REVIEW ARTICLE
Does vitamin D protect against respiratory viral infections?

K. J. BRYSON[1]*, A. A. NASH[1] AND M. NORVAL[2]

[1] *The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK*
[2] *Biomedical Sciences, University of Edinburgh Medical School, UK*

SUMMARY

The active form of vitamin D has effects on both innate and adaptive immune responses that may influence the outcome in many infectious diseases. Observational studies conclusively show that a low vitamin D status is associated with an increased occurrence of respiratory viral infections, which globally represent significant health and financial burdens. However, no consistent protective effects are evident in prospective clinical trials carried out to date where vitamin D was provided as a dietary supplement, except possibly in cases where the starting vitamin D status of the individual was considered deficient. Thus far, vitamin D has not been found to enhance the immune response to vaccines. The design of future prospective clinical trials assessing a role for vitamin D in respiratory viral infections requires very careful planning to avoid the uncertainties associated with the data available currently.

Key words: Influenza (seasonal), respiratory viral infections, respiratory syncytial virus, vitamin D.

Introduction

A number of diverse viruses are known to infect the respiratory tract (Fig. 1). In broad terms severe disease is restricted to infections of the lower respiratory tract (LRT), resulting in bronchiolitis and pneumonia. Such infections are the leading cause of hospitalization and deaths in infants and young children particularly in the developed world, [1], and of severe illness and death in subjects aged >65 years in industrialized countries. For example, respiratory syncytial virus (RSV) infections resulted in the hospitalization of 3·4 million young children, with about 133 000 deaths in 2005 worldwide [2]. Influenza virus infected 90 million and caused about 70 000 deaths in the same age group in 2008 [3]. In the elderly population of the USA, about 100 000 deaths due to RSV and 36 000 deaths due to influenza occur each year, according to the World Health Organization (WHO). Viral infections of the respiratory tract not only pose a significant global health burden, but also a significant financial burden due to healthcare costs and workdays lost. The WHO has estimated that 23 million workdays are lost per annum in the USA due to the common cold.

Outbreaks of respiratory viral infections, such as those caused by influenza A [4] and RSV [5], have long been associated with the changing seasons, reaching a peak in the winter months and a nadir in the summer months. Various factors have been proposed to explain this pattern, including increased survival and transmission of the viruses at low temperature and humidity, cold dry air reducing mucus in the nasal passages, and the tendency for indoor congregation during winter months [4], although human gathering

* Author for correspondence: Dr K. J. Bryson, The Roslin Institute and the Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian EH25 9RG, UK (Email: karen.bryson@roslin.ed.ac.uk)
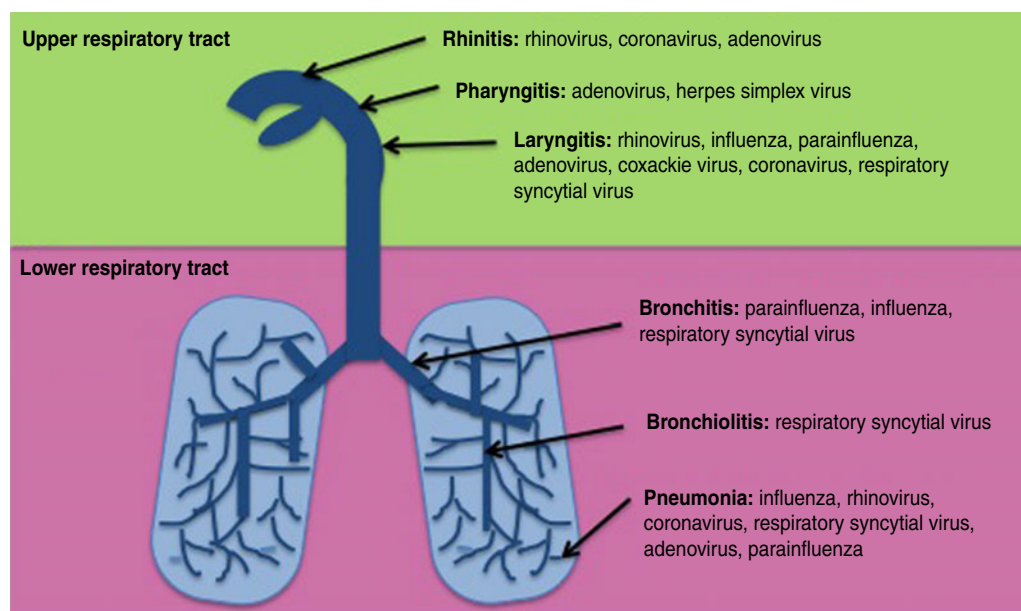
Fig. 1 [*colour online*]. Common viral infections of the respiratory tract.

occurs throughout the year, with public transport, schools and places of work likely to provide ample opportunity for viral transmission.

More than 30 years ago Hope-Simpson wrote:

latitude alone broadly determines the timing of epidemics [of respiratory infections] in the annual cycle, a relationship that suggests a rather direct effect of solar radiation acting positively or negatively upon the virus, the human host or their interaction [6].

Therefore solar ultraviolet (UV) radiation could influence the seasonality of respiratory infections at the majority of latitudes, providing most protection in the summer months when the UVB (280–315 nm) in sunlight is at a maximum. In the tropics where there is little fluctuation in solar UVB radiation, the seasonality of respiratory viral infections is blunted [4, 5], with influenza being more prevalent in the rainy season when sunlight is impaired by clouds and rain [5]. As vitamin D is produced following exposure of the skin to solar UVB, individuals living at mid to high latitudes frequently have lower vitamin D status in winter compared to summer, because of the lack of UVB radiation during the winter months. The seasonal fluctuation in vitamin D level thus has an inverse correlation with the seasonality of many respiratory viral infections. Solar flare activity has been noted as influencing respiratory disease rates, with a higher number of reported cases following solar flares [7]. Such flares increase atmospheric ozone, and hence reduce the UVB radiation reaching the earth's surface

and, in turn, the generation of vitamin D in human skin is lower. Therefore a protective role for vitamin D in determining susceptibility to respiratory viral infections has been proposed, providing an intriguing explanation for the seasonality of respiratory viral infections, as well as offering a potential prophylactic for reducing the global burden of these diseases.

