# Accepted Manuscript

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Please cite this article as: Sutaria N, Liu C-T, Chen TC, Vitamin D Status, Receptor Gene Polymorphisms, and Supplementation on Tuberculosis: A Systematic Review of Case-Control Studies and Randomized Controlled Trials, *Journal of Clinical & Translational Endocrinology* (2014), doi: 10.1016/j.jcte.2014.08.001.

# Vitamin D Status, Receptor Gene Polymorphisms, and Supplementation on Tuberculosis: A Systematic Review of Case-Control Studies and Randomized Controlled Trials

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Key Words: Vitamin D, Vitamin D Receptor, Polymorphism, Tuberculosis, Supplementation, Clinical Trials

Abbreviations: TB, Tuberculosis; 25(OH)D, 25-hydroxyvitamin D; $1\alpha,25(OH)_2D$, $1\alpha,25$-dihydroxyvitamin D; VDR, vitamin D receptor; WT homo, wild-type homozygous; HT, heterozygous; VR, variant recessive; TST, Tuberculin Skin Test

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Conflicts of Interest: None declared.

Highlights:

- Lower serum 25-hydroxyvitamin D increases susceptibility to tuberculosis.

- BsmI and FokI VDR gene polymorphisms may confer increased risk of acquiring tuberculosis.

- Vitamin D supplementation reduces tuberculosis incidence and increases recovery from tuberculosis.

## Abstract

**Objective:** To investigate the impacts of vitamin D status, supplementation and vitamin D receptor (VDR) gene polymorphisms on tuberculosis (TB).

**Methods:** We conducted a systematic review of published studies pertaining to case-control and randomized-control trials from 2002 to 2014 using the PubMed database.

**Results and conclusion:** Individuals with TB have lower vitamin D status than healthy individuals. Some VDR gene polymorphisms are associated with increased susceptibility to TB while others may not. Supplementation with vitamin D leads to improved clinical outcomes. However, further studies with a larger patient population and different ethnicities are needed to confirm these effects.

## Introduction

Tuberculosis (TB) remains a great burden throughout the world to this date. According to the latest World Health Organization data, an estimated 8.6 million individuals developed TB and 1.3 million died as a result of this disease in 2012. The majority of these cases occurred in South-East Asia (29%), Africa (27%), and the Western Pacific (17%). India and China accounted for 26% and 12% of the total cases, respectively. Furthermore, an estimated 1.1 million of the 8.6 million individuals who developed TB were also HIV-positive [1].

Vitamin D has been known to play an important role in bone health for almost a century [2]. However, the extra-skeletal roles of vitamin D have only received attention during the past two decades, including its role in human innate immunity [3]. This particular role of vitamin D is especially important in the body's defense against tuberculosis through its action on enhancing macrophage-mediated eradication of *M. tuberculosis* [4]. Vitamin D, either endogenously produced (vitamin $D_3$) or ingested (vitamin $D_2$ or vitamin $D_3$), must be activated in order to produce its effects (Figure 1) [5]. Upon entering the bloodstream, vitamin D is delivered to the liver, where it undergoes the first hydroxylation to become 25-hydroxyvitamin D (25(OH)D), the main circulating form of vitamin D. From the liver, the 25(OH)D travels to the kidney, where it undergoes

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another hydroxylation to become $1\alpha,25$-dihydroxyvitamin D ($1\alpha,25(OH)_2D$), which is the active form. Upon undergoing the second hydroxylation, vitamin D is now able to regulate transcription of genes throughout the body. However, new evidence has been accumulated indicating that $25(OH)D$ and $1\alpha,25(OH)_2D$ can be synthesized in tissues other than the liver and kidneys, respectively [5-7].

It has been shown that vitamin D deficiency [8] (serum $25(OH)D$ level <20 ng/mL or <50 nmol/L) and insufficiency [8] (serum $25(OH)D$ level <30 ng/mL or <75 nmol/L) are associated with a higher risk of active TB [9], suggesting that low serum $25(OH)D$ levels may also lead to prolonged clinical course of the disease if not corrected. Therefore, it is appropriate to surmise that individuals with higher levels of circulating $25(OH)D$ are associated with better outcomes with regard to TB, as reported in a previously conducted systematic review and meta-analysis of seven observational studies [10].

Along those lines, it can be assumed that increasing vitamin D intake would help protect against TB, but in reality this is not always the case. Vitamin D mediates its effect on the innate immune system via the vitamin D receptor (VDR) [Figure 2]. Upon binding to $1\alpha,25(OH)_2D$, the active form of vitamin D, or its analogs, the VDR complex moves into the nucleus where it regulates expression of gene products. Among its effects includes increased synthesis of components of the innate immune system, such as cathelicidin, which plays an important role against mycobacterial infections, as well as other antibacterial, antimycobacterial, and antiviral molecules [3,4]. Autophagy, the digestion of intracellular macromolecules and inclusions, is another important cellular function promoted by the activated VDR [3,4]. This is a particularly important function in clearing of intracellular pathogens, such as mycobacteria, as well as neoplastic cells. The activated VDR also plays a role in regulating the adaptive immune system by inhibiting lymphocyte proliferation and reducing production of pro-inflammatory cytokines to prevent excessive responses [11]. It has been previously shown that VDR gene polymorphisms exist [12], with some polymorphisms mediating stronger downstream effects than others. As a result, vitamin D's effect is dependent on the genotype of these

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receptors. As shown in several previous studies, certain VDR polymorphisms confer increased resistance to TB, while others make their hosts more susceptible [13,14].

