Vitamin D and solar ultraviolet radiation in the risk and treatment of tuberculosis

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Improved understanding of the association between tuberculosis and vitamin D is needed to inform clinical practice. Vitamin D has both immunostimulatory and immunosuppressive effects relevant to human antmycobacterial responses. Ultraviolet radiation, the main source of vitamin D, also induces immunomodulation and could affect the relation between vitamin D and tuberculosis. Clinical trials of vitamin D supplementation in patients with tuberculosis have produced largely negative results, prompting the review of dosing regimens—an explanation for low 25-hydroxyvitamin D status in patients with active tuberculosis is also needed. The reporting of vitamin D deficiency needs to address assay inaccuracies, rising thresholds to define sufficiency, and scarce knowledge of the concentrations needed for optimum immune responses. Future research to measure the effect of the inflammatory setting on serum concentrations of 25-hydroxyvitamin D, at tuberculosis diagnosis and during recovery, could help to account for 25-hydroxyvitamin D changes in these concentrations in patients with tuberculosis. Studies into the role of vitamin D supplementation in latent tuberculosis justify clinical trials in this population, but pose methodological challenges. Vitamin D trials in patients with active tuberculosis should be done in well selected populations using adequate vitamin D doses, although such doses remain undefined.

Introduction

Tuberculosis is the second most common cause of death from infection worldwide. Vitamin D deficiency is prevalent across broad geographical boundaries; an association between the two could be of major relevance to world health.

*Mycobacterium tuberculosis* is a human pathogen with efficient transmission and immune evasion strategies. After the HIV pandemic, tuberculosis re-emerged as a global emergency, peaking in 2006 to more than 9 million cases annually, then falling to an estimated 8–8 million by 2010. The Stop TB Partnership has an ambitious goal to eliminate tuberculosis by 2050 (to less than one case per 1 million every year). Traditionally classified as either latent or active disease, the outcome of infection with *M tuberculosis* is now perceived as a continuum. Prevention, diagnosis, and management of both active tuberculosis and latent infection remain challenging, despite substantial improvements in diagnostic tests, and new drugs for active tuberculosis, and new regimens for latent infection.

Drawbacks with tuberculosis treatment regimens, particularly rising drug resistance and HIV–tuberculosis co-infection, are driving the need for novel treatment approaches. Strategies to accelerate recovery and reduce treatment durations include the development of new antimicrobial agents and the investigation of adjunctive immunotherapies; both are priority areas of tuberculosis research. Mechanisms of action of potential adjunctive treatments include promotion of T-helper-1 (Th1) antmycobacterial immune responses (eg, administration of interferon gamma), upregulation of host innate (ie, macrophage) antmycobacterial immune responses (eg, vitamin D and nitric oxide), decrease of immunopathology mediated tissue damage because of excessive inflammatory responses (eg, corticosteroids), and alteration of the metabolic state of tuberculosis bacilli to shift them out of a non-replicative, antibiotic-resistant state (eg, tumour necrosis factor alpha (TNFα) inhibitors). There has been much hope that vitamin D might fulfil at least some of these actions as a potential adjunctive treatment in active or latent tuberculosis.

Vitamin D is derived from endogenous synthesis after exposure of the skin to solar ultraviolet radiation. Receptors for its active form, 1,25-dihydroxyvitamin D, are widely expressed in human cells, including monocytes and macrophages, dendritic, T cells, B cells, and natural killer cells. The effects of 1,25-dihydroxyvitamin D are immunostimulatory in monocytes and macrophages and immunosuppressive in dendritic and T cells. Ultraviolet radiation causes immune changes too, mainly downregulatory, in antigens encountered close to the time of the exposure.

Basic science, clinical research, and historical treatment practices (eg, phototherapy and cod-liver oil) suggest that inexpensive, accessible vitamin D could play an important part in the treatment of tuberculosis. Sufficiency in vitamin D has been hypothesised to decrease the risk of infection with tuberculosis after exposure, limits the progression from latent to active tuberculosis, and, as an adjunct to antimicrobial treatment, decreases the duration and improves the effectiveness of treatment.

Questions about the relation between tuberculosis, vitamin D, and ultraviolet radiation are largely unexplored. These include reconciliation of contradictory immunological actions of 1,25-dihydroxyvitamin D related to the balance between innate immunity (antimicrobial peptides and macrophages in particular) and adaptive immunity, understanding of the immunological effects of exposure to ultraviolet radiation (a potentially important confounder of the
vitamin D–tuberculosis relation), and consideration of alternative hypotheses that explain the common finding of low vitamin D status in patients with active tuberculosis. We reviewed the scientific literature to provide evidence, explore contradictions, and suggest alternative hypotheses.

