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 antimycobacterial 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.

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Introduction

Tuberculosis is the second most common cause of death from infection worldwide.[1] Vitamin D deficiency is prevalent across broad geographical boundaries;[2–5] 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.[6,7] After the HIV pandemic, tuberculosis re-emerged as a global emergency,[8] peaking in 2006 to more than 9 million cases annually, then falling to an estimated 8·8 million by 2010.[1] The Stop TB Partnership has an ambitious goal to eliminate tuberculosis by 2050 (to less than one case per 1 million every year).[9] Traditionally classified as either latent or active disease, the outcome of infection with *M tuberculosis* is now perceived as a continuum.[10,11] For clarity, in this Review we refer to latent tuberculosis infection and active tuberculosis. Prevention, diagnosis, and management of both active tuberculosis and latent infection remain challenging, despite substantial improvements in diagnostic tests,[12,13] new drugs for active tuberculosis,[14–16] and new regimens for latent infection.[17]

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.[18] Mechanisms of action of potential adjunctive treatments include promotion of T-helper-1 (Th1) antimycobacterial immune responses (eg, administration of interferon gamma),[19] upregulation of host innate (ie, macrophage) antimycobacterial immune responses (eg, vitamin D and nitric oxide),[20] decrease of immunopathology mediated tissue damage because of excessive inflammatory responses (eg, corticosteroids),[21] and alteration of the metabolic state of tuberculosis bacilli to shift them out of a non-replicative, antibiotic-resistant state (eg, tumour necrosis factor α [TNFα] inhibitors).[22] 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.[23] 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.[24,25]

Basic science,[26,27] clinical research,[28–30] population studies,[31] and historical treatment practices (eg, phototherapy and cod-liver oil)[32,33] 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,[34] limits the progression from latent to active tuberculosis,[35] and, as an adjunct to antimicrobial treatment, decreases the duration and improves the effectiveness of treatment.[20,28,30]

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 D_3, followed by a thermal reaction to form vitamin D_3 (cholecalciferol). Some foods contain vitamin D_3 or vitamin D_2, 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 D_2 and D_3 are interchangeable, although oral vitamin D_3 is more effective in raising serum 25-hydroxyvitamin D concentration than is D_2.[36] Vitamin D is fat soluble, and, as with the vitamin D metabolites, is carried in the circulation by hepatically 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.[37] 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.[38] 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.[39] 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),[40] clothing, dietary intake, body-mass index, serum cholesterol,[41] 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).[42]

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)[43] lack accuracy, reproducibility, and sensitivity.[44–46] Other assay methods, such as liquid chromatography-tandem mass spectrometry (LC-MS/MS) can distinguish 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 concentrations and are user dependent.[47] A vitamin D standardisation programme is underway, using LC-MS/MS to standardise measurements of serum 25-hydroxyvitamin D globally.[48]

In addition to drawbacks with measurement, there is no consensus on the reference values that define vitamin D sufficiency and deficiency (table 1).[54] Rising reference ranges have caused an increase in the prevalence of vitamin D deficiency, including in low-latitude settings.[2–5,58,59] In the 1990s, a common threshold for deficiency was less than 25 nmol/L, now, concentrations of up to 125 nmol/L are advocated.[56] 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.[54] In addition to the shifting targets, some evidence exists for an increase in vitamin D deficiency in populations.[54] Although samples were not run in parallel and assay drift is a recognised problem,[54] 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.[60]

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,[61] 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 10 000 IU of oral vitamin D.[62–65] 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.[66] Several UK studies show that recommended casual (short) sun exposure, even during summer, is generally insufficient to raise 25-hydroxyvitamin D to optimum concentrations,[67–69] particularly in deeply pigmented populations.[69] 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 D_3 requirement is 400 IU/day across all ages,[54,55] but treatment doses of 10 000 IU daily, or up to 600 000 IU

	Deficiency	Insufficiency	Adequacy	Optimal	Comment
Adults					
Harrison's Principles of Internal Medicine, 1998[49]			"Adequacy" varies according to assay and laboratory, from 20 to 200 nmol/L		..
Australian and New Zealand guidelines, 2005[50]	<12·5 nmol/L	12·5–50·0 nmol/L	>50 nmol/L	..	Extra categories of moderate and mild deficiency are summarised as insufficiency
Dawson-Hughes et al, 2005[51]	..	..	≥50 nmol/L, ≥70 nmol/L, ≥75 nmol/L, or ≥80 nmol/L	..	Consensus not reached by a panel of five experts
Hollis, 2005[52]	..	..	>80 nmol/L	..	..
Holick, 2007[53]	<50 nmol/L	50–75 nmol/L	>75 nmol/L	..	..
US Institute of Medicine, 2010[54]	..	..	≥50 nmol/L	..	..
Pearce and Cheetham, 2010[55]	<20 nmol/L	25–50 nmol/L	50–75 nmol/L	>75 nmol/L	..
Vitamin D Council, 2011[56]	..	..	>125 nmol/L	..	..
Children	..	..		..	..
Wagner and Greer, 2008[57]	..	..	≥50 nmol/L	..	Consensus not reached on concentration to define vitamin D insufficiency for infants and children

Data are serum 25-hydroxyvitamin.