Since TB has long been a burden throughout the world, many different treatments have been formulated and tested with varying results, including vitamin D. Several lines of evidence have led to the use of this seco-steroid. Way back in 1848, physicians at the Royal Brompton Hospital in London reported patients with consumption, an earlier term for TB, had a higher disease arrest (18% vs 5%) and survival (81% vs 67%) as compared to the standard regimen when cod liver oil was added to the standard therapy [15]. This finding clearly suggested that vitamin D could be the anti-TB principle as it was well-known that cod liver oil contained high concentrations of vitamin D, and was the source where vitamin D was discovered and named by McCollum EV in 1922 [16]. Further implication of the usefulness of vitamin D in treating TB came from the studies using heliotherapy during the period between 1928-1929 by Howson CR [17], Mekie EC [18], and Rollier A [19], and more recently by Hobday RA [20], Sabbatani S [21], Willis MD et al. [22], and Ralph AP et al. [23]. The connection of sunlight exposure, vitamin D and chronical diseases has been extensively discussed [24]. After the discovery of vitamin D and pure ergocalciferol (vitamin $D_2$) was available, a case report from 1947 notes its oral use in the successful treatment of a woman with cutaneous tuberculosis [25]. This highlights the fact that the anti-mycobacterial effects of vitamin D have been known for quite some time.  More recently, clinical trials have been conducted to explore the efficacy of vitamin D supplementation in individuals with TB.  Given the data collected from previous studies, one would expect that supplementation with vitamin D would lead to faster eradication of the disease.  A recent systematic review of randomized-controlled trials [26] showed mixed results with regard to vitamin D supplementation, urging for more data to be collected before any definitive conclusion could be reasonably made.

The purpose of this systematic review is to analyze newer data from the past ten years in order to gain insights into the following questions:
1) Do serum 25(OH)D levels impact the risk of TB?

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2) Do different VDR polymorphisms confer distinct degrees of protection or susceptibility to TB?

3) Does supplementation with vitamin D in patients with active TB result in improved outcomes?

## I. Discussion of Search and Analysis Methods

First, we conducted a systematic review of published studies that investigated the relationship between 25(OH)D serum levels and TB susceptibility, VDR polymorphisms and TB susceptibility, and vitamin D supplementation in the treatment of TB. We reviewed the medical literature in the PubMed online database from 2002 through 2014. We used a combination of the following search terms: "vitamin D, vitamin $D_2$, vitamin $D_3$, vitamin D analog, ergocalciferol, cholecalciferol, tuberculosis, respiratory illness, bacteria" to find studies pertaining to vitamin D status and vitamin D supplementation. For finding studies relating to VDR polymorphisms, the search terms "vitamin D receptor, polymorphism, tuberculosis, respiratory illness, bacteria" were used. The database was restricted to the articles published after 2002. The resulting abstracts were screened and cross-referenced to identify additional publications pertaining to the subject. Studies that were written in non-English languages were excluded.

## II. Vitamin D Levels and Tuberculosis

### A. Results

A total of three hundred and fifty-one articles were identified based on the initial PubMed search criteria. No additional articles were identified from any other databases. Studies were included if they were investigating 25(OH)D serum levels in a case-control fashion. Exclusion criteria included any studies without a control group or other form of comparison group and if subjects were previously treated for TB. A total of seven studies [27-33] met the inclusion and exclusion criteria (**Table 1**). A total of 6,553 individuals participated in the seven studies combined. All studies were published between 2007 and 2014, and all of them compared 25(OH)D levels in tuberculosis patients and controls.

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Four of the studies were based on indigenous Asian populations and two studies centered on indigenous African populations. The last study focused on a predominantly white population in Greenland, but also included a sizeable portion of the native Inuit population.

All studies investigated untreated TB patients who were to be initiated on anti-tuberculosis therapy. Six of the seven studies [27,28,30-33] recruited the case population either as inpatients or outpatients. Diagnosis was made predominantly by a positive sputum acid-fast bacilli culture in all of the studies. The remaining one study [29] was a cross-sectional study that selected only a control group, and then compared this group to TB patients from another study conducted in the same geographic location.
The control group selection procedures varied between the different studies. Talat et al. utilized household contacts of their TB patients [28]. Hong et al. selected healthy, matched controls based on a Korean nation-wide health survey [27]. Similarly, Mastala et al. selected control group from a random pool of participants who had completed a health questionnaire [29]. Ho-pham et al. recruited healthy controls from a previous study looking at vitamin D status in the same geographic location [31]. Nielsen et al. [32], Kim et al. [30], and Wejse et al. [33] all selected healthy controls from the same hospital/health center used to identify their TB patients.

Case subjects were matched to controls in six of the seven studies [27-32]. Information regarding potential confounders such as nutritional status and co-morbidities was provided across all seven studies. Blood samples were collected during same period for all subjects across all of the studies. Serum 25(OH)D levels were measured by radioimmunoassay [27,31,32], tandem mass spectrometry [30,33], or ELISA assay [28,29].

Three studies [28-30] reported mean serum 25(OH)D levels with corresponding standard deviation. Another study [27] reported median values with a corresponding range. The remaining three studies [31-33] investigated the prevalence of vitamin D insufficiency using the same cutoff value of 25(OH)D level <75nmol/L (<30ng/mL). One of the studies [31] looked at the differences in the prevalence of vitamin D insufficiency in men

versus women. One study [28] also investigated progression to fulminant TB in otherwise healthy household contacts of TB patients.

All seven studies demonstrated that patients with TB had a lower mean/median serum 25(OH)D level compared to the healthy controls. Of the three studies looking at the prevalence of vitamin D insufficiency, two studies [31,32] found that prevalence was higher in TB patients than healthy controls, while the third [32] found no difference. Interestingly, Ho-pham et al. [31] found that the prevalence of vitamin D insufficiency was higher in men with TB compared to healthy men, but determined that women with and without TB had a similar prevalence of vitamin D insufficiency. Talat et al. [28] illustrated that otherwise healthy contacts of TB patients had an 8% rate of development of TB, and found that the majority of this 8% had very low serum 25(OH)D levels.

## B. Discussion

Overall, the data analyzed finds that individuals with TB have lower serum levels of 25(OH)D than healthy, age-matched, sex-matched controls. Since anti-tuberculosis therapy may inadvertently cause a decline in 25(OH)D levels, we chose only studies that investigated untreated individuals.

The similar prevalence of vitamin D insufficiency between TB patients and healthy controls in the study by Wejse et al. may be due in part to the fact that the chosen controls were unmatched [33]. Therefore, it is possible that the control group was not representative of a healthy population without comorbidities. Additionally, per the authors' report, dietary intake of the subjects was not adequately monitored during the entirety of the trial, thus introducing another potential source of inaccuracy.

