

## Review Article

# Vitamin D in Early Childhood and the Effect on Immunity to *Mycobacterium tuberculosis*

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A potential role for vitamin D as a therapeutic immunomodulator in tuberculosis (TB) has been recognised for over 150 years, but has only recently returned to the centre of the research arena due to the increasing awareness of the global vitamin D deficiency epidemic. As early as birth a child is often deficient in vitamin D, which may not only affect their bone metabolism but also modulate their immune function, contributing to the increased susceptibility to many infections seen early in life. Recent studies have begun to explain the mechanisms by which vitamin D affects immunity. Antimicrobial peptides are induced in conjunction with stimulation of innate pattern recognition receptors enhancing immunity to particular infections. In contrast the role of vitamin D within the adaptive immune response appears to be more regulatory in function, perhaps as a mechanism to reduce unwanted inflammation. In this paper we focus on the effect of vitamin D on immunity to TB. Where much of the attention has been paid by past reviews to the role of vitamin D in adult TB patients, this paper, where possible, focuses on research in paediatric populations.

## 1. Introduction

Immune responses to *Mycobacterium tuberculosis* (MTB) are complex and remain incompletely understood. However, with recent advances in the field of immunology, we have learnt more about how MTB infects the human host and, in turn causes disease. There is increasing epidemiological evidence to support the role of vitamin D in the immune response to tuberculosis (TB) [1]. A recent meta-analysis included 7 studies with 531 participants and reported that low serum vitamin D levels were associated with a higher risk of active TB [1]. Additionally, an association of TB with season has been observed in many countries, including the UK where the incidence of TB is greater in the spring/summer months. The decreased vitamin D levels in the spring are thought to follow reduced sun exposure during winter months (the circulating form of vitamin D, 25-hydroxyvitamin D has an average half-life of 2–8 weeks [2–5]). Similar seasonality of TB [6] has been noted in Europe

[4, 7], South Africa [8, 9], and India [10]. Dietary factors also appear to influence vitamin D status and susceptibility to TB. In a study of Asian UK immigrants, the vegetarian diet, which is known to be low in vitamin D, was an independent risk factor for TB [11]. The mechanisms by which vitamin D may help to prevent or clear MTB infection and/or active TB are not completely clarified to date, but studies that have helped in the understanding of its role will be discussed in this paper.

## 2. Historical Context: The Road to Rediscovery

Cod-liver oil was traditionally used in the treatment of tuberculosis in the late nineteenth and early twentieth centuries [12]. The earliest case reports describing the effects of cod-liver oil in TB appeared in 1846 [13] and were followed subsequently by numerous cases that supported the notion that this dietary supplement could provide demonstrable improvements in the health of TB sufferers [14]. Later in

the nineteenth century, patients were frequently treated in sanatoriums, which were built in the countryside, and were designed to provide sufferers with therapeutic “fresh air” and notably, sunshine. Indeed the clinical use of sunlight exposure or “heliotherapy” gained significant momentum following the award of the Nobel prize for medicine in 1903 to Niels Ryberg Finsen: “*in recognition of his contribution to the treatment of diseases, especially lupus vulgaris (tuberculosis of the skin), with concentrated light radiation, whereby he has opened a new avenue for medical science*” [15].

It was not until later that vitamin D was discovered as the active ingredient in cod-liver oil [17]. Charpy, a physician from Dijon, France, appears to be one of the earliest individuals to effectively implement the clinical use of vitamin D<sub>2</sub> (calciferol) [18]. In 1945, he reported successfully using the formulation to treat 20 patients with lupus vulgaris. He obtained some “remarkable results” which were then reproduced by others in Europe and around the world [18–20]. By 1946 in London, Dr. Dowling and his colleagues had also used calciferol on a number of patients. They reported in the *Proceedings of the Royal Society of Medicine* that their experience could “*leave no room for doubt that calciferol in adequate dosage will cure a substantial proportion of cases of lupus*” [20].

The first reference to successful treatment of pulmonary TB with vitamin D appeared in the *Lancet* in 1947 [21]. The discovery by Alexander Fleming in 1928 of penicillin and its subsequent mass production and distribution by 1945 revolutionised medical treatment of infectious diseases, although not specifically TB, yet it appears that the benefits of vitamin D were somewhat overlooked in the wake of the antibiotic era [22].