Table 1: **Reference ranges for vitamin D status**

given as a one-off bolus in adults, have been advocated to treat deficiency in 2010 guidelines.[57] Because rickets, resulting from vitamin D deficiency in infants, continues to occur in high-resource settings,[57] 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.[52] 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[70] 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_3 boluses were given annually showed an association with increased risk of fall and fracture.[71] 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 *Fok*I F allele is more transcriptionally active than the f allele,[72–74] with increased responsiveness to 1,25-dihydroxyvitamin D.[73] 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 *Fok*I polymorphism, and decreased risk with the bb genotype of the *Bsm*I polymorphism.[75] 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[76] showed that TT or Tt alleles of *Taq*I were associated with increased tuberculosis risk only in people with vitamin D deficiency, and the ff allele of *Fok*I 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[77] or tt alleles[29] of *Taq*I, FF[77] or ff[78] alleles of *Fok*I, and the AA allele of *Apa*I.[78] 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 *GC*2/2 genotype is strongly linked with susceptibility to active tuberculosis compared with the *GC*1/1 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).[26,27,86,89,91] In brief, activation of macrophages via toll-like receptor[26] and interferon gamma,[27] reversal of phagosome maturation arrest,[86] and autophagy[27] 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.[82] 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)[92] 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.[38] 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.[30] A substantial improvement in antimycobacterial 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 antimycobacterial immunity, assessed by restriction of growth of BCG-lux, occurred.[93]

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

Adaptive responses (predominantly immunosuppressive)[82,83]
- Inhibits T-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 23[84]
- 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 death[26]
- Promotes autophagy via cathelicidin, which enters the autophagosome vacuole containing *M tuberculosis* to mediate cell death[85]
- Reverses *M tuberculosis*-induced phagosome maturation arrest via a phosphoinositide 3-kinase pathway[86] and via autophagy

Adaptive and innate responses
- Inhibits differentiation and maturation of dendritic cells[87]
- Increases number of invariant natural killer T cells and their cytokine production[88]
- Induces synthesis of antimicrobial peptides including LL-37, after macrophage activation is achieved in response to interferon gamma released from activated T cells[27]

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 can also inhibit MMP production in the lung, it could provide protection against tuberculosis-induced lung pathology[89]
- Vitamin D-binding protein has independent immune functions—eg, deglycosylated protein stimulates macrophages[90]

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

suppress immunity.[95] 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.[96]

Irradiation of human skin with ultraviolet light produces antimicrobial peptides, possibly through vitamin D, including β-defensin 2 and β-defensin 3.[97] 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.[98] Additionally, 1,25-dihydroxyvitamin D reduces ultraviolet-induced suppression in contact hypersensitivity in mice;[99] 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[100] 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[101] and New York.[102] 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.[102] 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.[103] Animals vaccinated with BCG, then irradiated with ultraviolet light, showed suppression of the delayed hypersensitivity response to purified protein derivative.[103] 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[104] showed that tuberculosis protection conferred by BCG increased with distance from the equator. Others have also noted that protection is reduced in tropical regions.[105] 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.[106] 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),[107] 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 radiation 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.[33,108] Although not a cure, a benefit in patients with tuberculosis was weight gain.[108] It was sometimes given in large doses (0·5–1·0 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[109] in the 1930s and the antirachitic component of cod-liver oil, identified in rats, was identical to vitamin D_3. One tablespoon of cod-liver oil contains about 1360 IU vitamin D_3, the highest level in any food, in addition to vitamin A and omega-3 fatty acids.[108]

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 D_2: 150 000 IU/day orally.[110] In one report, 56% of patients were cured and 20% were unresponsive after treatment, with hypercalcaemia as a recognised complication.[110] The toxicity threshold for vitamin D is now estimated at 10 000–50 000 IU/day.[54] In Jan 2012, hypercalcaemia was also noted in a case report after high-dose vitamin D supplementation in patients with tuberculosis.[111] 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.[112] 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.[32] A great advance was made by Finsen[32] 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.[113] Additionally, germicidal ultraviolet lamps, which emit short-wavelength UVC radiation and exert a direct bactericidal effect, have a proven effectiveness against airborne *M tuberculosis*.[114]