The finding by Ho-pham et al. [31] of the vitamin D insufficiency discrepancy between men and women with and without TB is notable. They state that the underlying etiology of this difference is unclear. Potential explanations include the fact that men have a higher risk of acquiring TB than women, due to socioeconomic and health care disparities between the two genders. Additionally, there is evidence showing that estrogen may

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possibly have protective effects against TB [34]. Furthermore, the prevalence of smoking in the male subjects with TB was much higher than in the male control group. No significant difference was found in the female subject groups with regard to smoking status. Smoking itself is a risk factor for TB, and smokers are more likely to have a lower vitamin D intake than non-smokers [35]. This could potentially account for the difference seen between both genders in this particular study.

While a relationship between TB and serum 25(OH)D levels has been established by these studies, it is still inconclusive whether low vitamin D status (low serum 25(OH)D level) increases the risk of acquiring TB, or whether TB patients have low vitamin D as a direct result of the infection. Given the thoroughly elucidated involvement of vitamin D in the innate immunity pathway, it is more likely that the former is true. However, the majority of these studies did not control for diet and/or sunlight exposure. Therefore, it is difficult to rule out whether the low serum vitamin D levels existed prior to infection with TB in these patients, or whether having TB led to poor nutritional intake and more time spent recuperating indoors, which in turn led to the low serum 25(OH)D levels (the reverse-causality effect).

Given the implication of vitamin D deficiency/insufficiency and risk for TB, many researchers have begun to investigate the effect of supplementation with vitamin D in tuberculosis patients. The addition of vitamin D supplements to the standard anti-tuberculosis drug regimen should theoretically lead to faster resolution of the disease. However, more information must be obtained before a conclusion can be made. Additionally, it would be beneficial to investigate the effect that vitamin D supplementation has on incidence of tuberculosis, particularly in high-risk areas around the world.

## III. Vitamin D Receptor Gene Polymorphisms and Tuberculosis

### A. Results
A total of sixty-five articles studying VDR single nucleotide polymorphisms (SNPs) in tuberculosis patients were identified based on the initial PubMed search criteria. No

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additional articles were identified from any other databases.  Studies were included in the analysis if they investigated VDR polymorphisms with regard to TB in case-control fashion.  Studies were excluded if they did not investigate the ApaI, BsmI, FokI, and TaqI polymorphisms. A total of seven studies, described in **Table 2**, met the inclusion and exclusion criteria [36-42].  A sum of 2,739 individuals participated in the seven studies combined.  All studies were published between 2007 and 2013, and all of them compared the prevalence of different vitamin D receptor polymorphisms between TB patients and healthy controls.

Four studies involved subjects of Asian ethnicity, with two [36,39] focusing on indigenous Chinese population, and the other two [37,40] focusing on the indigenous Indian population.  One study [38] was centered on the indigenous European population of Turkey, and yet another study [41] was centered on the indigenous population of Iran. One study [42] was conducted in South Africa, and involved mainly the indigenous African population, but also had a few subjects of Caucasian descent.  No other population groups were involved in these studies.

All studies enrolled TB patients from the primary site of the study.  All patients were newly diagnosed with TB, with the diagnosis made predominantly by sputum smear staining, sputum culture, radiographic findings, or a combination of these criteria.  One study further divided the case group based on multi-drug resistant strains of TB versus drug-susceptible strains of TB [37].  All of the studies focused on subjects with pulmonary TB, except for one study that looked at patients with spinal TB [36].

In five studies [36-40], controls were chosen from a healthy pool of age- and sex-matched individuals with similar demographics as the case group.  One of these five studies additionally recruited household contacts of the case group [40].  Another study recruited the control group from a pool of age- and sex-matched doctors, nurses, and TB support staff at the same facility where the TB patients were recruited [41].  The last study recruited healthy controls with the same demographics as the case group, but these individuals were not age-matched or sex-matched [42].

All seven studies investigated some combination of VDR polymorphisms. All seven studies investigated FokI. One study investigated ApaI [42], four studies investigated BsmI [37,38,40,41], and four studies investigated TaqI [37-39,42]. All seven studies utilized some form of genomic sequencing with restriction fragment length polymorphism markers to identify the target gene and determine the polymorphisms. Data collected was reported as number of individuals in each group with each VDR polymorphism, accompanied by an odds ratio in several of the studies.

Our analysis of the data from these seven studies was pooled together for each VDR polymorphism, similar to what previous VDR studies have done [13,14]. We divided the results into the subgroups for both the cases and controls – wild-type (WT), heterozygous (HT), and variant recessive (VR). Subsequently, an odds ratio was calculated from the pooled data comparing wild-type to heterozygotes and wild-type to variant recessives for each polymorphism and each population studied for that polymorphism. P-values were also calculated from the pooled data to determine significance of the odds ratios. Based on this analysis, we determined that only two of these polymorphisms play a significant role in the risk for tuberculosis [Table 3].

Analysis of the BsmI polymorphism showed that both the heterozygous and variant recessive polymorphisms confer an increased risk of TB as compared to the wild-type form (OR=1.70 and 1.55, respectively, $p<0.05$). Additionally, the FokI recessive polymorphism showed an increased risk of TB compared to wild-type (OR=1.93, $p<0.05$). However, the heterozygous form of FokI showed no significant difference over wild-type ($p>0.05$). The ApaI and TaqI heterozygous and recessive polymorphisms showed no significant difference in the risk of TB over their wild-type counterparts ($p>0.05$).