**Vitamin D**

**Background**

When 7-dehydrocholesterol in the plasma membrane of human keratinocytes is exposed to ultraviolet B (UVB) radiation, it is converted to previtamin D₃, followed by a thermal reaction to form vitamin D₃ (cholecalciferol). Some foods contain vitamin D₃ or vitamin D₂, which is synthesised by plants after UVB irradiation; however, these only contribute small amounts of the total vitamin D requirements in most individuals. The physiological effects of D₃ and D₂ are interchangeable, although oral vitamin D₃ is more effective in raising serum 25-hydroxyvitamin D concentration than is D₂. Vitamin D₂ is fat soluble, and, as with the vitamin D metabolites, is carried in the circulation by heptatically produced vitamin D-binding protein. In the liver, vitamin D undergoes hydroxylation by a 25-hydroxylase to form 25-hydroxyvitamin D, which is converted to the biologically active steroid hormone 1,25-dihydroxyvitamin D by 1α-hydroxylase enzyme. The actions of the hormone are mediated either through ligation with a nuclear vitamin D receptor (VDR) to regulate gene transcription, resulting in genomic responses, or via membrane rapid-response receptors. These receptors are distributed in most human tissues. Thus, 1,25-dihydroxyvitamin D can be generated by tissues expressing 1α-hydroxylase and act via local VDRs. The 1α-hydroxylase gene, *CPY27B1*, is primarily expressed in the kidney under normal conditions. Expression in a wide range of other human cells, including activated macrophages, is important in disease states. Production of 1,25-dihydroxyvitamin D in renal cells is under negative feedback control through induction by the hormone of 24-hydroxylase, which catabolises 25-hydroxyvitamin D as well as 1,25-dihydroxyvitamin D. This feedback does not happen in macrophages, perhaps because macrophages express a splice variant of the 24-hydroxylase gene. Thus, hypercalcaemia is possible in granulomatous diseases that are characterised by macrophage activation, such as sarcoidosis and tuberculosis.

Determinants of vitamin D status include exposure to UVB radiation emitted by the sun (which varies by latitude, albedo, altitude, season, time of day, pollution, and cloud cover), clothing, dietary intake, body-mass index, serum cholesterol, and genetic factors such as skin pigmentation and polymorphisms in or near genes encoding the VDR and enzymes of the vitamin D metabolic pathway (eg, 7-dehydrocholesterol reductase, 25-hydroxylase, 24-hydroxylase, and possibly 1α-hydroxylase).

**Measurement, reference ranges, and supplementation**

Vitamin D status is inferred from the concentration of total 25-hydroxyvitamin D in serum. The methods used to detect this concentration, however, (eg, chemiluminescence and radioimmunoassays) lack accuracy, reproducibility, and sensitivity. Other assay methods, such as liquid chromatography-tandem mass spectrometry (LC-MS/MS) can distinguish 25-hydroxyvitamin D₂ and 25-hydroxyvitamin D₃ concentrations and are user dependent. A vitamin D standardisation programme is underway, using LC-MS/MS to standardise measurements of serum 25-hydroxyvitamin D globally.

In addition to drawbacks with measurement, there is no consensus on the reference values that define vitamin D sufficiency and deficiency (table 1). Rising reference ranges have caused an increase in the prevalence of vitamin D deficiency, including in low-latitude settings. In the 1990s, a common threshold for deficiency was less than 25 nmol/L, now, concentrations of up to 125 nmol/L are advocated. The 2010 US Institute of Medicine statement defined vitamin D sufficiency as a serum concentration of 50 nmol/L 25-hydroxyvitamin D or higher for adults and children. In addition to the shifting targets, some evidence exists for an increase in vitamin D deficiency in populations. Although samples were not run in parallel and assay drift is a recognised problem, the mean serum 25-hydroxyvitamin D concentration in participants in the large US National Health and Nutrition Examination Surveys fell from 75 nmol/L in 1988–1994 to 60 nmol/L in 2001–2004, attributed in part to increased sun protection in response to skin cancer campaigns.

Evidence from several studies, often with small sample sizes or very specific populations or both (eg, elderly, epileptic, with psoriasis), which used small dose ranges of artificial ultraviolet radiation, suggest that whole-body exposure to ultraviolet radiation, sufficient to cause just-detectable erythema of the skin, is comparable, in raising 25-hydroxyvitamin D concentrations, to ingestion of 10000 IU of oral vitamin D. However, translation of these findings into natural sun exposure situations in which variable body surface areas are exposed, individuals are upright rather than horizontal (thus receiving around a third of the dose of ultraviolet radiation, depending on solar elevation), and the level of ambient UVB varies with latitude, season, time of day, cloud cover, and urban or rural environments is difficult. Several UK studies show that recommended casual (short) sun exposure, even during summer, is generally insufficient to raise 25-hydroxyvitamin D to optimum concentrations, particularly in deeply pigmented populations. Strategies to optimise vitamin D status by oral intake in adults differ widely, depending on whether physiological replacement or pharmacological dosing is desired, and whether daily or intermittent bolus treatment is preferred. The minimum vitamin D₃ requirement is 400 IU/day across all ages, but treatment doses of 10 000 IU daily, or up to 60 000 IU...
given as a one-off bolus in adults, have been advocated to treat deficiency in 2010 guidelines. Because rickets, resulting from vitamin D deficiency in infants, continues to occur in high-resource settings, supplementation (400 IU/day) is recommended as a routine for infants (aged 0–12 months).

Whether the 25-hydroxyvitamin D concentrations regarded as sufficient for bone health are applicable to other vitamin D functions such as immunity, remains unknown. Part of the drive to increase recommended reference ranges of vitamin D, rather than the use of population distributions, derives from studies of bone biomarkers such as parathyroid hormone. However, no such markers are available to estimate the optimum 25-hydroxyvitamin D concentration needed for the non-skeletal functions of vitamin D. The biological significance of the natural seasonal variation in serum 25-hydroxyvitamin D, which is common in people living outside tropical latitudes is poorly understood. Vieth hypothesises that such variation might be harmful, and that delays in cellular responses to changing concentrations could account for the increased risk of prostate and pancreatic cancers in people with high serum 25-hydroxyvitamin D living in settings with low ultraviolet exposure. Sudden changes in 25-hydroxyvitamin D from large doses of vitamin D could also be associated with adverse outcomes: a study in which 500 000 IU vitamin D bolus were given annually showed an association with increased risk of fall and fracture. These uncertainties, and the limitations of 25-hydroxyvitamin D assays and changing reference ranges, should be considered when associations between tuberculosis and vitamin D are examined.