In this review, the immune mechanisms by which vitamin D could influence the host response are outlined, and the evidence from observational studies and clinical trials discussed in relation to vitamin D and respiratory viral infections. The potential of vitamin D to improve the efficacy of vaccination against some respiratory viruses is also examined.

Vitamin D

The majority of vitamin D in most individuals is produced following exposure of the skin to the UVB component of sunlight (280–315 nm). The 7-dehydrocholesterol in the cutaneous keratinocyte membranes is converted in a series of steps to 25-hydroxyvitamin D_3 [25(OH)D_3, calcidiol] and then to the active form of vitamin D, 1,25-dihydroxyvitamin D_3 [1,25(OH)$_2$$D_3$, calcitriol] [8] (Fig. 2). The actions of 1,25(OH)$_2$$D_3$ are mediated through ligation with a nuclear vitamin D receptor (VDR), resulting in the regulation of the transcription of at least 1000 genes. 1,25(OH)$_2$$D_3$ also induces rapid membrane signalling through a specific membrane receptor, recently identified as a protein disulfide isomerase. The amount of circulating
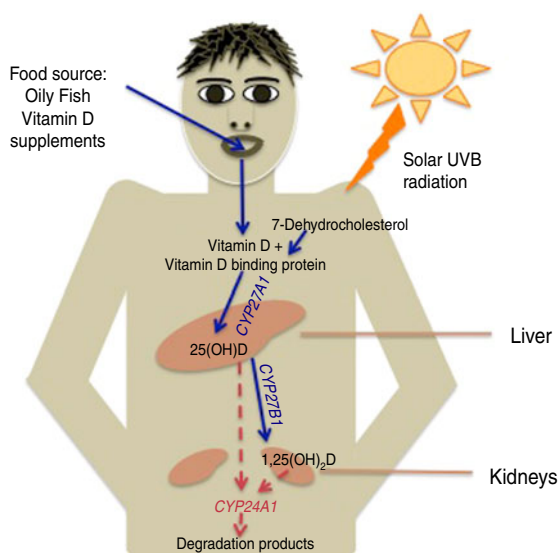
Fig. 2 [*colour online*]. Synthesis of 25-hydroxyvitamin D [25(OH)D] and 1,25-dihyroxyvitamin D [1,25(OH)$_2$D] following solar UVB radiation or ingestion of vitamin D.

1,25(OH)$_2$D$_3$ is tightly regulated by negative feedback control through induction by the hormone of 24-hydroxylase which catabolizes both 25(OH)D$_3$ and 1,25(OH)$_2$D$_3$ to various calcitronic acid products.

Dietary vitamin D, in the form of either D$_2$ or D$_3$, normally makes only a minor contribution to the total vitamin D requirement, and gets absorbed from the gastrointestinal tract into the circulation. Vitamin D is found in oily fish, egg yolk and irradiated food such as mushrooms, with the highest levels found in cod liver oil. A limited number of common staples are also fortified with vitamin D. Vitamin supplements in the form of D$_2$ or D$_3$, provide an alternative source. It has been reported that ingesting 10 000 IU vitamin D$_3$ is equivalent to whole-body UV radiation exposure, at a dose sufficient to cause just perceptible erythema. Such an estimate is unlikely to be accurate under natural outdoor conditions, and efforts have been made recently to construct models which provide a better understanding of how much sun exposure an individual requires under different climatic conditions to result in a particular vitamin D status [9, 10].

UVB makes up about 6% of ambient sunlight at most. The percentage depends on many external factors including latitude, season, time of day, altitude, ozone, cloud cover, air pollution and reflective surfaces. At latitudes above 45° (London is 51° N), there is essentially no solar UVB reaching the surface of the earth during the winter months, due to the decreased solar zenith angle. This is likely to result in a reduction in vitamin D status, which begins

to recover in the spring. For example, in the USA, the peak in vitamin D levels occurs in August and the trough in February, a lag of 8 weeks after the peak and trough, respectively, in ambient UVB [11]. Personal factors are also important in determining vitamin D production from solar UVB irradiation, such as skin type, age, amount of clothing worn and head cover, use of sunscreen, body mass index, serum cholesterol concentration, polymorphisms in the VDR and enzymes of the vitamin D metabolic pathway, and baseline 25(OH)D level. For example, to produce the same amount of pre-vitamin D$_3$, about a sixfold higher dose of UVB radiation is required for black skin compared to fair skin, due to absorption by melanin, and a twofold higher dose is required for an 80-year-old compared to a 20-year-old, due to a reduction in 7-dehydrocholesterol in older skin.

The vitamin D status of an individual is usually inferred by the concentration of 25(OH)D in the serum or plasma, but a number of caveats require consideration. In many studies only one sample per individual is assessed, frequently without taking into account the season, past history of sun exposure, place of residence and other environmental and personal factors. The 25(OH)D assay is known to lack accuracy, reproducibility and sensitivity [12], although this should improve with the recent implementation of a vitamin D standardization programme using liquid chromography-tandem mass spectroscopy (LC-MS/MS). There is also no consensus regarding what concentration of 25(OH)D constitutes 'satisfactory'. Although a level of at least 10 ng/ml 25(OH)D is thought necessary to promote bone mineralization and calcium homeostasis, this endpoint is not consistent between studies [13]. A range of 20 ng/ml (50 nmol/l) to 50 ng/ml may best provide the immunomodulatory effects of vitamin D [14], while concentrations >100 ng/ml may be harmful, although toxicity is rare below 200 ng/ml [15].

Immune responses to respiratory viral infection

Following entry into the respiratory tract and infection of predominantly epithelial cells, viruses trigger the innate immune responses including the inflammatory response. Neutrophils enter the lung parenchyma within hours of infection, followed by monocytes/macrophages, natural killer (NK) cells and then T cells within a few days of infection. The T cell infiltration peaks at 7 days post-infection, correlating with viral clearance from the lung. Neutralizing antibodies are also present around 7 days post-infection,

and are maintained in the host as a first line of defence against re-infection.

The innate immune cells elicit an early anti-viral response following recognition of the pathogen by pattern recognition receptors including Toll-like receptors (TLRs) and RIG-like receptors [16]. The engagement of these receptors induces signalling cascades that generate type I interferon (IFN) and pro-inflammatory cytokines. These molecules control the infection by mechanisms of viral cleavage and inhibition of viral fusion, replication and translation, by activating cytolytic cells and stimulating humoral factors including acute phase proteins, defensins, collectins and complement proteins. However, as these responses can lead to immunopathology in the lung [17] mediating the morbidity and mortality of respiratory viral infections, they require immune regulation.