When analyzing the results by ethnicity, it is clear that certain VDR polymorphisms play different roles in different populations. In the Asian population, the heterozygous and recessive forms of BsmI (OR=1.79 and 2.28, respectively, $p<0.05$), the recessive form of

FokI (OR=2.23, p<0.05), and the heterozygous form of TaqI (OR=1.35, p<0.05) confer increased susceptibility to TB when compared to the wild-type form. The heterozygous form of FokI, the recessive form of TaqI, and neither form of ApaI confer any increased susceptibility/resistance to TB over the wild-type form. In the European population, both the heterozygous and recessive forms of BsmI confer increased resistance to TB when compared to wild-type (OR=0.32 and 0.15, respectively, p<0.05). FokI and TaqI confer no additional increase in susceptibility/resistance compared to wild-type. ApaI was not studied in this population. In the Middle Eastern population, both the heterozygous and recessive polymorphisms of BsmI confer increased risk of TB over their wild-type counterparts (OR=5.93 and 3.07, respectively, p<0.05). Neither form of FokI confers any increased susceptibility/resistance to TB when compared to wild-type. The ApaI and TaqI polymorphisms were not studied in this population. Lastly, in the African population, there was no significant increase in susceptibility/resistance to TB between the heterozygous/recessive forms of ApaI, FokI, and TaqI and their wild-type counterparts. The BsmI polymorphism was not studied in this population.

**B. Discussion**
Our review finds that the individuals carrying BsmI and FokI VDR polymorphisms showed higher odds of acquiring TB while those with the ApaI and TaqI VDR polymorphisms did not. Both the heterozygous and recessive forms of BsmI confer an increased risk of acquiring TB compared to the wild-type form. Additionally, the FokI recessive form confers a higher risk compared to its wild-type counterpart while there is no difference between the heterozygous and wild-type forms. Notably, when looking at the pooled data, the FokI variant recessive polymorphism confers the highest risk of acquiring TB compared to wild-type.

As outlined in the previous section, we have observed that different ethnic populations may have different susceptibilities and resistance patterns with regard to the four polymorphisms analyzed. For instance, BsmI confers the highest risk to the Middle Eastern and Asian populations, FokI and TaqI confer their highest risk to the African cohort, and the European subjects show significant resistance when possessing BsmI.

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However, as described further below, it is still too early to draw a conclusion due to the small sample size of all the studied populations, excluding the Asian cohort.

In understanding the risks that these different VDR polymorphisms portend to the population with regard to TB, we may be able to effectively comprehend the impacts that vitamin D supplementation may have on treating TB. It is evident that several of these VDR polymorphisms lead to weaker downstream signaling, thus weakening the overall effect of vitamin D. Perhaps treatment with higher doses of vitamin D or its analogs to activate more receptors and lead to a stronger additive effect would be beneficial in these patients.

This particular review suffers from the fact that only seven studies were found that met the inclusion criteria. Due to this, the ethnic background of each study population limited the analysis of the different polymorphisms. For example, the ApaI gene was only investigated in one of the studies we analyzed, and this particular study was conducted in only the South African population. Therefore, we cannot conclude that the different ApaI polymorphisms will have a similar impact on individuals of other ethnic backgrounds. It is known that gene polymorphisms are influenced by different ethnic, geographical, and other factors [43]. Differences in genetic background among different races lead to differences in each races' respective gene pool. Hence, while a particular gene polymorphism predominates in one race, another polymorphism may prevail in another race. This makes it difficult to apply data from one race to all individuals across the world. Along these lines, the TaqI polymorphism was only studied across Asian and African populations, making it difficult to apply the results to other populations worldwide.

In order to remedy this shortcoming, it is important that further studies be conducted in more populations across the world, particularly those populations who are disproportionately affected by TB. Doing so would allow us to view the impact of these polymorphisms over a larger and more diverse group of individuals.

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In addition to VDR gene polymorphisms, polymorphisms of the gene responsible for vitamin D binding protein (DBP) biosynthesis may also potentially affect the cellular actions of vitamin D on TB. For example, Martineau and colleagues reported an association between Gc genotype and susceptibility to TB that is dependent on vitamin D status [44].

## IV. Vitamin D Supplementation and Tuberculosis

### A. Results

A total of three hundred and fifty-one articles were identified based on the initial PubMed search criteria. No additional articles were identified from any other databases. Studies were only included in the analysis if they were set up in a randomized controlled trial fashion, if there was a measurement of time to clearance of infection, and if patients were concurrently treated with a standard anti-tuberculosis drug regimen. Studies were excluded if the study subjects had any chronic diseases that would lead to altered vitamin D metabolism, and if the study was conducted in patients without TB. A total of seven studies [45-51] met the inclusion and exclusion criteria (**Table 4**). A total of 1,062 individuals participated in the seven studies combined. All studies were published between 2006 and 2013 and, and all of them were randomized-controlled trials investigating vitamin D supplementation versus placebo in addition to standard anti-tuberculosis drug regimens.

Two of the studies [46,49] were conducted with a predominantly white population in the United Kingdom. Another study included indigenous African population [50]. Yet another three studies [45,47,48] utilized the indigenous South Asian populations of Hyderabad, Karachi, and Jakarta. The final study recruited subjects from Mongolia [51]. Among those seven studies, six [45-50] were performed with adult populations while the seventh study [51] was conducted in schoolchildren.

All seven studies assessed time to clear TB infection as either the primary outcome or a secondary outcome, with six of the studies investigating sputum smear conversion [45-

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50] and the seventh looking at tuberculin skin test (TST) conversion [51]. In addition, several of the studies looked at other outcomes, such as change in weight, change in height, changes in TB score, time to resolution of chest radiograph abnormalities, and changes in expression of inflammatory markers. All of the studies utilized a different regimen of vitamin D for the treatment arm, ranging from daily administration of small doses, to administration of very large doses every few months.

Five of the seven studies [45,46,48,49,51] showed improvements in the primary outcome in the vitamin D arm as compared to the placebo arm. Time to sputum smear conversion was quicker, and the percentage of subjects with negative sputum smear by a certain number of weeks was greater when subjects received vitamin D in addition to their anti-TB drug regimen. One study showed no difference in the time to sputum smear conversion, but did show improvement in the mean number of lung zones involved in the vitamin D group versus the placebo group [47].