### 3. Vitamin D Biochemistry

The term vitamin D encompasses a number of steroid-like proteins: vitamins D<sub>2</sub>–D<sub>7</sub>. Vitamins D<sub>2</sub> and D<sub>3</sub> have known physiological significance in humans, with both undergoing hydroxylation steps to become active hormones in calcium and phosphate metabolism [23]. Their chemical structure is based on 4 steroid rings; 1 of which is broken. Vitamins D<sub>2</sub> and D<sub>3</sub> differ only by the nature of their side chains [24]. The 2 forms of vitamin D can be obtained from the diet, but predominantly, vitamin D is obtained in the D<sub>3</sub> form, from the action of UV light on a vitamin D precursor in the skin [25].

Vitamin D<sub>3</sub> undergoes two hydroxylation steps before becoming an active hormone: the first step occurs in the liver and results in the production of 25-hydroxyvitamin D (25[OH]D) [26]. The “25” of 25-hydroxyvitamin D refers to the location of a hydroxyl group on one of the side arms of the steroid rings. This form of vitamin D must undergo a further hydroxylation step to become physiologically active in the form of 1- $\alpha$ ,25-hydroxyvitamin D (1 $\alpha$ ,25[OH]2D). In the past, it had erroneously been assumed that this second hydroxylation step was only performed by the kidneys; however, it is now clear that a number of cells, particularly innate immune cells such as monocytes and macrophages, possess the machinery required to produce active 1 $\alpha$ ,25[OH]2D.

Indeed, since the discovery of vitamin D receptors (VDRs) in macrophages, the role of 1 $\alpha$ ,25[OH]2D as an immune modulator has become increasingly apparent [27].

In children, and neonates in particular, a C3-epimer of the 25[OH]D molecule, 3-epi-25[OH]D<sub>3</sub>, often constitutes a significant proportion of the total circulating 25[OH]D [28, 29]. It is therefore important, although not universal practice, to identify the proportion of the 3-epi-25[OH]D<sub>3</sub> when measuring 25[OH]D levels in children. The only method to reliably do this is the liquid chromatography-tandem mass spectroscopy method (LC-MS/MS) [28] enabling the epimer measurement to be removed from the final result if required. The RIA (radioimmune assay) method does not react with the epimer and the high performance liquid chromatography (HPLC) method cross-reacts with the epimer and causes interference without being able to discriminate between the isoforms making these methods unreliable when measuring vitamin D levels in infants [28]. The 3-epi-25[OH]D<sub>3</sub> differs from 25[OH]D by the asymmetrical arrangement of a hydroxyl group at the C3 position [28]. It is thought that this epimer may be the result of immature vitamin D metabolism and may display reduced efficacy in calcium-mediated bone metabolism [30]. Interestingly, the 3-epi-25[OH]D<sub>3</sub> form of vitamin D has a lower binding affinity for the VDR. However, this does not necessarily translate into reduced biological effects [31–33]. The overall impact of the 3-epi-25[OH]D<sub>3</sub> on immune health in infancy remains unknown. However, we can speculate that supplementation may be less effective in the infant cohort, because when the vitamin D supplement (cholecalciferol or ergocalciferol) undergoes the first hydroxylation step, the infant produces a large proportion of a physiologically less effective epimer of vitamin D, 3-epi-25[OH]D<sub>3</sub>. This may help to explain why a recent large-scale vitamin D supplementation trial in preterm neonates reported no significant effect on overall morbidity and mortality [34].

### 4. What Constitutes Vitamin D Sufficiency?

There is no agreed consensus on the optimal level for vitamin D status in the adult [26, 35–38] and particularly what constitutes vitamin D sufficiency in childhood. In the UK there are no up-to-date guidelines to define deficiency and insufficiency [36]. It is true that with 25[OH]D levels below <25 nmol/L children manifest clinical signs and symptoms of rickets [37]. However, children can be diagnosed with clinical rickets at higher 25[OH]D levels. Levels of 25[OH]D above the 25 nmol/L cut-off may be associated with other poor health outcomes [39], such as upper respiratory tract infections [40] and bronchiolitis [41]. A recent paper in the *British Medical Journal* summarises current opinion regarding adult vitamin D endocrine levels (Table 1) [37]. However, as mentioned previously the situation is further complicated in infancy by the presence of the C3-epimer of 25[OH]D which can contribute to erroneously high total 25[OH]D levels [28].

