized controlled trials; studies cited in this paper supplemented vitamin D in a broad range of forms, from 200 IU cholecalciferol 3 times/d (12 to 2000 IU/d (22), and including one-time supplements of cholecalciferol in 100,000-IU doses (78,100). Second, concentrations of 25(OH)D obtained from serum vary depending on the method of assay and reproducibility is poor (99,101). Third, concentrations of 25(OH)D are subject to change depending on levels of binding proteins and rapid fluid shifts, factors of concern in critically ill patients (99).

In 8 of 10 supplementation studies cited in this review that looked at respiratory illness as an outcome, serum vitamin D pre- and postsupplementation was not reported (12,14,17,22,92,93,100,102). Lack of vitamin D measurement in these studies makes interpretation of the results more difficult, because there is no way to demonstrate that participants in the supplementation group had significantly higher serum vitamin D than participants in the control group. In such studies, determining the biological relevance of a particular dose of vitamin D is also difficult. One study that supplemented vitamin D and measured vitamin D serum status postsupplementation found no significant difference in respiratory illness between the supplementation and control groups (18); one study found a significant difference in the level of mean 25(OH)D after a single mega-dose of cholecalciferol (78).

Limitations of current literature. Evidence from *in vitro*, *in vivo* animal, and *in vivo* human studies has been accumulating over the past decade, suggesting that vitamin D may influence the risk of respiratory infections, including influenza. However, much work remains to be done: there must be a focus on the application of laboratory and animal findings to human populations, because it has already been observed that the results of *in vitro* studies are not always replicated *in vivo* and the results of animal studies are not

always replicated in humans. It is important to note that several of the studies listed here were done in mouse models. Although mouse models are accepted for studying influenza infection, they are not ideal; mice do not possess a vitamin D response element for the production of cathelicidin (3) and other differences in cell signaling have been observed between mouse and human cells (42,103). However, a “humanized” mouse model is currently being developed that may prove useful in studying this mechanism more closely (39).

Several of the studies on vitamin D and respiratory illness cited here were conducted in settings where other micronutrient and macronutrient deficiencies are common (16,19,85,104,105). It is possible that other nutrient deficiencies act as effect modifiers or confounders for respiratory illness outcomes in such settings. Additionally, other infectious diseases with a higher prevalence in these settings than in other settings (e.g., malaria, multi-drug-resistant tuberculosis, and HIV/AIDS) may affect the outcome measurement. It is therefore important to keep in mind that such different settings limit generalizability of results.

The difference between the role of vitamin D during the acute initial infection state and after the initial infection state must be considered in future studies, because it is possible that the effects of vitamin D on the immune system change over time and between immune cell types. It is also important to differentiate between the effect of vitamin D status on influenza incidence and influenza severity, taking into special consideration the possibility that the serum 25(OH)D levels of critically ill patients may be subject to change more rapidly than in healthy individuals. Furthermore, a distinction must be made between “deficient” and “suboptimal” vitamin D status. Analyses considering not only vitamin D deficiency but the continuous range of serum vitamin D values will add to the literature. Finally, rigorous methodology, including a standardized serum vitamin D assay, consistent reporting of specific types of vitamin D [e.g., 1,25(OH)₂D as opposed to 25(OH)D] and other measures of immune response in addition to hemagglutinin antibody inhibition titers, such as cell-mediated immunity, are needed. PCR confirmation of influenza diagnosis is the gold standard for measuring outcome; if other methods are used, these methods should be clearly reported.

Conclusion

The evidence for an association between vitamin D and risk of influenza infection exists, albeit mainly in *in vitro* and animal studies describing the role of 1,25(OH)₂D in innate and adaptive immunity. Observational human studies of 25(OH)D deficiency and randomized controlled trials supplementing various forms of vitamin D have yielded mixed but promising results. More rigorous research studies with large populations and outcome measures including 25(OH)D serostatus post-supplementation are needed to further elucidate the possible relationships between vitamin D and risk of influenza infection. The establishment of a clear link between vitamin D status and influenza infection has broad implications for influenza research, especially in groups that are likely to have low

vitamin D levels, as well as the formulation of policy regarding vitamin D supplementation.

Acknowledgments

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