Vitamin D gene polymorphisms
A contribution of vitamin D insufficiency to worse outcomes after infection with tuberculosis could be supported by finding an association with genetic polymorphisms within vitamin D-related genes. Most studies have focused on VDR gene variants that could affect activity of the receptor and therefore its downstream functions. For example, the FokI F allele is more transcriptionally active than the f allele, with increased responsiveness to 1,25-dihydroxyvitamin D. A meta-analysis of 23 studies showed changes in risk of tuberculosis development associated with VDR polymorphisms in Asian populations: increased risk with the ff genotype of the FokI polymorphism, and decreased risk with the bb genotype of the BsmI polymorphism. Importantly, the effect of genotype within the context of vitamin D status has not been examined rigorously—ie, the effect of a less efficient VDR might be more apparent at low, compared with high, 25-hydroxyvitamin D concentrations. Wilkinson and colleagues showed that TT or Tt alleles of TaqI were associated with increased tuberculosis risk only in people with vitamin D deficiency, and the ff allele of FokI was associated with increased risk only in those with non-detectable 25-hydroxyvitamin D.

The response to treatment in relation to VDR genotype has been noted in several studies: rapid treatment responses occurred in patients with TT or tt alleles of TaqI, FF or Ff alleles of FokI, and the AA allele of Apal. No variation in treatment responses by VDR genotype in relation to serum 25-hydroxyvitamin D concentration has been investigated to date.

Few studies have examined polymorphisms in CYP27B1 (encoding 1α-hydroxylase) and GC (encoding
vitamin D binding protein) with respect to tuberculosis and vitamin D. For CYP27B1, polymorphisms are unrelated to susceptibility to active tuberculosis.79 Generally, GC genotypes are not associated with active tuberculosis,80 but in Gujarati Asians, the GC2/2 genotype is strongly linked with susceptibility to active tuberculosis compared with the GC1/1 genotype, although only in those with low (<20 nmol/L) 25-hydroxyvitamin D levels.81

Immunomodulatory effects of vitamin D
Most effects of 1,25-dihydroxyvitamin D are immunosuppressive, but some, important in controlling M tuberculosis infection, are immunostimulatory (panel 1).26,27,86,89 In brief, activation of macrophages via toll-like receptor26 and interferon gamma,27 reversal of phagosome maturation arrest,86 and autophagy27 are all crucial components of the immune response to M tuberculosis; each has been shown to use 1,25-dihydroxyvitamin D. Contrastingly, the suppression of acquired immune responses by 1,25-dihydroxyvitamin D could impair clearance of M tuberculosis, particularly through the downregulation of Th1 and Th17-mediated responses and generation of regulatory T cells.42 In peripheral blood mononuclear cells infected in vitro with M tuberculosis, 1,25-dihydroxyvitamin D changes the balance in cytokine production towards an anti-inflammatory profile (downregulation of interleukin 6, TNFα, and interferon gamma)39 through reduced expression of several pattern recognition receptors, such as TLR-2 and Dectin-1. How this can be reconciled with the increased risk of tuberculosis infection after inhibition of interferon gamma, interleukin 12, and TNFα is not clear.8 The mild T-cell suppression caused by 1,25-dihydroxyvitamin D, combined with its innate immunostimulatory effects, could be beneficial by mitigating cell-mediated immunopathology in active tuberculosis (akin to the use of corticosteroids in tuberculosis meningitis), while promoting M tuberculosis killing through activated macrophage pathways.

The effect of supplemental vitamin D on in-vivo immune responses to M tuberculosis is rarely studied. One report included 192 tuberculosis contacts, randomly assigned to receive one oral dose of vitamin D (2.5 mg, 100 000 IU) or placebo.40 A substantial improvement in anticyclobacterial immunity was noted in the vitamin D versus placebo group, as shown by the growth restriction of recombinant mycobacteria (BCG-lux assay) in whole blood taken from each patient. This response is regarded as a demonstration of innate immunity and, contrastingly, the acquired response (as measured by interferon gamma secretion after stimulation of blood cells with mycobacterial antigens in vitro) did not differ between the groups. Another study involved south Asian immigrants to the UK with low serum 25-hydroxyvitamin D concentrations who were UVB irradiated, resulting in an increase in these concentrations to about 50 nmol/L. However, no change in their anticyclobacterial immunity, assessed by restriction of growth of BCG-lux, occurred.93

Ultraviolet radiation-induced immunological effects and tuberculosis
Ultraviolet radiation is the major source of vitamin D and its immunological effects should therefore be assessed alongside those of vitamin D.94 Exposure of animals and human beings to ultraviolet light results in downregulation of T and B-cell response to various antigens, including tumour antigens, contact sensitisers, microorganisms, and alloantigens.25 Immunomodulation pathways vary depending on the antigen; the spectrum, dose and frequency of radiation exposure, time between exposure and application of the antigen, and the involvement of primary or memory immune responses. Differences exist between local immunosuppression, in which the antigen is applied directly to the irradiated body site, and systemic immunosuppression, in which the antigen is applied to a site distant from the irradiated area. The main outcome of local immunosuppression is the promotion of antigen-specific regulatory T and B cells in local lymph nodes that

Panel 1: Major immunomodulatory effects of 1,25-dihydroxyvitamin D

Adaptive responses (predominantly immunosuppressive)26,27,86,89
- Inhibits Th-helper (Th) 1 cytokines (eg, interleukin 2, interferon gamma)
- Promotes Th2 cytokines (eg, interleukin 4, 5, and 10)
- Suppresses antigen presentation with reduced interleukin 12 production
- Inhibits Th17 proliferation and interleukin 17 production
- Promotes number and function of regulatory T cells
- Inhibits differentiation and proliferation of B cells and production of immunoglobulin