TABLE 1: Serum 25-hydroxyvitamin D (25[OH]D) concentrations, health, and disease (modified from [37]).

25[OH]D concentration	Vitamin D status	Manifestation	Management
<25 nmol/L	Deficient	Rickets, Osteomalacia	Treat with high-dose calciferol
25–50 nmol/L	Insufficient	Associated with disease risk	Vitamin D supplementation
50–75 nmol/L	Adequate	Healthy	Lifestyle advice
>75 nmol/L	Optimal	Healthy	None

With increasing knowledge of the endocrine functions of vitamin D and more recent evidence of possible autocrine/paracrine functions, it is now important to also consider the concentration of vitamin D required to drive an appropriate immune response. At present this is not known but *in vitro* experiments suggest that at 98 nmol/L concentration IFN $\gamma$  can induce antimicrobial expression and can reduce growth of *Mycobacterium tuberculosis* whereas levels of 45 nmol/L cannot [42].

Physiological ranges for circulating 25[OH]D<sub>3</sub> can extend to beyond 200 nmol/L which is much greater than the considered average “norm” for a population. As a perspective, concentrations of 25[OH]D in nonhuman primates have a median value of 170 nmol/L and a lowest value >80 nmol/L [38], whereas modern humans in winter have a median value of 40 nmol/L and a maximum value of 70 nmol/L [38], a similar amount to that of rodents. In addition, a recent study of Masasai and Hadzabe hunter-gatherer traditional populations in Tanzania showed that the mean serum levels of 25[OH]D were 119 and 109 nmol/L, respectively, and none were below 50 nmol/L. These higher levels, only seen in Caucasian lifeguard populations that were exposed to more than 3 hours of sun per day for more than 5 day/week for at least 3 months, may serve as targets for further research [43].

With this lack of agreement on what levels of 25[OH]D constitute sufficiency, in turn there is variability in recommendations for supplementation. However a US study of newborns found that 78% had levels of 25[OH]D < 75 nmol/L and 17% had levels < 30 nmol/L suggesting a need for supplementation from birth [44]. The US Endocrine Society proposes that infants and children aged 0-1 year require at least 400 IU per day of vitamin D and that children 1 year and older require at least 600 IU per day to maximize bone health [5]. A recent study showed that in pregnant women supplementation of 4000 IU/d was safe and most effective in achieving sufficiency in women and their neonates, whereas the current estimated average requirement (200–400 IU/d) is comparatively ineffective at achieving adequate circulating 25[OH]D concentrations [45]. In the UK vitamin D supplementation for all mothers of breastfed infants is recommended and in infants greater than 6 months who are taking less than 500 mL of formula milk per day [46].

US guidelines recommend that supplementation directly to the breast fed (or partially breast fed) infant should commence in the first few days of life [25]. Partly because of the lack of agreement between health professionals, but also for a plethora of other reasons, compliance to these supplementation recommendations across countries remains poor.

## 5. Vitamin D as an Immunomodulator

The role of 1 $\alpha$ ,25[OH]2D in calcium and phosphate metabolism and bone health has been long established, but its immunomodulatory function remains poorly defined. In addition to TB as mentioned earlier, there is growing evidence that lower 25[OH]D levels are associated with a higher incidence of other infections, particularly of the respiratory tract [40, 52–55]. However, there remains a large degree of uncertainty in this area; for example, some studies find that vitamin D deficiency is associated with worse severity of infection in childhood [53, 56, 57], whilst others do not [58]. To help understand these discrepancies, the role of vitamin D in immunity, particularly with regards to TB, is further discussed.