Interest in natural exposure to sunlight as a tuberculosis treatment continued to develop. A 1903 study[32] of graded sun exposures showed that time-to-cure was shorter than 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,

	Design	Number of patients	25(OH)D cutoff	25(OH)D lower in active tuberculosis	Findings
Davies, UK, 1985[116]*	Case-control study of patients with tuberculosis and ethnically matched (related or unrelated) controls	50 cases; 50 controls	Not stated	Yes	Median 25(OH)D substantially lower in patients with tuberculosis than in controls (16·0 *vs* 27·2 nmol/L)
Grange et al, Indonesia, 1985 [117]*	Cross-sectional study in patients with smear-positive pulmonary tuberculosis and controls	40 cases; 38 controls	Not stated	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)
Davies et al, Kenya, 1987[118]*	Case-control study of patients with tuberculosis and related controls	15 cases; 15 controls	Not stated	Yes	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
Davies et al, Thailand, 1988[119]*	Case-control study of patients with tuberculosis and healthy (blood donor) controls	51 cases; 51 controls	Not stated	Yes	Mean 25(OH)D substantially lower in patients with tuberculosis cases than in controls (24·5 *vs* 29·3 nmol/L)
Chan et al, Hong Kong, 1994[120]*	Case-control study of patients with tuberculosis and controls receiving treatment for non-tuberculosis conditions	22 cases; 23 controls	Not stated	Yes, but difference not statistically significant	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)
Wilkinson et al, UK, 2000[76]*	Case-control study of patients with tuberculosis and tuberculosis contacts in people of Indian nationality	103 cases; 42 contacts	Deficient <10 nmol/L	Yes	25(OH)D low in both groups; median 25(OH)D substantially lower in patients with tuberculosis than in contacts (12 *vs* 17 nmol/L), and undetectable 25(OH)D more common in patients with tuberculosis than in contacts (20/103=19% *vs* 1/42=2%)
Sasidharan et al, India, 2002[121]*	Cross-sectional study in patients with tuberculosis and controls	35 cases; 16 controls	Deficient <22.5 nmol/L	Yes	Substantially lower mean 25(OH)D in patients with tuberculosis than in controls (26·7 *vs* 48·7 nmol/L); 16/35 tuberculosis cases and 0/16 controls with 25(OH)D <22·5 nmol/L
Wejse et al, Guinea-Bissau, 2007[58]	Cross-sectional study in patients with tuberculosis and unmatched healthy controls	362 cases; 494 controls	Insufficient (51–75 nmol/L); moderately deficient (26–50); severely deficient (≤25 nmol/L)	Yes, but deficiency more common in controls	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=13·2% *vs* 31/362=8·6%)
Sita-Lumsden et al, UK, 2007[122]	Cross-sectional case-control study of children and adults; controls were matched for age, sex, and skin colour	178 cases; 130 controls	Deficient:<21 nmol/L	Yes	25(OH)D substantially lower in patients with tuberculosis than in healthy controls (20·1 *vs* 30·8 nmol/L); more patients with tuberculosis in the deficient category (64% *vs* 31%); seasonal variation in 25(OH)D in controls, but not in patients with tuberculosis
Gibney et al, Australia, 2008[34]	Retrospective	40 cases or past tuberculosis; 81 latent tuberculosis	Moderately to severely deficient <25 nmol/L	Yes	25(OH)D lower in patients with latent tuberculosis than in patients with no tuberculosis (37·3 *vs* 54·6 nmol/L); 25(OH)D lower in patients with current (17·1 nmol/L) or past (13·3 nmol/L) tuberculosis than in patients with latent tuberculosis (37·3 nmol/L)
Friis, Tanzania, 2008[123]	Cross-sectional study of patients with suspected tuberculosis	506 culture-positive tuberculosis; 129 culture-negative tuberculosis	Insufficient (50–75 nmol/L); mildly deficient (25–49); severely deficient (<25 nmol/L)	Marginally lower in culture-positive tuberculosis than in culture-negative tuberculosis	Mean 25(OH)D sufficient, but lower in patients with culture-positive tuberculosis than in patients with culture-negative tuberculosis (85·5 *vs* 92·9 nmol/L)