Innate responses (predominantly immunostimulatory)
- Inhibits toll-like receptor-mediated production of interleukin 12 and interleukin 2346
- Upregulates expression of the cathelicidin gene CAP18 (ie, CAMP) in macrophages, leading to the production of the antimicrobial peptide, LL-37, which mediates Mycobacterium tuberculosis cell death46
- Promotes autophagy via cathelicidin, which enters the autophagosome vacoule containing M tuberculosis to mediate cell death46
- Reverses M tuberculosis-induced phagosome maturation arrest via a phosphoinositide 3-kinase pathway86 and via autophagy

Adaptive and innate responses
- Inhibits differentiation and maturation of dendritic cells27
- Increases number of invariant natural killer T cells and their cytokine production48
- Induces synthesis of antimicrobial peptides including LL-37, after macrophage activation is achieved in response to interferon gamma released from activated T cells27

Other
- Inhibits production of matrix metalloproteinases (MMPs) in human peripheral blood mononuclear cells. MMP production in the lung is induced by M tuberculosis and could contribute to cavity formation; if 1,25-dihydroxyvitamin D can also inhibit MMP production in the lung, it could provide protection against tuberculosis-induced lung pathology96
- Vitamin D-binding protein has independent immune functions—eg, deglycosylated protein stimulates macrophages94
suppress immunity. In systemic immunosuppression, mediators such as prostaglandins might be released from the irradiated site and a systemic increase in regulatory T cells and activation of natural killer T cells that promote tolerance could occur.

Irradiation of human skin with ultraviolet light produces antimicrobial peptides, possibly through vitamin D, including β-defensin 2 and β-defensin 3. Thus, although exposure to ultraviolet light suppresses adaptive immunity, it can also foster innate mechanisms that could be important in controlling bacterial infections, especially on epidermal surfaces. In human skin cells in vitro and mouse skin in vivo, the topical application of 1,25-dihydroxyvitamin D after exposure to ultraviolet radiation can reduce DNA damage and apoptosis of skin cells via the rapid non-genomic pathway. Additionally, 1,25-dihydroxyvitamin D reduces ultraviolet-induced suppression in contact hypersensitivity in mice; thus, 1,25-dihydroxyvitamin D is protective against several of the immunosuppressive effects of ultraviolet radiation.

Different approaches point to an effect of exposure to ultraviolet radiation in tuberculosis transmission. First, tuberculosis has an annual seasonal pattern in many countries. Fares reviewed 12 studies involving data from 11 countries or regions and recorded a peak of cases (defined as time of notification, frequently about 3 months after onset of symptoms) in spring and summer. Similar findings of a spring peak have been reported from Cape Town and New York. This pattern could be attributed to high M tuberculosis transmission risk during winter months and low vitamin D status at this time of the year leading to impaired innate immune responses and perhaps reactivation of latent infection. Additionally, cough during winter could erroneously be attributed to non-tuberculous causes, with tuberculosis diagnoses being delayed until spring. Alternatively, infection in winter could be controlled, allowing the ultraviolet-induced immunosuppression after high exposure to solar ultraviolet radiation in spring to be sufficient to enable active disease.

Second, ultraviolet irradiation can reduce the immune response to M tuberculosis in a guineapig model. Animals vaccinated with BCG, then irradiated with ultraviolet light, showed suppression of the delayed hypersensitivity response to purified protein derivative. On challenge with an aerosol of live M tuberculosis, the pulmonary microbial load was increased, and in-vitro lymphoproliferative response to purified protein derivative reduced, in irradiated compared with non-irradiated animals. Third, in the only meta-analysis of vaccine efficacy that considered location, Colditz and colleagues showed that tuberculosis protection conferred by BCG increased with distance from the equator. Others have also noted that protection is reduced in tropical regions. Ultraviolet-induced immunosuppression, greatest in equatorial areas, could account for this finding although many additional changes occur with latitude such as exposure to other pathogens, diet, clothing, temperature, and daylight hours. The moderating role of skin pigmentation in ultraviolet-induced vitamin D production could also be relevant. Finally, ultraviolet irradiation reduces memory responses to BCG in humans. Individuals immunised with BCG were exposed to suberythemal solar simulated ultraviolet radiation before challenge with purified protein derivative on the irradiated and a distant non-irradiated site—the Mantoux reaction was suppressed at the irradiated site but not at the distant site.

Effects of exposure to ultraviolet radiation and vitamin D are difficult to examine separately in studies of human beings. Such research would necessitate assessment of ultraviolet radiation exposure with personal ultraviolet dosimeters (frequently worn as wristbands), categorisation of skin type, and measurement of serum 25-hydroxyvitamin D concentration. Both experimental studies on animals and epidemiological studies in human beings suggest independent effects for ultraviolet radiation and vitamin D in the autoimmune disease multiple sclerosis, but we are unaware of any research specifically assessing antimycobacterial responses.

Studies reviewed here suggest that ultraviolet irradiation could have a predominantly immunosuppressive role in tuberculosis through the downregulation of acquired immunity, while potentially promoting the production of vitamin D.

Historical perspective

In the 19th century, cod-liver oil was used in Europe to prevent childhood diseases such as rickets and tuberculosis. Although not a cure, a benefit in patients with tuberculosis was weight gain. It was sometimes given in large doses (0.5–1 pint every 4–8 days or one or two tablespoons two to four times daily). The chemical structures of the vitamins D were discovered by Windaus and colleagues in the 1930s and the antirachitic component of cod-liver oil, identified in rats, was identical to vitamin D₃. One tablespoon of cod-liver oil contains about 1360 IU vitamin D₃, the highest level in any food, in addition to vitamin A and omega-3 fatty acids.