There is significant biological plausibility for a clinical association between low 25[OH]D levels and infection with many studies describing the direct effect of 1 $\alpha$ ,25[OH]2D on innate immunity [16, 59, 60]. Many immune cells express the VDR, including T and B cells [61], dendritic cells [62], as well as macrophages [63]. Initial studies found that 1 $\alpha$ ,25[OH]2D stimulates antimicrobial activity [57, 58], but it is only recently that the possible mechanism has been described. Ligation of the innate immune pattern recognition receptors, Toll-like receptors (TLRs) on human macrophages, causes upregulation of the intracellular VDR and vitamin D<sub>1</sub> hydroxylase genes, resulting in induction of cathelicidin [64] and/or  $\beta$  defensin [65], both of which are potent antimicrobial peptides (Figure 1). It appears that this action of 1 $\alpha$ ,25[OH]2D may be dependent on the presence of interferon gamma (IFN $\gamma$ ) [42, 66] suggesting a link with adaptive immunity.

In 1986 Rook et al. found that incubation of monocytes with 1 $\alpha$ ,25[OH]2D inhibited growth of *MTB* [67]. Although this appeared to be independent of IFN $\gamma$ , the addition of IFN $\gamma$  along with 1 $\alpha$ ,25[OH]2D resulted in a synergistic effect on mycobacterial growth inhibition [67]. In addition Denis showed that in the presence of IFN $\gamma$  and TNF $\alpha$ , 1 $\alpha$ ,25[OH]2D promoted increased intramonocyte killing of *MTB* [68]. These effects are thought to be exerted through the release of antimicrobial peptides as described previously [65, 69]. Indeed, the more recent paper by Liu et al. in 2006 shows that monocyte TLR ligation promotes conversion of 25[OH]D to 1 $\alpha$ ,25[OH]2D and subsequent cathelicidin release. Cathelicidin induces fusion of the phagolysosome, which is essential for the containment, degradation and subsequent killing of *MTB* [64] (Figure 1).

It seems that 1 $\alpha$ ,25[OH]2D also exerts its effects on innate immune responses by the promotion of autophagy [70–72] and the suppression of tissue remodelling and lung matrix breakdown [73]. Autophagy is a potent mechanism

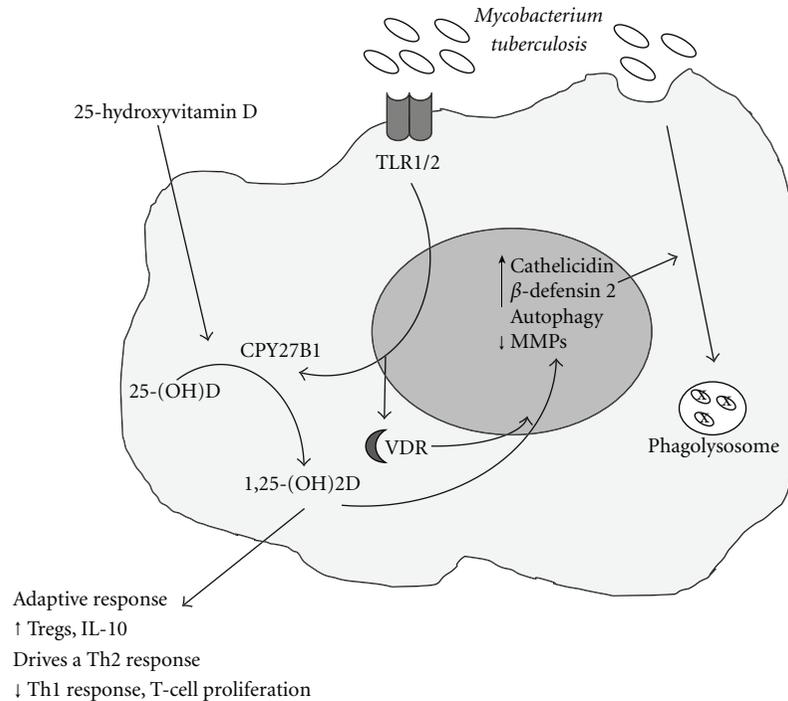


FIGURE 1: Mechanism of vitamin-D-induced immunity to *Mycobacterium tuberculosis* (modified from [16]). Stimulation of monocyte Toll-like receptors (TLR1/2) by *Mycobacterium tuberculosis* (MTB) results in transcriptional induction of the vitamin D receptor (VDR) and 1 $\alpha$ -hydroxylase (CYP27B1). Circulating 25-hydroxyvitamin D (25[OH]D) enters the cell and is converted to 1,25-dihydroxyvitamin D (1,25[OH]2D) by the CYP27B1 enzyme. VDR-bound 1,25(OH)2D then induces expression of cathelicidin and  $\beta$ -defensin 2 (DEFB4). In addition 1,25(OH)2D induces autophagy and downregulating metalloproteinases (MMPs), all of which help in the formation of phagolysosomes and the killing of Mtb. 1,25(OH)2D also affects the adaptive immune system and leads to an upregulation of regulatory responses and a skewing towards a Th2 response. IFN $\gamma$  is thought to induce the expression of the CYP27B1 enzyme suggesting a feedback mechanism between the innate and adaptive response to vitamin D.

