Immunoregulatory effects and therapeutic potential of Vitamin D in multiple sclerosis

Running title: Therapeutic potential of vitamin D in multiple sclerosis

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ABSTRACT

Initially recognised as an important factor for bone health, Vitamin D is now known to have a range of effects on the immune system. Vitamin D deficiency is associated with an increased risk of multiple sclerosis (MS), a chronic immune-mediated demyelinating disease of the central nervous system (CNS). In this Review, we explore the links between vitamin D deficiency, MS risk and disease activity. We also discuss the known immune effects of vitamin D supplementation and the relevance of these observations to the immunopathology of MS. Finally, we review the existing evidence for vitamin D supplementation as an MS therapy, highlighting several recent clinical studies and trials.

KEYWORDS

Vitamin D, cholecalciferol, multiple sclerosis, immune, transcriptome, genetic, supplementation, treatment, therapeutic

1. INTRODUCTION

Vitamin D refers to a group of lipid-soluble secosteroid compounds. Its two major forms are vitamin D_2 (ergocalciferol), produced by plants and fungi, and vitamin D_3 (cholecalciferol), synthesised in animals (Figure 1). Vitamin D is best known for its roles in skeletal health as well as calcium and phosphate homeostasis. Its clinical importance in bone health is illustrated by diseases associated with severe vitamin D deficiency, rickets and osteomalacia. These are diseases of bone matrix demineralisation in children and adults, respectively (Holick, 2007).

The extra-skeletal actions of vitamin D include immunomodulatory effects in both innate and adaptive immune cells. Vitamin D deficiency increases the risk of developing a number of autoimmune diseases, including multiple sclerosis (MS), type 1 diabetes mellitus and Crohn's disease (Munger et al., 2006; Ananthakrishnan et al., 2012; Gorham et al., 2012). Based on the strong inverse association of vitamin D levels and risk of MS, it has been proposed that vitamin D supplementation could have a therapeutic role in this disease. Here, we review the known effects of vitamin D on immune cells, and provide a summary of recently completed and on-going studies of vitamin D supplementation for the treatment and/or prevention of MS.

2. MULTIPLE SCLEROSIS

2.1. What is Multiple Sclerosis?

Multiple sclerosis (MS) is a chronic autoimmune demyelinating disease of the CNS and is a leading cause of disability affecting young adults (Wallin et al., 2019). The MS disease course exists on a spectrum. The most common phenotype is relapsing-remitting MS. Relapsing-remitting MS is defined by relapses (attacks). During a relapse, focal or multifocal neurological symptoms develop over hours to days. Symptoms persist for days to weeks and then improve, either fully or partially. Relapsing-remitting MS accounts for at least 85% of all diagnoses (Weinshenker, 1994). 5-15% of patients have a phenotype referred to as primary progressive MS. Primary progressive MS is characterised by a gradual progressive course from symptom onset. In both relapsing-remitting and primary progressive MS, disability accumulates over time. The mean age of onset in relapsing-remitting MS is 28.5 years (Scalfari et al., 2010). The female-to-male ratio is 2-3:1 in relapsing-remitting MS,

but 1:1 in primary progressive MS (Koch-Henriksen and Sørensen, 2010; Ribbons et al., 2015).

Magnetic Resonance Imaging (MRI) is a key diagnostic and prognostic tool in MS. Typical features on MRI have become an integral part of the diagnostic criteria of MS, and development of new MRI lesions is a key measure of disease activity in trials and clinical practice (Barkhof et al., 2009). MRI-visible MS lesions are hyperintense on T2-weighted sequences and typically involve white matter regions of the brain and spinal cord. In MS, the target of the autoimmune attack is principally the myelin sheath of CNS axons, though a target antigen remains elusive after many decades of research (Wootla et al., 2012).

2.2. Multiple Sclerosis Pathology

Both adaptive and innate arms of the immune system have been implicated in MS pathogenesis. The innate immune response is rapid and non-antigen specific, whereas the adaptive immune system elicits an antigen-specific response and carries an antigen-specific memory. Innate immune cells include myeloid lineage-derived cells such as monocytes, macrophages and Dendritic Cells (DC), as well as lymphoid-derived Natural Killer cells. Monocytes, macrophages and DC recognise pathogens through pattern recognition receptors. Pathogen detection leads to upregulation of co-stimulatory molecules such as CD80 and CD86 on their surface. These co-stimulatory molecules are required for activation of T cells during antigen presentation, the key interaction between innate and adaptive immune cells in the immune response.

Adaptive immune cells include B and T lymphocytes. B cells are responsible for antibody production (when terminally differentiated into plasma cells) and also have roles in antigen presentation and cytokine production. T lymphocytes can be divided into two main types: $CD4^+$ T cells and $CD8^+$ cytotoxic T lymphocytes. These cells express antigen receptors that bind to specific epitopes. $CD4^+$ T cells can differentiate into several functionally distinct subsets, including T helper (Th) 1 and Th2 and Th17 subsets, depending on the cytokines they are exposed to during maturation. A small number of both T and B cells are so-called regulatory lymphocytes. They suppress T cell responses and promote a tolerogenic immune state - important in preventing

autoimmunity and limiting the extent of any immune response to avoid severe self-injury (Sakaguchi, 2011).