The adaptive immune response is also responsible for the recovery from viral respiratory infection. Antigen-presenting cells, including macrophages and dendritic cells, process and present the viral antigens to CD4$^+$ and CD8$^+$ T cells in the draining lymph node. The T cells then migrate and accumulate in the infected tissue to mediate cytolytic and pro-inflammatory effects. CD4$^+$ T helper cells are central: they promote the B cell response including proliferation, differentiation to plasma cells, immunoglobulin class switching to produce IgG and IgA antibodies, affinity maturation and memory B cell induction, and they also promote cytolytic CD8$^+$ T cell responses [16]. Antibodies produced by B cells can prevent viral entry into cells and promote phagocytosis of the virus by innate immune cells. IgA is particularly important in blocking transmission of a virus between hosts.

The innate and adaptive immune responses thus have interdependent roles in protection against respiratory viral infections.

Modulation of the innate immune response to respiratory viral infections by vitamin D

The VDR is widely expressed on cells of the immune system and on epithelial cells [18], with a single nucleotide polymorphism in the VDR correlating with the risk of RSV infection [19]. 1,25(OH)$_2$D stimulates neutrophils, macrophages, and NK cells of the respiratory tract, as well as epithelial cells, to produce antimicrobial peptides (AMPs), including defensins and cathelicidins [20]. These AMPs have anti-viral activity with hCAP18/LL-37 in particular having anti-influenza effects [21].

1,25(OH)$_2$D also influences the innate immune response by increasing TLR and CD14 expression [20], macrophage maturation and oxidative burst capacity [22]. It therefore enhances the antimicrobial activity of macrophages. The importance of macrophages and neutrophils during the initial interaction of the virus with the host has been demonstrated in mice by increased disease severity following depletion of these cells prior to influenza infection [23]. However, despite the enhanced activity of macrophages, autophagy is reduced during the infection [24]. Autophagy, an important process in cellular homeostasis, has a role in cytokine induction. In influenza A virus infection, autophagy is associated with the production of IFN-α and CXCL10, and with increased viral replication [25]. Thus inhibition of autophagy by vitamin D may be a further mechanism for the control of respiratory viral infections, in addition to controlling pathology in the lung. The effects of vitamin D on the innate immune response also extend to promoting the migration of myeloid dendritic cells to lymphoid organs distant from draining lymph nodes where they activate antigen-specific CD4$^+$ T and B cells [26, 27].

Vitamin D inhibits the production of pro-inflammatory cytokines. During influenza A infection, IFN-β, tumour necrosis factor (TNF)-α, interleukin (IL)-8, IL-6 and RANTES are all reduced in lung epithelial cells in response to treatment with 1,25 (OH)$_2$D [24]. During RSV infection of airway epithelial cells, vitamin D induces IκBα, thus inhibiting NFκB responses [28]. Although such effects may appear counter-productive in the induction of effective host anti-respiratory viral responses, it is recognized that the pathogenicity of respiratory viruses is associated with hypercytokinaemia [29], also referred to as cytokine storm, which is a potentially fatal self-perpetuating cycle of inflammatory responses. In particular, infection with the more pathogenic strains of influenza results in increased viral replication, neutrophil infiltration and cytokine and chemokine levels, relative to the less pathogenic strains [23]. Thus controlling pro-inflammatory responses is beneficial to the host exposed to respiratory viruses. In support, it has been shown that the levels of IL-1β, IL-6, TNF-α, IFN-γ and IL-10 are suppressed on TLR stimulation of human peripheral blood mononuclear cells in the summer months, when cases of respiratory viral infection are at their lowest, compared to the responses in winter [26].

Modulation of the adaptive immune response to respiratory viral infections by vitamin D

The intriguing immunomodulating effects of vitamin D extend to the adaptive immune response, especially to T cells. $1,25(OH)_2D$ downregulates the induction of both T helper (Th)1- and Th2-associated cytokines when applied during T helper cell activation; only the Th1-associated cytokines are downregulated if $1,25(OH)_2D$ is applied after the establishment of the activated T helper cell subsets [30]. This inhibition is at the level of cytokine transcription rather than any effect on cell cycle or Th1-/Th2-associated transcription factors. T cells in VDR knockout mice develop normally but over-produce the pro-inflammatory Th1- [31] and Th17-associated cytokines [32], thus substantiating the role of vitamin D in regulating inflammatory cytokines.

Vitamin D can also activate T regulatory (Treg) cell subsets. The VDR binds to a conserved non-coding region of the Treg cell-associated gene *FoxP3*, and promotes the expression of this gene in T helper cells [33]. This increases the number of Treg cells and their suppressor activity. Furthermore, increased production of the anti-inflammatory cytokine IL-10 by $CD4^+$ T cells has been noted in response to moderate levels of $1,25(OH)_2D$ [34]. Even topical administration of $1,25(OH)_2D$ confers an increase in the suppressive activity of $CD4^+CD25^+$ T cells, as reported in an asthma model [35]. Adoptive transfer of Treg cells to lymphocyte-deficient mice, which cannot generate an adaptive immune response, resulted in suppressed innate immune-mediated pathology during influenza infection [36]. The recipient mice had delayed weight loss and prolonged survival following infection. Therefore vitamin D has the potential to confer protective responses during respiratory viral infection by its effects on Treg cells.

Vitamin D can modulate both innate and adaptive immune responses by the mechanisms outlined above and VDR polymorphism has been correlated with susceptibility to respiratory viral infections, thus suggesting that vitamin D could provide a therapeutic target for such diseases. The role of vitamin D has been investigated in clinical studies and the results to date are now described.