by which the host defends against mycobacterial infection, by degradation of a cells' own components through the lysosomal machinery [74]. Fabri and Modlin have shown that monocytes cultured in vitamin D sufficient sera and stimulated with IFN $\gamma$  display autophagy as well as secrete antimicrobial properties against *MTB* [72]. It has been shown *in vitro* that 1 $\alpha$ ,25[OH]2D downregulates matrix metalloproteinases (MMPs) and upregulates tissue inhibitor of metalloproteinase 1 (TIMP1) in peripheral blood mononuclear cells (PMBCs) in the presence of live *MTB* [73]. *MTB* induces significant pathological effects through tissue remodelling and breakdown of extracellular matrix in the lung, and therefore it is possible that 1 $\alpha$ ,25[OH]2D may protect the host against this effects.

It appears that downstream adaptive immunity can also be modified by 1 $\alpha$ ,25[OH]2D [27, 75, 76], which is evidenced by its effects on human B-cell differentiation [77] and antigen presentation [78]. Interestingly 1 $\alpha$ ,25[OH]2D has antiproliferative effects on CD4+ T cells [79] and appears to inhibit Th1 cytokine production [80–83], whilst promoting T regulatory function [84], and potentially upregulating Th2 cytokine production [85]. Culturing peripheral blood mononuclear cells from TB patients in the presence of 1 $\alpha$ ,25[OH]2D, Vidyarani et al. recently showed that 1 $\alpha$ ,25[OH]2D suppressed IL-12p40 and IFN $\gamma$  production

in response to *MTB* antigens [83]. However, for *MTB* to be maintained in a latent state, Th1-cytokine-driven granuloma formation is actually required, and inhibition of Th1 cytokines could therefore be detrimental to the host. Indeed, a recent study using *in vitro* analysis of sera of patients with pulmonary TB has shown that coculture of T cells with 1 $\alpha$ ,25[OH]2D reduces the number of Th1 cytokine expressing cells (specifically IFN $\gamma$  and TNF $\alpha$ ) [86]. The authors propose that 1 $\alpha$ ,25[OH]2D “may play a dual role in the immunity against tuberculosis by eliminating infection as well as reducing inflammation at the site of infection”.

The effects of vitamin D on Th2 cytokine responses are less well understood, and certainly how changes in the Th2 cytokine profile may affect *MTB* infection in humans has not been extensively studied. We do know that the classical Th2 cytokines, IL-4, and IL-13 are potent inhibitors of autophagy, which is an essential immune pathway in the host defence against *MTB* infection although in contrast autophagy has been shown to be induced by vitamin D through the innate response as mentioned earlier [64–66]. Animal studies suggest that production of IL-4, is upregulated in the presence of 1 $\alpha$ ,25[OH]2D [85]. Indeed, Boonstra et al. [87] have demonstrated that 1 $\alpha$ ,25[OH]2D induces Th2 cell development and IL-4, IL-5, and IL-10 production *in vitro*. However, it is prudent to say that

the effects of vitamin D on Th2 cytokine production remain unclear [88], and how changes to the Th2 cytokine profile may affect the pathophysiology of *MTB* infection needs further elucidation.

## 6. Vitamin D and Infant Immunity to TB

In children, infections remain a major cause of morbidity and mortality around the world [89, 90]. Many epidemiological studies have shown that vitamin D is associated with respiratory disease and viral infections including HIV [91]. There is very little literature describing the role of vitamin D in immunity to TB in infants one such paper examined vitamin D status in children with active TB and found that 86% were vitamin D deficient ( $25[\text{OH}]\text{D} < 20 \text{ nmol/L}$ ) or insufficient ( $25[\text{OH}]\text{D} < 75 \text{ nmol/L}$ ) [92].