The use of cod-liver oil in the treatment of tuberculosis fell during the early 20th century because of its unpleasant taste and the increased popularity of heliotherapy (sun exposure) and phototherapy (exposure to an artificial light source). In the 1940s, however, several physicians treated lupus vulgaris (cutaneous tuberculosis) with large doses of vitamin D₃: 150 000 IU/day orally. In one report, 56% of patients were cured and 20% were unresponsive after treatment, with hypercalcaemia as a recognised complication. The toxicity threshold for vitamin D is now estimated at 10 000–50 000 IU/day. In Jan 2012, hypercalcaemia was also noted in a case report after high-dose vitamin D supplementation in patients with tuberculosis. Vitamin D was also used in the treatment
of disseminated tuberculosis: a 1948 case report described success after oral administration of 100 000 IU cholecalciferol daily. Nevertheless, vitamin D was replaced by antibiotics from the second half of the 20th century, until its return recently. Heliotherapy was introduced in the mid-1800s with the opening of a thermal treatment station in Slovenia. A great advance was made by Finsen who used filtered sunlight in 1893 to treat cutaneous tuberculosis, and in 1901, created a carbon arc lamp that emitted concentrated ultraviolet radiation—the first source of phototherapy. Antimicrobial peptides induced in keratinocytes by exposure to ultraviolet light, via a vitamin D-related mechanism, have been postulated to provide an explanation for this result. However, the spectrum of the lamp was recorded recently and contained no UVB, hence vitamin D production in patients irradiated using this technique is unlikely; an alternative suggestion is that the longer UVA rays might kill *M tuberculosis* by a photodynamic mechanism because the bacteria contains coproporphyrin III. Additionally, germicidal ultraviolet lamps, which emit short-wavelength UVC radiation and exert a direct bactericidal effect, have a proven effectiveness against airborne *M tuberculosis.*

Interest in natural exposure to sunlight as a tuberculosis treatment continued to develop. A 1903 study of graded sun exposures showed that time-to-cure was shorter when carbon arc lamps were used. From the 1920s until the antibiotic era, heliotherapy became the most popular method of tuberculosis treatment in Europe and the USA.

### Findings

<table>
<thead>
<tr>
<th>Design</th>
<th>Number of patients</th>
<th>25(OH)D cutoff</th>
<th>25(OH)D lower in active tuberculosis</th>
<th>Findings</th>
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<tbody>
<tr>
<td>Davies, UK, 1985[^{114}][^{115}]</td>
<td>Case-control study of patients with tuberculosis and ethnically matched (related or unrelated) controls</td>
<td>50 cases; 50 controls</td>
<td>Not stated</td>
<td>Median 25(OH)D substantially lower in patients with tuberculosis than in controls (16·0 vs 27·2 nmol/L)</td>
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<td>Grange et al, Indonesia, 1985[^{116}][^{117}]</td>
<td>Cross-sectional study in patients with smear-positive pulmonary tuberculosis and controls</td>
<td>40 cases; 38 controls</td>
<td>Not stated</td>
<td>No Similar bimodal distribution of 25(OH)D in patients with tuberculosis and controls, with similar median values (65·6 nmol/L vs 69·4 nmol/L)</td>
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<tr>
<td>Davies et al, Kenya, 1987[^{118}][^{119}]</td>
<td>Case-control study of patients with tuberculosis and related controls</td>
<td>15 cases; 15 controls</td>
<td>Not stated</td>
<td>Median 25(OH)D substantially lower in patients with tuberculosis than in controls (39·7 vs 65·4 nmol/L), median 1,25D similar in cases and controls</td>
</tr>
<tr>
<td>Davies et al, Thailand, 1988[^{120}][^{121}]</td>
<td>Case-control study of patients with tuberculosis and healthy (blood donor) controls</td>
<td>51 cases; 51 controls</td>
<td>Not stated</td>
<td>Mean 25(OH)D substantially lower in patients with tuberculosis cases than in controls (24·5 vs 29·3 nmol/L)</td>
</tr>
<tr>
<td>Chan et al, Hong Kong, 1994[^{122}][^{123}]</td>
<td>Case-control study of patients with tuberculosis and controls receiving treatment for non-tuberculosis conditions</td>
<td>22 cases; 23 controls</td>
<td>Not stated</td>
<td>Yes, but difference not statistically significant</td>
</tr>
<tr>
<td>Wilkinson et al, UK, 2000[^{124}][^{125}]</td>
<td>Case-control study of patients with tuberculosis and tuberculosis contacts in people of Indian nationality</td>
<td>103 cases; 42 contacts</td>
<td>Deficient &lt;10 nmol/L</td>
<td>Yes</td>
</tr>
<tr>
<td>Sasidharan et al, India, 2002[^{126}][^{127}]</td>
<td>Cross-sectional study in patients with tuberculosis and controls</td>
<td>35 cases; 16 controls</td>
<td>Deficient &lt;22·5 nmol/L</td>
<td>No substantial difference between patients with tuberculosis and controls in either 25(OH)D (46·4 vs 52·2 nmol/L, respectively) or 1,25D (45·9 vs 45·9 nmol/L, respectively)</td>
</tr>
<tr>
<td>Weise et al, Guinea-Bissau, 2007[^{128}][^{129}]</td>
<td>Cross-sectional study in patients with tuberculosis and unmatched healthy controls</td>
<td>362 cases; 494 controls</td>
<td>Insufficient (51·575 nmol/L); moderately deficient (26–50); severely deficient (&lt;25 nmol/L)</td>
<td>Yes, but deficiency more common in controls</td>
</tr>
<tr>
<td>Sita-Lumsden et al, UK, 2007[^{130}][^{131}]</td>
<td>Cross-sectional case-control study of children and adults; controls were matched for age, sex, and skin colour</td>
<td>178 cases; 130 controls</td>
<td>Deficient &lt;21 nmol/L</td>
<td>Median 25(OH)D substantially lower in patients with tuberculosis than in controls (77·5 vs 83·0 nmol/L); moderate to severe deficiency (≥50 nmol/L) more common in healthy controls than in patients with tuberculosis, (65/494 vs 3/362; 8·6% vs 0·8% respectively)</td>
</tr>
<tr>
<td>Gibney et al, Australia, 2008[^{132}]</td>
<td>Retrospective</td>
<td>40 cases or past tuberculosis; 81 latent tuberculosis</td>
<td>Moderately to severely deficient &lt;25 nmol/L</td>
<td>Yes</td>
</tr>
<tr>
<td>Fris, Tanzania, 2008[^{133}][^{134}]</td>
<td>Cross-sectional study of patients with suspected tuberculosis</td>
<td>506 culture-positive tuberculosis; 129 culture-negative tuberculosis</td>
<td>Insufficient (50–75 nmol/L); mildly deficient (25–49); severely deficient (&lt;25 nmol/L)</td>
<td>Marginally lower in culture-positive tuberculosis than in culture-negative tuberculosis (85·5 vs 92·9 nmol/L)</td>
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although it was contraindicated for pulmonary tuberculosis in some studies. 