Observational evidence associating vitamin D status with protection against respiratory viral infections

A number of studies published in the past 10 years have investigated the correlation between vitamin D status, as assessed by 25(OH)D concentration in the serum, and the occurrence of respiratory viral infections. A selection of these publications is outlined in Table 1. The studies were carried out in various countries and settings, with numbers of subjects ranging from fewer than 50 to many thousands. In most instances, the lower the concentrations of 25(OH)D, the higher the risk of respiratory viral infections. However, in the majority of cases, the population examined already had symptoms of the infection at the time of the 25(OH)D measurement. Thus whether low vitamin D status is a contributing factor or a consequence of the infection cannot be distinguished. Indeed in one study involving large numbers of subjects, it was found that a reduction in the risk of infection preceded an increase in 25(OH)D levels [45]. In addition, the infections were rarely diagnosed by laboratory tests but subjectively by self- or parental reporting or by clinical symptoms, making it impossible to attribute any effect of vitamin D status specifically to a viral pathogen. Finally, no studies have examined the possibility that the infections themselves could have an effect on catabolism of 25(OH)D and $1,25(OH)_2D$, resulting in an apparent vitamin D deficiency.

Whether the risk of respiratory viral infection in a child could be affected by the vitamin D status of the mother has also been investigated. Karatekin *et al.* [48] found that serum 25(OH)D levels were lower in newborns hospitalized with acute LRT infections and their mothers, than in healthy newborns and their mothers. Both Camargo *et al.* [49] in New Zealand and Belderbos *et al.* [50] in The Netherlands have monitored 25(OH)D in cord blood and related this prospectively to the occurrence of respiratory infections in the child. In the former study, 882 newborns with <10 ng/ml 25(OH)D in cord blood were twice as likely to develop a respiratory infection (as reported by the mothers) at 3 months compared to those newborns with ⩾30 ng/ml. In the latter study, 156 newborns with <20 ng/ml 25(OH)D in cord blood were six times more likely to develop a LRT infection (as reported by the mother and by the occurrence of RSV-RNA in nose-throat swabs) in the first year of life compared to those with >30 ng/ml 25(OH)D. In addition a multicentre study based in Spain showed that the offspring of mothers, whose 25(OH)D levels were in the highest quartile, had a 23% reduction in their risk of LRT infection during their first year compared to the offspring of mothers in the lowest quartile [51].

Table 1. *Observational studies associating vitamin D status with risk of respiratory viral infections*

Study population and place	Investigation	Result	Ref.
443 infants, hospitalized for acute illnesses (Amman, Jordan)	Cause of admission, clinical signs of rickets	LRT diseases in 85% children with rickets, in 10% children without rickets	[37]
80 infants hospitalized with acute LRT infections, 70 healthy controls (Indapur, India)	Serum 25(OH)D levels	25(OH)D levels >9 ng/ml associated with lower risk of acute LRT infections	[38]
64 infants hospitalized with acute LRT infections, 65 healthy controls (Edmonton, Canada)	Serum 25(OH)D levels	No association between 25(OH)D levels and acute LRT risk (mean 30·8 ng/ml in cases, 30·9 ng/ml in controls)	[39]
18 883 subjects aged ⩾12 years (Third National Health and Nutrition Examination Study, USA)	Self-reported recent URT infections, serum 25(OH)D levels	25(OH)D levels inversely associated with recent URT infections. Stronger association in those with respiratory tract diseases	[40]
25 young children hospitalized with acute LRT infections, 25 matched controls (rural Bangladesh)	Serum 25(OH)D levels	Mean 25(OH)D lower in acute LRT infection cases than in controls (mean 11·6 ng/ml cases, 15·6 ng/ml controls)	[41]
195 healthy adults (Connecticut, USA)	Serum 25(OH)D levels monthly over autumn and winter, acute viral respiratory tract infections monitored over same period (H1N1 influenza, rhinovirus, RSV, parainfluenza)	25(OH)D levels of ⩾38 ng/ml associated with twofold reduction in risk of acute respiratory tract infections, and reduction in days ill	[42]
282 infants hospitalized with wheeze, median age 1·6 years (Turku, Finland)	Serum 25(OH)D levels, nasopharyngeal aspirates tested for 18 different viruses	Serum 25(OH)D levels inversely associated with occurrence of RSV and rhinoviruses, but not with other viruses	[43]
32 institutionalized adults aged >65 years (Sendai, Japan)	Serum 25(OH)D level at start, incidence of febrile respiratory infections and pneumonia followed for next 2 years	Those with lower 25(OH)D (34·4 vs. 66·4 ng/ml) at significantly higher risk of febrile respiratory infection but not of pneumonia	[44]
6789 adults aged 45–47 years (UK)	Serum 25(OH)D levels adjusted for season, self-reported respiratory infections in preceding 3 weeks	Each 4 ng/ml increase in 25(OH)D associated with 7% lower risk of respiratory infection. Seasonal rise in 25(OH)D levels with concurrent seasonal decrease in prevalence of respiratory infections	[45]
105 healthy hospital employees (mean age 35 years) (Bucharest, Romania)	25(OH)D levels in winter, occurrence of URT infections at same time (some self-reported)	No correlation between 25(OH)D level and risk of URT infection	[46]
2070 adults, aged ⩾65 years (England)	25(OH)D level at time of personal interview; self-reported respiratory disease in the past	Increased risk of respiratory disease (including influenza and pneumonia) if 25(OH)D <20 nmol/ml	[47]

LRT, Lower respiratory tract; RSV, respiratory syncytial virus; URT, upper respiratory tract; 25(OH)D, 25-hydroxyvitamin D.

These findings demonstrate that a low maternal vitamin D status could adversely affect the development of the fetal immune system, possibly leading to a less effective antiviral response after birth. In addition if the vitamin D content of the mother's breast milk is low, this will result in an insufficient vitamin D status in the child during breastfeeding. Alternatively a higher vitamin D status in the mother may represent a marker for more sun exposure that may have positive effects independent of vitamin D production. It is clearly important to monitor the 25(OH)D levels of children in early life to clarify these possibilities and to make informed recommendations regarding vitamin D supplements during pregnancy and in the first months after birth.