The predominant cytokine essential for mycobacterial immunity is IFN $\gamma$  as shown in studies of patients who lack the IFN $\gamma$  and IL-12 receptor that leads to a predisposition to mycobacterial infections [93]. The dual role of IFN $\gamma$  in enhancing the effects of vitamin D *in vitro*, although, being in itself reduced by addition of vitamin D, may be quite different when considering a neonate. In neonates NK production of IFN $\gamma$  has a dominant role to play in response to *MTB* antigens rather than T cells as observed in older infants and adults [94]. There are many other distinct qualities of the immature immune response, in particular reduced Th1 adaptive responses and attenuated innate immunity [95, 96] which suggests that vitamin D may have different effects in the immune system of infants than that of adults. *In vitro* supplementation of  $25[\text{OH}]\text{D}$  in cord blood cultures showed increased TLR-induced cathelicidin expression suggesting that supplementation in neonates may improve antimicrobial activity [44]. It is known that infants and young children are particularly susceptible to severe TB [97, 98] and therefore the role of vitamin D in childhood TB warrants further investigation.

Trying to understand the varying effects of BCG worldwide one study has looked at the association of vitamin D with BCG vaccination. Lalor's recent observational study of UK infants found that those that were BCG vaccinated had higher vitamin D levels at 3 and 9 months of age, compared to unvaccinated controls [99]. Interestingly infants with higher vitamin D levels had lower IFN $\gamma$  responses to the *Mycobacterium-tuberculosis*-purified protein derivative (*MTB* PPD). As previously discussed, *in vitro*,  $1\alpha,25[\text{OH}]\text{D}$  has been shown to have dual effects by, on the one hand, dampening Th1 responses [81], yet on the other stimulating antibacterial peptide secretion to aid clearance of *MTB* [64]. Traditionally IFN $\gamma$  response to vaccination is used as an indicator of the effectiveness of the vaccine. However, Lalor suggests that in the context of BCG vaccination, a dampened IFN $\gamma$  response may be beneficial to the host, in preventing unnecessary inflammation in response to the *Mtb* PPD which needs to remain present to provide protection against TB [99]. A recent animal study supports this finding

with reduced IFN $\gamma$  and IL-17F gene expression in PPD-stimulated blood of BCG vaccinated cattle after addition of vitamin D [100].

## 7. It Is in the Genes

Genetic factors clearly play a role: specific vitamin D receptor (VDR) polymorphisms are associated with a higher risk of TB [101]. The association between TB incidence and other polymorphisms varies widely across different ethnic groups. For example, the *FokI ff* genotype of the VDR appears to be most consistently associated with increased susceptibility to TB among Asians, but not Africans [102], whereas the *tt* genotype of *TaqI* has been shown in Gambian men to be associated with a higher risk of TB [103]. No studies have looked at VDR polymorphisms and incidence of TB in children, but a recent Canadian study reported an association between the *FokI ff* genotype of the VDR and acute lower respiratory tract infection in young children [104].

## 8. Vitamin D Supplementation and TB Treatment

There are disparate reports in the literature regarding a role of vitamin D supplementation in the treatment of TB infection (Table 2). Early studies reported a favourable response [47, 51] to vitamin D supplementation. In Jakarta in 2006, Nursyam et al. randomised 67 patients and found that 100% of the vitamin D group versus only 76.7% of the placebo group had sputum conversion at 12 weeks ( $P = 0.002$ ) [47]. However, Wejse et al. found no effect of supplementation on disease outcome [49]. The study addressed the use of 100,000 IU of vitamin D given as an adjunctive treatment at the time of commencement of anti-TB therapy, and again at 5 and 8 months after starting treatment. The authors note that the dose may have been insufficient, and the response to vitamin D dependent on the immune status of the individual patient [49].

A recent large-scale trial of vitamin D supplementation in the treatment of adult TB was carried out by Martineau and colleagues in London, UK [50]. Patients who were receiving standard anti-TB chemotherapy and had been supplemented with vitamin D displayed sputum clearance almost 1 week earlier than those taking placebo (from 43.5 to 36.0 days). However the difference between the intervention and control group did not reach statistical significance. Intriguingly, in a subset of patients with the *TaqI* VDR polymorphism, time to sputum conversion was significantly quicker than that in controls, indicating that these patients would benefit from supplementation.