Exposure to the sun and dietary vitamin D were thus thought to help in the treatment of specific forms of tuberculosis, although other components of these strategies might have contributed to any true therapeutic effects. There were also substantial changes in social environments in high-income countries, which reduced the risks of *M tuberculosis* transmission.

**Observational studies of serum 25-hydroxyvitamin D concentration**

In-vivo studies in human beings have been unable to clearly address whether vitamin D status affects susceptibility to tuberculosis infection, development of active disease from latency, or treatment response. This is because most studies of serum 25-hydroxyvitamin D concentration in patients with active tuberculosis are cross-sectional, and are therefore unable to show whether low 25-hydroxyvitamin D levels are a result of, rather than a risk factor for, the disease process (table 2). Moreover, diagnostic tests for latent tuberculosis infection (ie, Mantoux test and in-vitro interferon gamma production) assess T-cell responses to mycobacterial antigens, responses that themselves might be affected by vitamin D status.

Patients with tuberculosis have insufficient 25-hydroxyvitamin D concentrations that are lower than in comparator populations (table 2). A meta-analysis of seven international studies showed that low 25-hydroxyvitamin D levels were associated with high active tuberculosis risk. In a study from Greenland, where consumption of sea mammal liver, similar to cod-liver oil use, can produce high serum 25-hydroxyvitamin D concentrations, an unexpected U-shaped curve was identified, with both higher and lower 25-hydroxyvitamin D concentrations recorded in patients with tuberculosis compared with controls. High concentrations of potentially immunosuppressive omega-3 fatty acids and vitamin A in liver were suggested to explain this association, although Viet’s hypothesis, or harm from high 25-hydroxyvitamin D concentrations, might be important considerations too.

### Table 2: Studies reporting serum 25-hydroxyvitamin D concentrations in patients with tuberculosis

<table>
<thead>
<tr>
<th>Design</th>
<th>Number of patients</th>
<th>25(OH)D cutoff</th>
<th>25(OH)D lower in active tuberculosis</th>
<th>Findings</th>
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<tbody>
<tr>
<td>Tostman et al, Tanzania, 2010</td>
<td>81 cases</td>
<td>Insufficient (50–75 nmol/L); deficient (&lt;50 nmol/L)</td>
<td>No comparison group</td>
<td>Sufficient 25(OH)D in most patients (67/81=83%), both at diagnosis and at 2-month follow-up; small but substantial rise in median 25(OH)D (from 24.0–101.0 nmol/L) at follow-up despite no supplementation</td>
</tr>
<tr>
<td>Nielsen et al, Greenland, 2010</td>
<td>72 cases; 72 controls</td>
<td>Insufficient (50–75 nmol/L); mildly deficient (25–49 nmol/L); severely deficient (&lt;25 nmol/L)</td>
<td>Yes, but also in higher group</td>
<td>Both high (&gt;140 nmol/L) and low (&lt;49 nmol/L) 25(OH)D associated with patients with tuberculosis cases, creating a U-shaped curve; serum for 25(OH)D collected either at diagnosis of tuberculosis or during treatment</td>
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<tr>
<td>Banda et al, Malawi, 2011</td>
<td>161 cases</td>
<td>Deficient &lt;5 nmol/L</td>
<td>No comparison group</td>
<td>Deficient 25(OH)D in most patients (120/161=74.5%); 25(OH)D &lt;25 nmol/L in 21 patients (13%)</td>
</tr>
<tr>
<td>Nansera et al, Uganda, 2011</td>
<td>50 tuberculosis–HIV; 50 HIV; 50 controls</td>
<td>Deficient &lt;30 nmol/L</td>
<td>No, but deficiency more common in tuberculosis-HIV than in controls</td>
<td>No difference in mean 25(OH)D between controls, HIV and patients with tuberculosis-HIV; more patients with tuberculosis-HIV had 25(OH)D &gt;30 nmol/L (38% vs 20%) or &lt;12 nmol/L (12% vs 0%) than controls</td>
</tr>
<tr>
<td>Talat et al, Pakistan, 2000</td>
<td>100 household contacts; 28 current or past tuberculosis</td>
<td>Insufficient (45–67 nmol/L); deficient (&lt;45 nmol/L)</td>
<td>Yes</td>
<td>Median 25(OH)D low overall; similar in patients with tuberculosis and contacts (19.7 vs 24.0 nmol/L); but significantly lower when tuberculosis, past tuberculosis, and treated tuberculosis were grouped together vs contacts; 8/92 household contacts followed for 4 years developed tuberculosis, seven in the lowest, one in the middle, and none in the higher tertile of 25(OH)D; relative risk of progression to tuberculosis in lowest 25(OH)D group vs 1</td>
</tr>
<tr>
<td>Martineau et al, UK, 2011</td>
<td>126 cases</td>
<td>Profoundly deficient &lt;20 nmol/L</td>
<td>No comparison group</td>
<td>Mean 25(OH)D in patients with tuberculosis at baseline about 21 nmol/L; majority of patients (75/126) &lt;20 nmol/L at baseline, and almost all (122/126) &lt;75 nmol/L</td>
</tr>
<tr>
<td>Martineau et al, South Africa, 2011</td>
<td>192 cases; 178 latent tuberculosis</td>
<td>Deficient &lt;50 nmol/L</td>
<td>Yes</td>
<td>In HIV-negative patients, deficient 25(OH)D more common in patients with active tuberculosis than in patients with latent tuberculosis (75% vs 37%); in HIV-positive subjects, deficient 25(OH)D more common in patients with active tuberculosis than in patients with latent tuberculosis (86% vs 52%)</td>
</tr>
</tbody>
</table>