Aside from quicker clearance of TB, supplementation with vitamin D led to other beneficial results as well. Salahuddin et al. [47] found that vitamin D supplementation improved mean weight gain during anti-TB therapy and an improvement in the TB score. Ganmaa et al. [51] found that supplementation with vitamin D improved growth rates in schoolchildren.

One study [50] showed no significant difference in time to sputum smear conversion between the vitamin D and placebo groups. This study also showed no difference in changes in TB score or 12-month mortality rates.

**B. Discussion**

Our review finds that supplementation with vitamin D in patients with TB undergoing treatment leads to improved clinical outcomes. The addition of vitamin D to standard anti-tuberculosis drug regimen results in faster clearance of the infection. Participants of these clinical trials experienced no significant side effects from the vitamin D supplements, thus indicating that vitamin D at the doses used are safe.

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It should be noted that each clinical trial utilized different doses and administration regimens. It is notable that regardless of the dose, the majority of the studies showed benefits from vitamin D supplementation. Thus, vitamin D will produce a noticeable effect regardless of the dosing scheme. For instance, according to the studies reviewed, dosing at 10,000 IU (0.25 mg) daily will have similar effects on treating tuberculosis infection as dosing at 60,000 IU (1.5 mg) every week. This allows the dosing to be modified to fit the particular individual's preferences while still being effective.

The study by Wejse et al. [50] showed no benefit to treatment with vitamin D in patients with TB. In this trial, subjects were randomized to receive 100,000 IU (2.5 mg) of vitamin D at initiation, 5 months, and 8 months. The authors of this study speculate that this particular dosing regimen was not effective. They further stated that either the dose concentration was suboptimal or the dosing interval was too long, thus failing to allow circulating 25(OH)D to reach its effective level. This, coupled with the fact that serum 25(OH)D levels were only measured prior to administration of the next dose, may have resulted in a lack of clinical effect from vitamin D.

While six of the seven studies looked at vitamin D supplementation in addition to anti-tuberculosis therapy in adults, the seventh study [51] investigated the effect of vitamin D supplementation in schoolchildren on risk of acquiring tuberculosis. TST conversion was less frequent in children receiving vitamin D versus those receiving placebo. This finding correlates with a previously conducted study in adults [52] showing that TST conversion was less frequent among adults with a sufficient serum 25(OH)D level. With this information, we can further decrease the incidence of TB by supplementing children and adult alike with vitamin D, especially in areas heavily burdened by this disease.

## Conclusions

The implication of vitamin D on TB infection has a long history. Our analysis reported here demonstrates the impacts of serum 25(OH)D levels, VDR polymorphisms, and vitamin D supplementation on the risk of acquiring TB and on the outcomes of TB

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infections.  An increased risk of TB was observed among the individuals with lower serum vitamin D levels.  Additionally, certain VDR polymorphisms, including the heterozygous and autosomal recessive forms of the four SNPs (ApaI, BsmI, FokI, and TaqI) that we analyzed, have shown an association with the increased risk of acquiring TB.  Finally, vitamin D supplementation appears to have a positive effect on TB outcomes, particularly with regard to the length of infection.  Due to the fact that studies analyzed did not report their results in a uniform manner and no raw data are available for these studies, we were unable to conduct a meta-analysis of the data.  While the findings in this review show a potential impact of vitamin D on TB infection, more well-designed clinical trials are needed in order to fully characterize the effect, particularly with regard to the VDR polymorphisms and vitamin D supplementation [53].  Vitamin D has been identified as an important agent in preventing many different infections and oncologic processes in the human body.  Further characterization of its effects and impacts on these illnesses will allow us to develop improved treatment methods for TB in the future, as well as other chronic diseases.

## Acknowledgements

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**Figure Legends**

Figure 1.  Photosynthesis and cytochrome P-450 enzyme-dependent metabolism of vitamin $D_3$. Humans receive most of their vitamin D requirement from the exposure of their skin to sunlight while a minor portion may obtained from dietary sources, such as fortified milk and oily fish. Upon exposure to ultraviolet B wavelengths between 290-315 nm, 7-dehydrocholecalciferol (7-DHC) in the skin is photolyzed to form a 9,10-seco-sterol pre-vitamin $D_3$ (Pre-$D_3$), which undergoes a heat-dependent isomerization to form vitamin $D_3$ ($D_3$). $D_3$ produced is specifically translocated by the vitamin D-binding protein (DBP) into the circulation and then to the liver for hydroxylation at carbon-25 to form 25-hydroxyvitamin $D_3$ ($25D_3$) mainly by two cytochrome P-450 enzymes, CYP2R1 and CYP27A1. 25D synthesized is then transported to the kidneys after binding to DBP in the blood stream. In the kidneys, $25D_3$ is hydroxylated in the presence of CYP27B1 to $1\alpha,25$-dihydroxyvitamin $D_3$ ($1,25D_3$), the active form of vitamin D, which serves as a hormone to regulate a variety of cellular functions in other organs, or acts inside the kidneys in an autocrine and/or paracrine fashion, and then hydroxylated further by CYP24A1 at carbon-24 to form $1\alpha,24,25$-trihydroxyvitamin $D_3$ ($1,24,25D_3$). The hydroxylation at carbon-24 by CYP24A1 is the first step of 1,25D catabolism to terminate its actions, which leads to the formation of calcitroic acid, a water soluble metabolite, and excreted into the urine.

Figure 2. Vitamin D-mediated activation of the innate immune system in macrophage (adapted from Adams and Hewison, Nat Clin Pract Endocrinol Metab. 2008;4(2):80-90. Ref. 3)  Innate immune systems provide immediate and the first line of defense against pathogen infection. Upon pathogen attack, such as Mycobacterium tuberculosis (M. Tuberculosis), toll-like receptors (TLR) on the macrophage membrane are activated to induce transcriptional up-regulation of vitamin D receptor (VDR) and CYP27B1 expression, leading to the increased synthesis of 1,25D and VDR, two essential components responsible for the VDR-dependent regulation of a variety of genes including the up-regulation of cathelidicin expression. Incorporation of cathelidicin  into phagosomes containing internalized M. Tuberculosis enables the peptide to function as an antimicrobial agent to kill the invading pathogen.