Clinical trials of dietary vitamin D supplements and protection against respiratory viral infections

While studies associating 25(OH)D levels with the risk of respiratory viral infections are described above, this section examines clinical trials of vitamin D_3 supplementation in the prevention of respiratory infections. Eight such investigations are summarized in Table 2. In common with the observational studies (Table 1), these reports include a wide range of subjects, in some cases with underlying health problems, living in various parts of the world, who were given vitamin D_3 supplements at different doses and frequencies over varying time periods. There was also considerable variation in the measured endpoints with self-reported symptoms being frequent and laboratory identification of specific infectious agents uncommon. In brief, no consistent difference in the risk of respiratory viral infection between those given the supplement and those given the placebo was found. However, there was some evidence, such as reported by Camargo et al. [58], that the supplement could have a protective effect in some individuals with a low baseline vitamin D status. This could indicate that boosting the 25(OH)D level from deficiency activates various innate and adaptive immune responses that are critical in the control of some respiratory viral infections, while, in contrast, boosting from a higher starting level of 25(OH)D provides no additional benefit.

A novel approach involved subjecting a group of young adults to suberythemal sunbed exposure three times weekly during the winter, while another group ingested vitamin D supplements (1000 IU) daily, and a control group had no intervention [60].

The serum 25(OH)D level dropped in the control group, but rose in the other two groups. However, the percentage of subjects in each group who developed a self-reported upper respiratory tract infection did not differ. Thus it was concluded that neither the UVB exposures nor vitamin D supplements lowered the risk of such infection.

Vitamin D and the efficacy of vaccination

As discussed above, $1,25(OH)_2D_3$ has the capacity to induce some anti-viral immune responses and therefore may have the potential to improve the efficacy of vaccination, such as by macrophage activation, monocyte chemotaxis, and migration of myeloid dendritic cells from the site of cutaneous vaccination to multiple lymphoid organs where they stimulate antigen-specific T and B cell responses [27]. Experiments in adult mice showed that co-administration of $1,25(OH)_2D_3$ with inactivated influenza virus enhanced both the antibody response against the viral haemagglutinin and mucosal immunity [61]. This approach has been tested in a limited number of human studies thus far, details of which are shown in Table 3. In some cases, the 25(OH)D level in the subjects was assessed, and in other cases vitamin D supplements were administered in controlled trials. In general, the outcomes indicated that neither the induction of seropositivity, nor its degree, were determined by vitamin D status or by ingesting vitamin D supplements, although it should be noted that in all cases the number of subjects was small and frequently they were not healthy. Also serum rather than mucosal immunity was assessed, the latter being of greater importance in the initial interaction between a respiratory virus and the host.

Conclusions

It is clear that $1,25(OH)_2D$ has many immunomodulating properties which could potentially lessen the risk of a symptomatic respiratory viral infection. The majority of the observational studies indicate that low levels of 25(OH)D increased the risk of respiratory viral infections but, in most instances, it was not possible to determine the timing of the infection with respect to the vitamin D status of the individual. In the prospective clinical trials to date, almost all have not demonstrated conclusively that vitamin D supplements influence the risk of respiratory viral infections. However, where the starting

Table 2. *Prospective randomized double-blind trials of vitamin D supplementation and protection against respiratory viral infections*

Study population and place	Investigation	Result	Comments	Ref.
162 adults, mean age about 58 years (Long Island, USA)	2000 IU vitamin D_3 daily or placebo during 12 weeks of winter; incidence and severity of URT infections by questionnaire bi-weekly	25(OH)D level increased in vitamin group from 25·7 to 35·4 ng/ml, unchanged in placebo group; no difference in incidence, duration or severity of URT infections between the two groups	A higher dose of vitamin D supplement could have been used or started earlier in the year; most subjects have sufficient vitamin D levels at start; short duration of study	[52]
164 male conscripts aged 16–25 years (South-west Finland)	400 IU vitamin D_3 daily or placebo during winter months; number of days absent due to acute RT infections plus self-reported symptoms of acute RT infections	No increase in 25(OH)D levels in supplemented group, decrease in placebo group from 29·8 to 20·5 ng/ml; days absent slightly lower and proportion of subjects without any days absent higher in vitamin D group	High dropout rate; supplement not sufficient to raise 25(OH)D level	[53]
167 children, aged 6–15 years (Jikei, Japan)	1200 IU vitamin D_3 daily or placebo during winter months; incidence of influenza A, B and flu-like illness as determined by antigen detection in nasopharyngeal swabs	Reduction in incidence of influenza A in vitamin D group but no difference between groups for influenza B and flu-like illnesses	Small sample size; 25(OH)D not measured; no reduction in incidence of influenza A in children with asthma in vitamin D group; antibody levels to influenza viruses not assessed	[54]
453 children in hospital with pneumonia, aged 1–36 months (Kabul, Afghanistan)	100 000 IU vitamin D_3 orally or placebo plus antibiotics; time to recovery, and repeat episodes of pneumonia in following 3 months	No difference between groups in mean days to recovery; risk of repeat episode of pneumonia within 90 days lower in vitamin D group	Cause of pneumonia not determined; no measurement of 25(OH)D status; children likely to be severely vitamin D deficient	[55]
322 healthy adults (Christchurch, New Zealand)	200 000 IU vitamin D_3 orally, then 200 000 IU 1 month later, then 100 000 IU monthly or placebo at same times, over 18 months. Number, duration and severity of URT infections as assessed by questionnaire and PCR tests of nasopharyngeal swabs for range of respiratory viruses	Increase in 25(OH)D from 29 to about 50 ng/ml in vitamin D group while placebo group remained at about 28 ng/ml; no difference between groups in number of URT infections per subject, number of missed days at work, duration or severity of symptoms	Large sample size; long duration of study; high dose of vitamin D supplement; laboratory-confirmed diagnosis of viral infection; most subjects had sufficient 25(OH)D levels throughout	[56]
3046 children, aged 1–11 months (Kabul, Afghanistan)	100 000 IU vitamin D_3 orally or placebo every 3 months for 18 months; first episode of pneumonia, defined by clinical symptoms and by chest radiography in some cases	Low baseline 25(OH)D in both groups, then 25(OH)D level in vitamin D group consistently higher (about 32 ng/ml) than in placebo group (about 10 ng/ml); no difference in incidence of first or only pneumonia between vitamin D and placebo groups	Most children were malnourished; vitamin D supplied as high bolus at 3-monthly intervals; large sample size; low loss to follow-up	[57]

Table 2 (*cont.*)