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	Design	Number of patients	25(OH)D cutoff	25(OH)D lower in active tuberculosis	Findings
(Continued from previous page)					
Tostman et al, Tanzania, 2010[124]*	Prospective study of 25(OH)D levels in patients with tuberculosis at diagnosis and 2-month follow-up	81 cases	Insufficient (50–75 nmol/L); deficient (<50 nmol/L)	No comparison group	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
Nielsen et al, Greenland, 2010[125]	Matched case-control study	72 cases; 72 controls	Insufficient (50–75 nmol/L); mildly deficient (25–49 nmol/L); severely deficient (<25 nmol/L)	Yes, but also in higher group	Both high (>140 nmol/L) and low (<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
Banda et al, Malawi, 2011[59]	Cross-sectional study of patients with tuberculosis (82% HIV-positive)	161 cases	Deficient <75 nmol/L	No comparison group	Deficient 25(OH)D in most patients (120/161=74·5%); 25(OH)D <25 nmol/L in 21 patients (13%)
Nansera et al, Uganda, 2011[126] *†	Cross-sectional study in patients with HIV, tuberculosis–HIV and controls (none with tuberculosis alone)	50 tuberculosis–HIV; 50 HIV; 50 controls	Deficient <30 nmol/L	No, but deficiency more common in tuberculosis–HIV than in controls	No difference in mean 25(OH)D between controls, HIV and patients with tuberculosis–HIV; more patients with tuberculosis–HIV had 25(OH)D <30 nmol/L (38% *vs* 20%) or <12 nmol/L (12% *vs* 0%) than controls
Talat et al, Pakistan, 2000[127]	Prospective study of household tuberculosis contacts	100 household contacts; 28 current or past tuberculosis	Insufficient (45–67 nmol/L); deficient (<45 nmol/L)	Yes	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=5·1
Martineau, UK, 2011[29] *†	Randomised controlled trial of vitamin D in tuberculosis including baseline measurement of 25(OH)D	126 cases	Profoundly deficient <20 nmol/L	No comparison group	Mean 25(OH)D in patients with tuberculosis at baseline about 21 nmol/L; majority of patients (75/126) <20 nmol/L at baseline, and almost all (122/126) <75 nmol/L
Martineau et al, South Africa, 2011[101]	Cross-sectional study of HIV-positive and HIV-negative adults with active or latent tuberculosis	192 cases; 178 latent tuberculosis	Deficient <50 nmol/L	Yes	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%)

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.

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

although it was contraindicated for pulmonary tuberculosis in some studies.[115] Notably, long-term rest and a nourishing diet were deemed to be as essential as sun exposure.

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.[31] In a study from Greenland,[125] 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,[125] although Vieth's hypothesis,[70] or harm from high 25-hydroxyvitamin D concentrations, might be important considerations too.

We identified only one prospective study that provides evidence for the importance of vitamin D deficiency as an antecedent to development of active tuberculosis.[127] 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[140] concluded that benefits were uncertain because the studies were of poor quality, often used vitamin D_2, 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.[143] 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 neccessary concentrations or in the appropriate cellular compartments. Lappe and Heaney[144] 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.[26,27,34]
- Vitamin D deficiency contributes to progression from latent to active tuberculosis.[127]
- 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.[124,129]
- 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,[131,132] and 1,25-dihydroxyvitamin D is raised in patients with tuberculosis compared with controls;[133] 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.[120]
- 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.[135,136]
- A factor common to both tuberculosis and low 25-hydroxyvitamin D—eg, vitamin D-binding protein might be lower at diagnosis of tuberculosis,[137] its function could be changed and affect vitamin D binding,[138] 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 mycobactericidal 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

	Design	Number of patients	Intervention	Primary outcomes	Findings and comments
Nursyam et al, Indonesia, 2006[141]	Placebo controlled (unclear whether randomised)	Intervention (34); placebo (33)	10 000 IU (0·25 mg/day) vitamin D (D2 or D3 not stated) orally for 6 weeks	Clearance of acid-fast bacilli from sputum and chest radiograph improvement at 6 weeks	Substantial benefit from vitamin D, but methodological problems noted (mode of tuberculosis diagnosis and randomisation and masking processes not described)
Wejse et al, Guinea-Bissau, 2009[129]	Randomised, double blind, placebo controlled	Intervention (187); placebo (180)	100 000 IU cholecalciferol by injection at 0, 5, and 8 months	Composite clinical score at 2-monthly time points and 12-month mortality	No substantial benefit from vitamin D, but the dose was low (similar serum 25-hydroxyvitamin D at 2 and 8 months in both study groups); non-significant reduction in 1-month smear positivity in the vitamin D group; 60% of planned sample recruited
Martineau et al, UK, 2011[29]	Randomised, double blind, placebo controlled	Intervention (62); placebo (64)	100 000 IU cholecalciferol orally at 0, 2, 4, and 6 weeks	Time to culture negativity	No substantial benefit from vitamin D overall, but TaqI vitamin D receptor genotype status affected response: time to culture conversion was much faster in patients with TaqI tt genotype randomised to vitamin D *vs* placebo; further analyses from this study, restricted to 95 patients fulfilling criteria for per-protocol analysis, found substantially faster sputum smear conversion and immunological impacts from supplemental vitamin D[142]

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

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.

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.

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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