25(OH)D=25-hydroxyvitamin D; 1,25D=1,25-dihydroxyvitamin D. *Serum 25(OH)D measured before start of tuberculosis treatment. †Liquid chromatography-tandem mass spectrometry method used to measure 25(OH)D.
We identified only one prospective study that provides evidence for the importance of vitamin D deficiency as an antecedent to development of active tuberculosis.237 In this study, household contacts of tuberculosis patients in Pakistan (who did not receive preventive treatment) were followed up for up to 4 years. The risk of progression to active tuberculosis was substantially higher (relative risk 5.1) among patients with the lowest 25-hydroxyvitamin D concentrations (table 2).

The main hypothesis to explain low vitamin D status in people with active tuberculosis is that a fall in serum 25-hydroxyvitamin D concentration precedes the diagnosis of tuberculosis, since this fall allows latent disease to become activated, as suggested by studies of migrants moving from high to low ultraviolet exposure settings.76,128 However, other hypotheses merit consideration (panel 2).

Clinical trials of vitamin D supplementation

Although observational studies justify trials of vitamin D supplementation to inhibit progression from latent to active tuberculosis, such studies pose difficulties because they need large numbers of patients, long follow-up, and would need to show a clear additional benefit over evidence-based treatments for latent infection (eg, isoniazid preventive treatment and antiretroviral therapy for patients with HIV). Of note, vitamin D supplementation does not decrease the risk of tuberculosis in patients with renal dialysis.139

By contrast with the absence of prevention trials to date, administration of vitamin D in active disease has been reported over many decades. A review in 2006 of three trials and ten case series of treatment of patients with tuberculosis with vitamin D concluded that benefits were uncertain because the studies were of poor quality, often used vitamin D3, and did not examine the effect of vitamin D supplementation on outcomes. Subsequent trials show predominantly negative results (table 3). Such findings also characterise trials of vitamin D supplementation for other diseases. For example, despite a clear role for this vitamin in bone health, and vitamin D being commonly recommended for fracture prevention, most studies have failed to show an independent benefit of vitamin D in hip fracture prevention.44 Reasons that trials may fail to support findings from observational studies could include uncontrolled confounding: that vitamin D is indeed beneficial, but trials had inadequate power or used suboptimal doses; that the 25-hydroxyvitamin D concentration examined in observational studies is a proxy for sun exposure and physical activity; or that host determinants, such as expression of 1α-hydroxylase and vitamin D binding protein, affect results. Furthermore, increases in serum 25-hydroxyvitamin D through vitamin D supplementation might not result in increased 1,25-hydroxyvitamin D to the necessary concentrations or in the appropriate cellular compartments. Lappe and Heaney44 describe potential reasons for false-negative findings from vitamin D trials, including that the dose–response curve for vitamin D is sigmoid, so only a few patients towards the centre of the curve will experience substantial effects from a given supplementary dose. Recent results suggest more promising effects of vitamin D supplementation in tuberculosis than had previously been shown (table 3).142

Panel 2: Hypotheses to explain the association between serum 25-hydroxyvitamin D and tuberculosis

- Vitamin D deficiency increases the risk of tuberculosis after exposure.90,123,124
- Vitamin D deficiency contributes to progression from latent to active tuberculosis.125
- Vitamin D deficiency is a consequence of active tuberculosis due to low exposure to ultraviolet radiation and low dietary vitamin D, supported by the finding of spontaneous improvement in serum [25(OH)D] during tuberculosis treatment in some instances.124,125
- 25-hydroxyvitamin D is low in patients with active tuberculosis owing to the effect of inflammatory processes on vitamin D metabolism. This hypothesis is supported by the finding that serum [25(OH)D] can fall during development of tuberculosis immune restoration inflammatory syndrome, in inverse proportion to serum cytokines, suggesting that low [25(OH)D] may be a consequence of immunological activation.130
- Low 25-hydroxyvitamin D suggests a normal anti-tuberculosis immune response because upregulation of 1α-hydroxylase in activated macrophages causes available 25-hydroxyvitamin D to be converted to 1,25-dihydroxyvitamin D. In support of this hypothesis, 1,25-dihydroxyvitamin D provides negative feedback on 25-hydroxyvitamin D,90,123 and 1,25-dihydroxyvitamin D is raised in patients with tuberculosis compared with controls;125 note also 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D are related directly in tuberculosis patients but inversely in controls, suggesting changes in vitamin D metabolism in tuberculosis.125
- Calcium deficiency in low-resource, tuberculosis-endemic settings contributes to raised serum 1,25-dihydroxyvitamin D and thus to low serum 25-hydroxyvitamin D.132
- Low serum cholesterol in under-nourished patients with tuberculosis contributes to low 25-hydroxyvitamin D concentrations in patients with active tuberculosis.41,134
- If vitamin D assays are done after the start of anti-tuberculosis treatment, low 25-hydroxyvitamin D concentrations could be due to isoniazid or rifampicin.95,135
- A factor common to both tuberculosis and low 25-hydroxyvitamin D—eg, vitamin D-binding protein might be lower at diagnosis of tuberculosis,91 its function could be changed and affect vitamin D binding,90 it can stimulate macrophages, and transports immunoactive molecules.90