Study population and place	Investigation	Result	Comments	Ref.
247 children, aged 8–11 years (Ulaanbaatar, Mongolia)	300 IU vitamin D_3 daily in milk or unfortified milk during 7 weeks in winter; parent-reported acute respiratory infections over 3 months	Very low baseline 25(OH)D levels (median 7 ng/ml); after 7 weeks, median 25(OH)D 18·9 ng/ml in vitamin D group and 7·2 ng/ml in placebo group; fewer (about half) acute respiratory infections in vitamin D group compared to placebo group	Low vitamin D status even in vitamin D group after 7 weeks; short time period of study; no clinical or laboratory diagnosis of infection	[58]
140 patients, aged 18–75 years, with antibody deficiency or increased susceptibility to respiratory tract infections (Huddubge, Sweden)	4000 IU vitamin D_3 orally (as oil drops) or placebo for 1 year; infectious score based on symptoms, malaise and antibiotic usage, reported daily by each patient	Mean 25(OH)D at start about 20 ng/ml, rising to about 52 ng/ml in vitamin D group and remaining relatively unchanged in placebo group; infectious score reduced by 23% in vitamin D group compared to placebo group; no difference in levels of antimicrobial peptides between groups in nasal fluid	Small sample size; selected patient group; long time period of study; high daily dose of vitamin D; laboratory identification of bacteria and fungi but not of viruses; self-reported symptoms	[59]

RT, Respiratory tract; URT, upper respiratory tract; 25(OH)D, 25-hydroxyvitamin D.

25(OH)D levels were very low, there was some indication of a protective effect from the supplements. The limited studies carried out thus far do not show, in general, that vitamin D status affects the immune response to vaccination.

While many of the studies reviewed here do not demonstrate a clear unambiguous role for vitamin D in protection against respiratory viral infections, the approaches taken provide pointers for the design of future trials. Thus consideration needs to be given to the health, vitamin D status and sample size of the subject group. For example, if the vitamin D status of the group is already sufficient, then it is unlikely that dietary supplements will have any effect. It is also possible that polymorphisms in the VDR, vitamin D binding protein or hydroxylase enzymes could affect the ability of an individual to respond to the vitamin D supplement, thus genotyping may be required to distinguish subgroup effects. In addition the administration and quantity of the dietary supplement is of importance – whether it should be given intermittently at high dose, on a monthly basis, or more frequently at lower dose, on a weekly or daily basis [68]. The length of the study period needs to be assessed, taking into account the seasonal variation in 25(OH)D levels at many latitudes, and that there may be a low frequency of respiratory viral infections in the study population. In large-scale prospective trials not due to be completed for at least 4 years, doses range from 2000 IU daily to 100 000 (2·5 mg) IU vitamin D_3 each month. Laboratory-based methods to identify the microorganisms causing the clinical symptoms are required and to explore the possibility that vitamin D might be protective against one type of virus but not another. Finally it is possible, as has been suggested in several recent reports that solar UV radiation may have beneficial effects on health other than through the production of vitamin D [69, 70]

It remains a tantalizing, but so far unfulfilled, hope that increasing the vitamin D status of a population could be an effective and inexpensive prophylactic against a range of respiratory viral infections, particularly in those subjects most at risk of severe symptoms, such as young children and the elderly.

Table 3. *Summary of studies relating vitamin D status to the efficacy of vaccination*

Study population	Intervention	Result	Limitations	Ref.
175 healthy subjects (Utah, USA)	Co-administration calcitriol or placebo with trivalent influenza vaccine	No difference in influenza antibody titre between groups	No assessment of vitamin D status throughout; calcitriol and vaccine given at same time; pre-existing influenza antibodies in almost all subjects	[62]
26 haemodialysis patients (Indiana, USA)	Paricalcitriol or placebo for 12 weeks, hepatitis B vaccine booster at 8 weeks	No difference in antibody response between groups	No assessment of vitamin D status throughout; small sample size	[63]
35 prostate cancer patients (New York, USA)	Trivalent influenza vaccination	High 25(OH)D level associated with more frequent serological response to the vaccine	Cancer patients; high mean baseline 25(OH)D level (45 ng/ml); no association between 25(OH)D level and serological response to the three individual influenza strains	[64]
298 HIV-seropositive subjects (aged 8–60 years), 89% on highly active anti-retroviral therapy (Vancouver, Canada)	About 30% taking vitamin D supplements. Trivalent influenza vaccination	No difference in seroconversion rate or seroprotection rate with use of vitamin D supplements	Patients with HIV and treated with anti-retroviral therapy; no assessment of 25(OH)D levels throughout	[65]
90 adults with controlled HIV disease, previously unexposed to H1N1 (Philadelphia, USA)	Influenza H1N1 vaccination	25(OH)D level did not affect whether a subject responded to the vaccine, or the degree of seropositivity induced	Small sample size; patients with HIV and treatment with anti-retroviral therapy	[66]
116 children with history of recurrent otitis media, previously unexposed to influenza vaccine (Milan, Italy)	1000 IU vitamin D orally each day or placebo for 4 months, trivalent influenza vaccine at start and 1 month later	No difference in seroconversion or seroprotective rates or antibody titre to any influenza vaccine antigens between groups	Small sample size	[67]

Declaration of Interest

None.