Prevention of TB with vitamin D supplementation is still debated [13]. In one study TB contacts were given a single dose of 100,000 IU vitamin D. *In vitro* analysis revealed those receiving the supplement had enhanced immunity to mycobacteria, demonstrated through ability of participants' whole blood to restrict luminescence in the BCG-lux assay [95]. These promising laboratory results need to be addressed in a Randomised Control Trial (RCT) to fully

TABLE 2: Vitamin D single dose (dosage concentration not reported).

Subjects ( <i>n</i> )	Country	Vitamin D supplement	Findings	Reference
<i>Adult studies</i>				
TB patients with pulmonary TB aged 15–59 (67)	Jakarta, Indonesia	0.25 mg per day for 6 weeks	100% of vitamin D group had sputum conversion at 12 weeks after supplementation versus 76.7% of the placebo group	[47]
TB contacts (192)	London, UK	2.5 mg single dose vitamin D <sub>2</sub>	Those given the vitamin D had enhanced immunity to TB using the lux <i>in vitro</i> assays but did not affect IFN $\gamma$ production after ESAT-6/CFP-10 stimulation	[48]
TB patients with pulmonary TB (367, 136 completed trial in vitamin-D-supplemented group, 145 completed in placebo group)	Bissau, Guinea Bissau	100,000 IU of vitamin D given at time of anti-TB treatment, then at 5 and 8 months later	No differences in clinical severity between groups and no differences in mortality 12 months later	[49]
TB (146)	London, UK	2.5 mg vitamin D <sub>2</sub> given at time of Tb treatment plus 14, 28 and 42 days later	Those on Vitamin D supplementation displayed sputum clearance at 36 days after treatment versus 43.5 days but this was not statistically significant but it did significantly hasten sputum culture conversion in participants with the <i>tt</i> genotype of the <i>TaqI</i> vitamin D receptor polymorphism	[50]
<i>Paediatric studies</i>				
Children aged between 1.5 and 13 years of age with TB (24), 13 extra thoracic, 7 intrathoracic, and 4 mixed	Egypt	Vitamin D single dose unable to obtain information regarding ( <i>dosage concentration not reported</i> )	8 weeks after supplementation greater clinical improvement was observed in vitamin-D-supplemented group	[51]

NB: 25 mg = 1,000 IU.

support these results. However, there is evidence that vitamin D supplementation can have beneficial effects on immune health in general, and particularly in childhood. A RCT in school children 6–18 years of age showed that oral vitamin D supplementation reduced the incidence of influenza A [92] and a further trial found promise in the use of vitamin D supplementation in the prevention of recurrent pneumonia in children between the ages of 1–36 months [105]. However no specific recommendations exist for the use of vitamin D supplementation to improve immune health outcomes in childhood.

## 9. Future Directions

Recent reports suggest that vitamin D deficiency has become a global health epidemic with 20–100% of US, Canadian, and European elderly men and women being vitamin D deficient [106]. Children and younger adults are equally at risk with vitamin D deficiency common in Australia, the Middle East, India, Africa, and South America [106]. Historically humans probably obtained their vitamin D requirement from prolonged sunlight exposure as “hunters and gatherers”. However, current lifestyle practices, particularly in the developed world, often dictate infrequent sun exposure and a subsequent propensity to deficiency, which is confounded by diets low in vitamin D.

With the expanding evidence that vitamin D not only affects bone metabolism but also may play a large role in immune modulation, these global statistics are worrying. Even neonates at birth are often deficient in vitamin D and if vitamin D can affect immunity to many infectious diseases including TB, then further research in this area is required.

A number of RCTs are currently under way to assess the role of vitamin D in the treatment of tuberculosis and the results are eagerly awaited [107–113]. Of particular interest for child health professionals is the ongoing study in California to determine whether a single oral dose of vitamin D given to infants prior to BCG vaccination will enhance the immune response to BCG vaccination [114].

This paper draws attention to the lack of quality research exploring the role of vitamin D in TB or other infections in early childhood. As we now begin to understand the differences in immune function between adults and children more must be done to address this cohort with respect to vitamin D deficiency and potential benefits of vitamin D supplementation.

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