**Table 1: Serum vitamin D level study characteristics and results**

| Article | Study Population | Design | Cases | Controls | Measured Parameter | Results (serum 25(OH)D level) |
|---|---|---|---|---|---|---|
| Hong et al. (2013) [27] | Indigenous Korean population | Case-control | 94 untreated patients with culture-positive pulmonary TB. Mean age 35.4 years. | 282 age and sex-matched controls. Mean age 35.4 years. | Serum 25(OH)D levels | Cases: median (range) = 9.86 ng/mL (7.19-14.15) or 24.65 nmol/L (17.98-35.38) Controls: median (range) = 16.03ng/mL (12.38-20.30) or 40.08 nmol/L (30.95-50.57) P<0.001 |
| Talat et al. (2010) [28] | Indigenous Pakistani population | Case-control and prospective cohort | 20 untreated patients with sputum-positive pulmonary TB. | 109 household contacts of those TB patients. | Serum 25(OH)D levels | Cases: mean = 7.9 ng/mL (19.75 nmol/L) Controls: mean = 9.6 ng/mL (24 nmol/L) Progression to TB in 8% of household contacts, with majority of those individuals having 25(OH)D level <7 ng/mL (<17.5 nmol/L) |
| Mastala et al. (2013) [29] | Indigenous Malawi population | Cross-sectional study matched to cases from previous study | 161 patients with untreated pulmonary TB. Mean age 35.1 years. | 157 inpatients and outpatients without TB. Mean age 38.9 years. | Serum vitamin D levels | Cases: mean = 23.88 ng/ml (59.7 nmol/L) Controls: mean = 33.68 ng/ml (84.2 nmol/L) |
| Kim et al. (2013) [30] | Indigenous Korean population | Case-control | 165 untreated patients with predominantly culture-positive pulmonary TB. Median age 46 years. | 197 age and sex-matched controls. Median age 50 years. | Serum 25(OH)D levels | Cases: mean =13.2±8.63 ng/mL or 33 ±21.55 nmol/L Controls: Mean = 18.7±8.33 ng/mL or 46.75±20.82 nmol/L |
| Ho-pham et al. (2010) [31] | Indigenous Vietnamese population | Case-control | 166 (113 males, 53 females) untreated patients with | 219 (113 males, 106 females) age and sex- | Serum 25(OH)D levels | Prevalence of vitamin D insufficiency 35.4% in men with TB and |

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|---|---|---|---|---|---|---|
| | | | smear- and culture-positive pulmonary TB. Mean age 49 years. | matched controls. Mean age 49 years. | | 19.5% in controls. P=0.01<br><br>No significant difference in women with regard to serum 25(OH)D levels and prevalence of vitamin D insufficiency |
| Nielsen et al. (2010) [32] | Greenland, unspecified | Case-control | 72 untreated patients with predominantly culture-positive pulmonary TB. Mean age 39 years. | 72 age and sex-matched controls. Mean age 39 years. | Serum 25(OH)D levels | Vitamin D insufficiency (25(OH)D <30 ng/ml or <75 nmol/L) in 35% of patients with TB versus 17% of controls. |
| Wejse et al. (2007) [33] | Indigenous West African population | Unmatched case-control | 363 untreated patients with pulmonary TB. Mean age 37.4 years. | 494 unmatched controls. Mean age 37.3 years. | Serum 25(OH)D levels | Hypovitaminosis D (25(OH)D ≤30 ng/ml or ≤75 nmol/L) similar prevalence in cases and controls<br><br>Cases had lower average serum 25(OH)D level than controls |

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**Table 2: VDR Polymorphism study characteristics**

| Article | Country | Ethnicity | TB Group | Control Group | SNPs and TB |
|---|---|---|---|---|---|
| Zhang et al. (2009) [36] | China | Asian | 110 previously treated individuals with pathologically diagnosed spinal TB. Mean age 33.8 years. | 102 healthy, age and sex-matched volunteers. Mean age 32.2 years. | FokI VDR gene may be associated with spinal TB. |
| Rathored et al. (2012) [37] | India | Asian | 692 individuals with newly diagnosed pulmonary TB via sputum smear positivity. Mean age 27.5 years. Further divided into two arms based on drug-susceptibility testing:<br><br>354 individuals with MDR-TB<br><br>338 individuals with DS-PTB | 205 healthy age and sex-matched volunteers. Mean age 29.0 years. | FokI, TaqI and BsmI may predispose to TB. |
| Ates et al. (2010) [38] | Turkey | European | 128 individuals with pulmonary and extra-pulmonary TB. Mean age 47.8 years. | 80 healthy, age and sex-matched individuals. Mean age 54.1 years. | BsmI, not FokI and TaqI, variants are associated with susceptibility to TB. |
| Wu et al. (2013) [39] | China | Asian | 213 individuals with predominantly smear or culture positive pulmonary TB. | 211 healthy, age and sex-matched individuals | FokI, not TaqI, is associated with Susceptibility to TB. |
| Joshi et al. (2013) [40] | India | Asian | 110 individuals with newly diagnosed pulmonary TB. | 110 household contacts of TB cases<br><br>115 healthy, age-matched controls | FokI and BsmI are associated with TB susceptibility |
| Merza et al. (2009) [41] | Iran | Middle-Eastern | 117 individuals with newly diagnosed pulmonary TB. | 60 age, sex, and nationality-matched nurses, doctors, and TB staff with +PPD, but no clinical signs or symptoms of TB | BsmI, not FokI, is associated with susceptibility to TB. |
| Babb et al. (2007) [42] | South Africa | Mixed, predominantly African | 249 individuals with newly diagnosed pulmonary TB. | 352 healthy individuals from same population group, high-risk suburb, and socio-economic status | No association between VDR genotypes (FokI, ApaI, TaqI) and TB. |