Conclusions

The interpretation of studies on vitamin D needs an appreciation of assay variability and the controversies surrounding appropriate serum 25-hydroxyvitamin D reference ranges. Vitamin D has both immunosuppressive and immunostimulatory effects, and ultraviolet radiation, an important confounder, has mainly immunosuppressive effects. Whereas historical literature on phototherapy supports the use of vitamin D in patients with tuberculosis, direct mycobacterial actions of exposure to ultraviolet light could have contributed to such findings, if indeed real benefits occurred. Low serum 25-hydroxyvitamin D concentrations could reflect an appropriate immune response of activated macrophages to M tuberculosis, because this molecule is consumed in
the production of 1,25-hydroxyvitamin D, a mediator of important antimycobacterial effects. Because low baseline serum 25-hydroxyvitamin D in tuberculosis increases spontaneously over time, there is clearly a need for improved understanding of vitamin D metabolism in acute disease, especially disease characterised by macrophage activation, and in which protein synthesis, including vitamin D binding protein, could be impaired. The main trials of vitamin D in people with active tuberculosis to date have not been able to show major benefits overall.

The classification of the relation between vitamin D and tuberculosis risk through prospective surveillance of populations with latent infection or a clinical trial of supplementary vitamin D in this group, would need ambitious sample sizes. People at increased risk of developing active tuberculosis, such as HIV-positive individuals recently infected with tuberculosis, already need isoniazid preventive therapy with or without antiretrovirals; thus, benefits of vitamin D would need to be shown in addition to these optimised background treatment strategies. If HIV-negative individuals were selected, the sample size needed to detect a decrease in active tuberculosis rates from 5% to 3% during 2 years of follow-up, in people given placebo versus vitamin D, would be more than 4000. While such studies are not implausible, in the meanwhile, important scope exists for further investigation in patients with active tuberculosis to shed light on the vitamin D-tuberculosis association.

Research to address the hypotheses outlined in panel 2 and to improve our understanding of vitamin D metabolism in active tuberculosis could include investigation of 25-hydroxyvitamin D concentration in relation to measured ultraviolet radiation exposure, 1,25-hydroxyvitamin D, parathyroid hormone, vitamin D binding protein, and VDR polymorphisms, at tuberculosis diagnosis and during recovery. Further supplementary trials in patients with active tuberculosis, which could incorporate these assessments, would best be done in vitamin D-deficient populations or subgroups selected on the basis of VDR polymorphisms, using adequate vitamin D doses that remain to be identified. Accessible, safe, and inexpensive measures to reduce the population burden of tuberculosis would be welcomed. Any major therapeutic role for vitamin D in tuberculosis remains an unproven but tantalising concept.

**Contributors**

The authors made equal contributions to the literature searching and writing of the Review. Lead authors on separate components of the paper were APR for tuberculosis, RML for vitamin D, and MN for ultraviolet radiation.

**Conflicts of interest**

We declare that we have no conflicts of interest.

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**References**


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### Table 3: Clinical trials of vitamin D supplementation in adults with pulmonary tuberculosis, 2006–11

<table>
<thead>
<tr>
<th>Design</th>
<th>Number of patients</th>
<th>Intervention</th>
<th>Primary outcomes</th>
<th>Findings and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuryam et al, Indonesia, 2006</td>
<td>Placebo controlled (unclear whether randomised)</td>
<td>Intervention (34); placebo (33)</td>
<td>10 000 IU (0.25 mg/day) vitamin D (D2 or D3 not stated) orally for 6 weeks</td>
<td>Clearance of acid-fast bacilli from sputum and chest radiograph improvement at 6 weeks</td>
</tr>
<tr>
<td>Wejse et al, Guinea-Bissau, 2009</td>
<td>Randomised, double blind, placebo controlled</td>
<td>Intervention (187); placebo (180)</td>
<td>100 000 IU cholecalciferol by injection at 0, 5, and 8 months</td>
<td>Composite clinical score at 2-monthly time points and 12-month mortality</td>
</tr>
<tr>
<td>Martineau et al, UK, 2011</td>
<td>Randomised, double blind, placebo controlled</td>
<td>Intervention (62); placebo (64)</td>
<td>100 000 IU cholecalciferol orally at 0, 2, 4, and 6 weeks</td>
<td>Time to culture negativity</td>
</tr>
</tbody>
</table>

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**Search strategy and selection criteria**

We searched PubMed for articles published in English from 1913 to Nov 2012, using the search terms “tuberculosis”, “vitamin D”, and “ultraviolet radiation”. We sourced further articles from our personal databases, and from references cited in papers identified through the process above.


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