REFERENCES

1. **Reed G, et al.** Epidemiology and clinical impact of parainfluenza virus infections in otherwise healthy infants and young children <5 years old. *Journal of Infectious Diseases* 1997; **175**: 807–813.
2. **Nair H, et al.** Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. *Lancet* 2010; **375**: 1545–1555.
3. **Nair H, et al.** Global burden of respiratory infections due to seasonal influenza in young children: a systematic review and meta-analysis. *Lancet* 2011; **378**: 1917–1930.
4. **Juzeniene A, et al.** The seasonality of pandemic and non-pandemic influenzas: the roles of solar radiation and vitamin D. *International Journal of Infectious Diseases* 2010; **14**: e1099–1105.
5. **Shek LP, Lee BW.** Epidemiology and seasonality of respiratory tract virus infections in the tropics. *Paediatric Respiratory Reviews* 2003; **4**: 105–111.
6. **Hope-Simpson RE.** The role of season in the epidemiology of influenza. *Journal of Hygiene* 1981; **86**: 35–47.
7. **Hayes DP.** Influenza pandemics, solar activity cycles, and vitamin D. Impact of vitamin D administration on immunogenicity of trivalent inactivated influenza vaccine in previously unvaccinated children. *Medical Hypotheses* 2010; **74**: 831–834.
8. **Holick MF.** Vitamin D: a d-lightful solution for health. *Journal of Investigative Medicine* 2011; **59**: 872–880.
9. **Kazantzidis A, et al.** Calculations of the human vitamin D exposure from UV spectral measurements at three European stations. *Photochemical and Photobiological Sciences* 2009; **8**: 45–51.
10. **Diffey BL.** Modelling vitamin D status due to oral intake and sun exposure in an adult British population. *British Journal of Nutrition* 2013; **110**: 569–577.
11. **Kasahara AK, Singh RJ, Noymer A.** Vitamin D (25OHD) Serum Seasonality in the United States. *PLoS One* 2013; **8**: e65785.
12. **Fraser WD, Milan AM.** Vitamin D assays: past and present debates, difficulties, and developments. *Calcified Tissue International* 2013; **92**: 118–127.
13. **Cranney A, et al.** Effectiveness and safety of vitamin D in relation to bone health. *Evidence Report/Technology Assessment (Full Report)* 2007; **158**: 1–235.
14. **Ross AC, et al.** (eds). *Dietary Reference Intakes for Calcium and Vitamin D.* Washington, DC: The National Academies Press, 2011.
15. **Heaney RP.** Assessing vitamin D status. *Current Opinion in Clinical Nutrition & Metabolic Care* 2011; **14**: 440–444.
16. **Kreijtz JH, Fouchier RA, Rimmelzwaan GF.** Immune responses to influenza virus infection. *Virus Research* 2011; **162**: 19–30.
17. **Nakano M, et al.** Type I interferon induces CX3CL1 (fractalkine) and CCL5 (RANTES) production in human pulmonary vascular endothelial cells. *Clinical and Experimental Immunology* 2012; **170**: 94–100.
18. **Milde P, et al.** Expression of 1,25-dihydroxyvitamin D3 receptors in normal and psoriatic skin. *Journal of Investigative Dermatology* 1991; **97**: 230–239.
19. **Kresfelder TL, et al.** Confirmation of an association between single nucleotide polymorphisms in the VDR gene with respiratory syncytial virus related disease in South African children. *Journal of Medical Virology* 2011; **83**: 1834–1840.
20. **Beard JA, Bearden A, Striker R.** Vitamin D and the anti-viral state. *Journal of Clinical Virology* 2011; **50**: 194–200.
21. **Barlow PG, et al.** Antiviral activity and increased host defense against influenza infection elicited by the human cathelicidin LL-37. *PLoS One* 2011; **6**: e25333.
22. **Cannell JJ, et al.** Epidemic influenza and vitamin D. *Epidemiology and Infection* 2006; **134**: 1129–1140.
23. **Tumpey TM, et al.** Pathogenicity of influenza viruses with genes from the 1918 pandemic virus: functional roles of alveolar macrophages and neutrophils in limiting virus replication and mortality in mice. *Journal of Virology* 2005; **79**: 14933–14944.
24. **Khare D, et al.** Calcitriol [1,25[OH]$_2$D$_3$] pre- and post-treatment suppresses inflammatory response to influenza A (H1N1) infection in human lung A549 epithelial cells. *European Journal of Nutrition* 2013; **52**: 1405–1415.
25. **Zhou Z, et al.** Autophagy is involved in influenza A virus replication. *Autophagy* 2009; **5**: 321–328.
26. **Khoo AL, et al.** Regulation of cytokine responses by seasonality of vitamin D status in healthy individuals. *Clinical and Experimental Immunology* 2011; **164**: 72–79.
27. **Enioutina EY, Bareyan D, Daynes RA.** TLR ligands that stimulate the metabolism of vitamin D3 in activated murine dendritic cells can function as effective mucosal adjuvants to subcutaneously administered vaccines. *Vaccine* 2008; **26**: 601–613.
28. **Hansdottir S, et al.** Vitamin D decreases respiratory syncytial virus induction of NF-kappaB-linked chemokines and cytokines in airway epithelium while maintaining the antiviral state. *Journal of Immunology* 2010; **184**: 965–974.
29. **de Jong MD, et al.** Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. *Nature Medicine* 2006; **12**: 1203–1207.
30. **Staeva-Vieira TP, Freedman LP.** 1,25-dihydroxyvitamin D3 inhibits IFN-gamma and IL-4 levels during *in vitro* polarization of primary murine CD4$^+$ T cells. *Journal of Immunology* 2002; **168**: 1181–1189.
31. **Froicu M, et al.** A crucial role for the vitamin D receptor in experimental inflammatory bowel diseases. *Molecular Endocrinology* 2003; **17**: 2386–2392.
32. **Bruce D, et al.** Converging pathways lead to overproduction of IL-17 in the absence of vitamin D signaling. *International Immunology* 2011; **23**: 519–528.
33. **Kang SW, et al.** 1,25-Dihyroxyvitamin D3 promotes FOXP3 expression via binding to vitamin D response elements in its conserved noncoding sequence region. *Journal of Immunology* 2012; **188**: 5276–5282.

34. **Urry Z, et al.** The role of 1alpha,25-dihydroxyvitamin D3 and cytokines in the promotion of distinct Foxp3+ and IL-10+ CD4+ T cells. *European Journal of Immunology* 2012; **42**: 2697–2708.

35. **Gorman S, Judge MA, Hart PH.** Topical 1,25-dihydroxyvitamin D3 subverts the priming ability of draining lymph node dendritic cells. *Immunology* 2010; **131**: 415–425.

36. **Antunes I, Kassiotis G.** Suppression of innate immune pathology by regulatory T cells during influenza A virus infection of immunodeficient mice. *Journal of Virology* 2010; **84**: 12564–12575.

37. **Najada AS, Habashneh MS, Khader M.** The frequency of nutritional rickets among hospitalized infants and its relation to respiratory diseases. *Journal of Tropical Pediatrics* 2004; **50**: 364–368.

38. **Wayse V, et al.** Association of subclinical vitamin D deficiency with severe acute lower respiratory infection in Indian children under 5 y. *European Journal of Clinical Nutrition* 2004; **58**: 563–567.

39. **Roth DE, et al.** Vitamin D status is not associated with the risk of hospitalization for acute bronchiolitis in early childhood. *European Journal of Clinical Nutrition* 2009; **63**: 297–299.