**Table 3: Pooled results from all VDR Polymorphism studies**

| SNP | Ethnicity | Genotype | Cases | Controls | OR | p-value | SNP | Ethnicity | Genotype | Cases | Controls | OR | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ApaI | Total | WT homo | 101 | 116 | 1 | | BsmI | Total | WT homo | 142 | 124 | 1 | |
| | | HT | 108 | 173 | 0.72 | 0.07 | | | HT | 397 | 204 | 1.70 | <0.05 |
| | | VR homo | 40 | 63 | 0.73 | 0.2 | | | VR homo | 234 | 132 | 1.55 | <0.05 |
| | African | WT homo | 101 | 116 | 1 | | | Asian | WT homo | 107 | 106 | 1 | |
| | | HT | 108 | 173 | 0.72 | 0.07 | | | HT | 262 | 145 | 1.79 | <0.05 |
| | | VR homo | 40 | 63 | 0.73 | 0.2 | | | VR homo | 159 | 69 | 2.28 | <0.05 |
| FokI | Total | WT homo | 571 | 581 | 1 | | | European | WT homo | 28 | 5 | 1 | |
| | | HT | 510 | 447 | 1.16 | 0.09 | | | HT | 68 | 38 | 0.32 | <0.05 |
| | | VR homo | 184 | 97 | 1.93 | <0.05 | | | VR homo | 32 | 37 | 0.15 | <0.05 |
| | Asian | WT homo | 314 | 308 | 1 | | | Middle-Eastern | WT homo | 7 | 13 | 1 | |
| | | HT | 300 | 256 | 1.15 | 0.23 | | | | HT | 67 | 21 | 5.93 | <0.05 |
| | | VR homo | 157 | 69 | 2.23 | <0.05 | | | VR homo | 43 | 26 | 3.07 | <0.05 |
| | European | WT homo | 58 | 35 | 1 | | TaqI | Total | WT homo | 517 | 500 | 1 | |
| | | HT | 60 | 37 | 0.98 | 0.95 | | | HT | 322 | 281 | 1.11 | 0.31 |
| | | VR homo | 10 | 8 | 0.75 | 0.81 | | | VR homo | 89 | 67 | 1.28 | 0.16 |
| | Middle-Eastern | WT homo | 67 | 35 | 1 | | | Asian | WT homo | 332 | 280 | 1 | |
| | | HT | 46 | 25 | 0.96 | 0.9 | | | HT | 163 | 102 | 1.35 | <0.05 |
| | | VR homo | 4 | 0 | - | - | | | VR homo | 56 | 34 | 1.39 | 0.16 |
| | African | WT homo | 132 | 203 | 1 | | | European | WT homo | 49 | 30 | 1 | |
| | | HT | 104 | 129 | 1.24 | 0.21 | | | HT | 65 | 39 | 1.02 | 0.95 |
| | | VR homo | 13 | 20 | 1.00 | 1 | | | VR homo | 14 | 11 | 0.78 | 0.59 |
| | | | | | | | | African | WT homo | 136 | 190 | 1 | |
| | | | | | | | | | HT | 94 | 140 | 0.94 | 0.72 |
| | | | | | | | | | VR homo | 19 | 22 | 1.21 | 0.57 |

Abbreviations: WT Homo = wild-type homozygous, HT = heterozygous, VR = variant recessive

**Table 4: Vitamin D supplementation study characteristics and results**

| Article | Ethnicity | Study Population | Main Endpoints | Intervention | Outcomes |
|---------|-----------|------------------|----------------|--------------|----------|
| Kota et al. (2011) [45] | South Asian | 30 individuals age >15, newly diagnosed pulmonary TB, uncontrolled DM and 25(OH)D D <20ng/mL<br><br>n1 = 15 (oral cholecalciferol 60,000U/wk)<br>n2 = 15 (no vitamin D) | Time to achieve sputum smear conversion | 60,000 IU* of cholecalciferol per week plus quadruple therapy for TB or placebo plus quadruple therapy | Sputum smear conversion in 6 weeks for vitamin D group versus 8 weeks for placebo group |
| Martineau et al. (2011) [46] | Mixed – Asian, African, and Caucasian | 126 individuals age >18, without hypercalcemia, with newly diagnosed pulmonary TB<br><br>n1 = 62 (vitamin D$_3$)<br>n2 = 64 (placebo) | Time to achieve sputum smear conversion | 4 doses of 2.5mg of vitamin D given at 7, 14, 28, and 42 days in addition to anti-TB regimen or placebo in similar doses as above in addition to anti-TB regimen | Mean time to sputum smear conversion was 36 days in the vitamin D group versus 43.5 days in the placebo group |
| Salahuddin et al. (2013) [47] | South Asian | 259 individuals age >16 with newly diagnosed pulmonary TB<br><br>n1 = 132 (vitamin D$_3$)<br>n2 = 127 (placebo) | Weight gain<br><br>Resolution of chest radiograph abnormalities<br><br>Whole blood cell antigen-stimulated IFN-γ response<br><br>Percent sputum smear conversion by 12 weeks<br><br>Improvements in TB score | 2 doses of 600,000 IU at initiation and 1 month of vitamin D or placebo in similar doses as above in addition to anti-TB regimen | Mean weight gain 4.02kg in vitamin D arm versus 2.61kg in placebo arm (p<0.05)<br><br>Mean number of lung zones involved by CXR 1.35 in vitamin D arm versus 1.82 in placebo arm (p<0.05)<br><br>MTB-stimulated IFN-γ levels 3092pg/mL in vitamin D arm versus 2987pg/mL in placebo arm (p=0.5)<br><br>Sputum smear conversion by |

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| | | | | | 12 weeks 81.8% in vitamin D arm versus 81.2% in placebo arm (not statistically significant)<br><br>TB score reduction by 3.19 in vitamin D arm versus 2.79 in placebo arm |
|---|---|---|---|---|---|
| Nursyam et al. (2006) [48] | South Asian | 67 individuals aged 15-59 with newly diagnosed pulmonary TB<br><br>n1 = 34 (vitamin D)<br>n2 = 33 (placebo) | Percent sputum smear conversion by 6 weeks | 0.25mg/day of vitamin $D_3$ or placebo during initial 6 weeks of treatment with anti-TB medication | Sputum smear conversion by 6 weeks was 100% in vitamin D arm versus 76.7% in the placebo arm (p=0.002) |
| Coussens et al. (2012) [49] | Unspecified | 95 individuals with newly diagnosed pulmonary TB<br><br>n1 = 44 (vitamin D)<br>n2 = 51 (placebo) | Time to sputum smear conversion | 2.5mg of vitamin $D_3$ given every 2 weeks for a total of 4 doses or placebo, in addition to standard anti-TB regimen | Sputum smear conversion by 23 days in vitamin D arm versus 36 days in placebo arm (p=0.04) |
| Wejse et al. (2009) [50] | African | 365 individuals with newly diagnosed pulmonary TB were studied. 281 subjects completed the 12 months follow-up.<br>n1 = 136 (vitamin D)<br>n2 = 145 (placebo) | TB score reduction<br><br>12-month mortality<br><br>Time to sputum smear conversion | 100,000 IU of vitamin $D_3$ at initiation, 5 months, and 8 months or placebo, in addition to standard anti-TB medications | No difference in TB score, 12-month mortality, or sputum smear conversion times between the two arms |
| Ganmaa et al. (2012) [51] | Asian | Initially, 120 schoolchildren aged 12-15 were studied. However, only 117 children completed the 6 months follow-up.<br><br>n1 = 59 (vitamin D) | TST conversion<br><br>Change in serum 25(OH)D levels<br><br>Height | 800 IU vitamin $D_3$ or placebo daily for 6 months | TST conversion in 5 children (11%) in vitamin D arm versus 11 (27%) in placebo arm<br><br>Increase in serum 25(OH)D by |

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| | | | | | |
|---|---|---|---|---|---|
| | | n2 = 58 (placebo) | | | mean of 12.7 in vitamin D arm versus 2.3 in the placebo arm<br><br>Mean increase in stature 2.9 ± 1.6cm in vitamin D arm versus 2.0 ± 1.7cm in placebo arm |

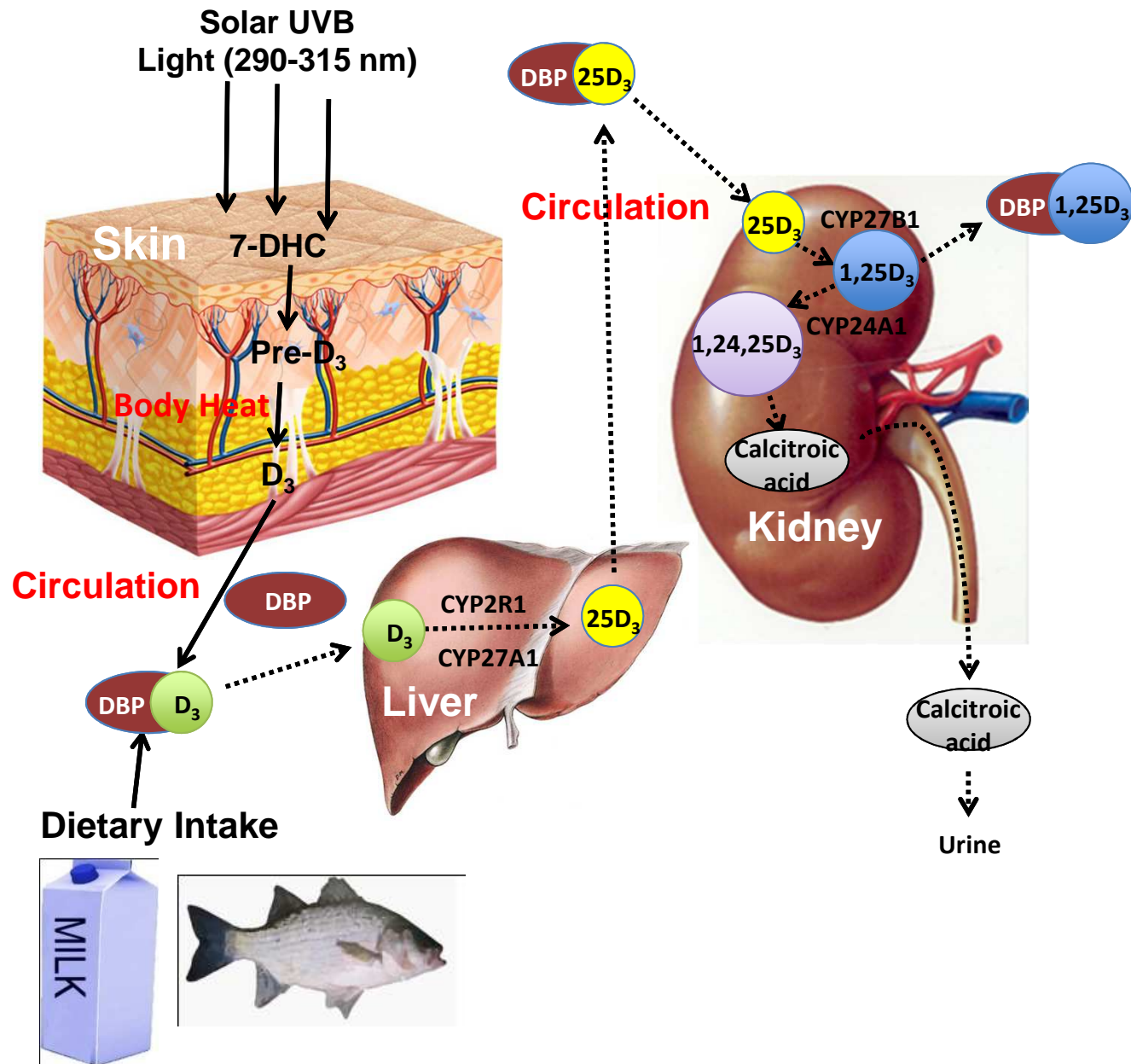*40 IU is equivalent to 1μg of cholecalciferol

# Figure 1: Photosynthesis of Vitamin $D_3$ and Its Metabolism

# Figure 2. Vitamin D – Mediated Activation of the Innate Immune System in Macrophase