40. **Ginde AA, Mansbach JM, Camargo CA.** Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. *Archives of Internal Medicine* 2009; **169**: 384–390.

41. **Roth DE, et al.** Vitamin D status and acute lower respiratory infection in early childhood in Sylhet, Bangladesh. *Acta Paediatrica* 2010; **99**: 389–393.

42. **Sabetta JR, et al.** Serum 25-hydroxyvitamin D and the incidence of acute viral respiratory tract infections in healthy adults. *PLoS One* 2010; **5**: e11088.

43. **Jartti T, et al.** Low serum 25-hydroxyvitamin D levels are associated with increased risk of viral coinfections in wheezing children. *Journal of Allergy and Clinical Immunology* 2010; **126**: 1074–1076.

44. **Asamura T, et al.** Low serum 1,25-dihydroxyvitamin D level and risk of respiratory infections in institutionalized older people. *Gerontology* 2010. **56**: 542–543.

45. **Berry DJ, et al.** Vitamin D status has a linear association with seasonal infections and lung function in British adults. *British Journal of Nutrition* 2011; **106**: 1433–1440.

46. **Porojnicu AC, et al.** Vitamin D status in healthy Romanian caregivers and risk of respiratory infections. *Public Health Nutrition* 2012; **15**: 2157–2162.

47. **Hirani V.** Associations between vitamin D and self-reported respiratory disease in older people from a nationally representative population survey. *Journal of the American Geriatric Society* 2013; **61**: 969–973.

48. **Karatekin G, et al.** Association of subclinical vitamin D deficiency in newborns with acute lower respiratory infection and their mothers. *European Journal of Clinical Nutrition* 2009; **63**: 473–477.

49. **Camargo CA, et al.** Cord-blood 25-hydroxyvitamin D levels and risk of respiratory infection, wheezing, and asthma. *Pediatrics* 2011; **127**: e180–187.

50. **Belderbos ME, et al.** Cord blood vitamin D deficiency is associated with respiratory syncytial virus bronchiolitis. *Pediatrics* 2011; **127**: e1513–1520.

51. **Morales E, et al.** Maternal vitamin D status in pregnancy and risk of lower respiratory tract infections, wheezing, and asthma in offspring. *Epidemiology* 2012; **23**: 64–71.

52. **Li-Ng M, et al.** A randomized controlled trial of vitamin D3 supplementation for the prevention of symptomatic upper respiratory tract infections. *Epidemiology and Infection* 2009; **137**: 1396–1404.

53. **Laaksi I, et al.** Vitamin D supplementation for the prevention of acute respiratory tract infection: a randomized, double-blinded trial among young Finnish men. *Journal of Infectious Diseases* 2010; **202**: 809–814.

54. **Urashima M, et al.** Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. *American Journal of Clinical Nutrition* 2010; **91**: 1255–1260.

55. **Manaseki-Holland S, et al.** Effects of vitamin D supplementation to children diagnosed with pneumonia in Kabul: a randomised controlled trial. *Tropical Medicine and International Health* 2010; **15**: 1148–1155.

56. **Murdoch DR, et al.** Effect of vitamin D3 supplementation on upper respiratory tract infections in healthy adults: the VIDARIS randomized controlled trial. *Journal of the American Medical Association* 2012; **308**: 1333–1339.

57. **Manaseki-Holland S, et al.** Effect on the incidence of pneumonia of vitamin D supplementation by quarterly bolus dose to infants in Kabul: a randomised controlled superiority trial. *Lancet* 2012; **379**: 1419–1427.

58. **Camargo CA, et al.** Randomized trial of vitamin D supplementation and risk of acute respiratory infection in Mongolia. *Pediatrics* 2012; **130**: e561–567.

59. **Bjorkhem-Bergman L, et al.** Serum levels of 25-hydroxyvitamin D and the CYP3A biomarker 4beta-hydroxycholesterol in a high-dose vitamin D supplementation study. *Drug Metabolism and Disposition* 2013; **41**: 704–708.

60. **de Gruijl FR, Pavel S.** The effects of a mid-winter 8-week course of sub-sunburn sunbed exposures on tanning, vitamin D status and colds. *Photochemical and Photobiological Sciences* 2012; **11**: 1848–1854.

61. **Daynes RA., et al.** Steroids as regulators of the mammalian immune response. *Journal of Investigative Dermatology* 1995; **105**(1 Suppl): 14S–19S.

62. **Kriesel JD, Spruance J.** Calcitriol (1,25-dihydroxyvitamin D3) coadministered with influenza vaccine does not enhance humoral immunity in human volunteers. *Vaccine* 1999; **17**: 1883–1888.

63. **Moe SM, et al.** A placebo-controlled trial to evaluate immunomodulatory effects of paricalcitol. *American Journal of Kidney Diseases* 2001; **38**: 792–802.

64. **Chadha MK, et al.** Effect of 25-hydroxyvitamin D status on serological response to influenza vaccine in prostate cancer patients. *Prostate* 2011; **71**: 368–372.

65. **Cooper C, Thorne A.** Vitamin D supplementation does not increase immunogenicity of seasonal influenza vaccine in HIV-infected adults. *HIV Clinical Trials* 2011; **12**: 275–276.

66. **Momplaisir F, et al.** Vitamin D levels, natural H1N1 infection and response to H1N1 vaccine among HIV-infected individuals. *Journal of AIDS and Clinical Research* 2012; **3**: 152–159.

67. **Principi N, et al.** Impact of vitamin D administration on immunogenicity of trivalent inactivated influenza vaccine in previously unvaccinated children. *Human Vaccines and Immunotherapeutics* 2013; **9**: 969–974.

68. **Hollis BW, Wagner CL.** The role of parent compound vitamin D with respect to metabolism and function: why clinical dose intervals can affect clinical outcomes. *Journal of Clinical Endocrinology & Metabolism* 2013; **98**: 4619–4628.

69. **Hart PH, Gorman S, Finlay-Jones JJ.** Modulation of the immune system by UV radiation: more than just the effects of vitamin D? *Nature Reviews Immunology* 2011; **11**: 584–596.

70 **Milliken SV, et al.** Effects of ultraviolet light on human serum 25-hydroxyvitamin D and systemic immune function. *Journal of Allergy and Clinical Immunology* 2012; **129**: 1554–1561.