Pathological studies of actively demyelinating MS lesions have identified inflammatory infiltrates characterised by a large number of activated macrophages, as well as $CD4^+$ and $CD8^+$ T cells, B cells, activated microglia and scant plasma cells (Lucchinetti et al., 2000). Some plaques exhibit complement and immunoglobulin deposition and oligodendrocyte apoptosis, implicating both innate (complement) and adaptive (B cell production of immunoglobulin) immune system involvement. Axonal injury is common, but widely believed to be secondary to the inflammatory demyelination. CNS myelin can regenerate to some extent, whereas CNS axons in humans cannot, thus any axonal injury is likely to be a significant contributor to irreversible injury and, ultimately, disability. Though MS is most often thought of as a demyelinating disease affecting white matter, it also involves demyelinating lesions in the cerebral cortex, deep nuclei and spinal cord grey matter (Lucchinetti et al., 2011).

2.3. Multiple Sclerosis risk factors

Both genetic and environmental risk factors have been implicated in the development of MS (van der Mei et al., 2016; International Multiple Sclerosis Genetics Consortium, 2019). The presence of a latitudinal gradient of MS prevalence and incidence, increasing with distance away from the equator, is a key environmental risk factor. A recent meta-analysis revealed that the latitudinal variation of MS prevalence has increased over time (Simpson, Jr. et al., 2019). The key candidates to explain these findings are UVB exposure and vitamin D status. Infection with the Epstein-Barr virus (EBV) in adolescence (as symptomatic glandular fever) and smoking are other known risk factors for MS (Levin et al., 2010; Hedström et al., 2016). Childhood and adolescent obesity is weakly linked with MS risk (Langer-Gould et al., 2013; Jacobs et al., 2020).

3. VITAMIN D METABOLISM AND PHYSIOLOGICAL FUNCTIONS

In humans, the three major sources of vitamin D are UVB-mediated production in the skin, dietary intake of Vitamin D-rich foods and pharmacological supplementation. Dietary intake is generally insufficient to meet requirements. Synthesis via UVB (290-315 nm wavelength) exposure is the predominant source of vitamin D. Whole

body UVB exposure of one minimal erythemal dose, the dose of UV radiation required to cause mild reddening of skin, produces an amount equivalent to ingestion of 10,000 IU vitamin D (Holick, 1995). Both intrinsic and extrinsic factors can influence the degree of vitamin D_3 production, including age, area of exposed skin, skin pigmentation, geographical latitude and season (Holick, 1995). Vitamin D-rich foods are few, but include fatty fish, in particular salmon, cod liver oil and shiitake mushrooms (Holick, 2007). Supplementation and fortification of foods (eg. margarine, milk) are often used when UVB exposure and diet are inadequate to achieve vitamin D sufficiency, particularly in more polar regions.

UVB-mediated synthesis begins with the precursor 7-dehydrocholesterol, found in the epidermis. UVB exposure transforms 7-dehydrocholesterol to previtamin D_3, which is then converted to vitamin D_3 via temperature-dependent isomerisation (Figure 2) (Tian et al., 1993). There is an upper limit to the amount of vitamin D_3 produced, following which previtamin D_3 and excess vitamin D_3 are photoconverted to inactive metabolites including lumisterol, tachysterol, suprasterol I and suprasterol II (Webb et al., 1989). Thus vitamin D toxicity from UVB exposure does not occur unless a concomitant medical condition such as primary hyperparathyroidism is present.

Vitamin D binds to vitamin D-binding protein in the circulation and, to a lesser extent, lipoproteins (Haddad et al., 1993). Vitamin D is inactive and must undergo two steps to be transformed into its active metabolite. It is first carried to the liver where 25-hydroxylases, which include CYP2R1, CYP27A1 and CYP3A4, add a hydroxy group to form 25-hydroxyvitamin D (25(OH)D) (Zhu and DeLuca, 2012). 25(OH)D re-enters the circulation and is carried to the kidneys. It is transported into renal tubular cells where 1-alpha-hydroxylase (CYP27B1) hydroxylates 25(OH)D to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), also known as calcitriol, the active form of vitamin D (Miller and Portale, 2000). 24-alpha-hydroxylase (CYP24A1) is also present in renal tubular cells and inactivates both $1,25(OH)_2D$ and 25(OH)D to calcitroic acid (Makin et al., 1989). CYP24A1 is induced by calcitriol in an autoregulatory loop protecting against calcitriol toxicity. The $25(OH)D_3$ metabolite has a half-life of 2-4 weeks, in contrast to the much shorter half-life of $1,25(OH)_2D_3$ of 4-6 hours (Gray et al., 1978; Jones et al., 2014). The half-life of $25(OH)D_2$ is shorter than that of $25(OH)D_3$. $25(OH)D_3$ provides the most accurate measure of

vitamin D stores and is the form of vitamin D routinely measured to assess vitamin D status.

Calcitriol ($1,25(OH)_2D$) is carried in the circulation by vitamin D-binding protein and acts on target cells in an endocrine fashion. It passes into the cell nucleus and binds the vitamin D receptor (VDR). The $1,25(OH)_2D$-VDR complex heterodimerises with retinoid X receptor and binds to vitamin D response elements of the DNA, and together with other proteins acts as a transcription factor to alter gene expression. The most common vitamin D response element is the DR3-type element which consists of two hexameric repeated motifs separated by three nucleotides (Heikkinen et al., 2011).

Many cell types express VDR, including cells of the innate and adaptive immune systems, and can therefore respond to vitamin D (Provvedini et al., 1983). Additionally, multiple cell types express the enzymes required for vitamin D metabolism, including CYP27B1 and CYP24A1, allowing them to respond to vitamin D in an autocrine or paracrine fashion. These include T and B lymphocytes, DC, monocytes and neural cells (including neurons and microglia) (Kreutz et al., 1993; Hewison et al., 2003; Chen et al., 2007; Baeke et al., 2010; Landel et al., 2018). Responses to vitamin D vary by cell type and are dependent on factors such as activation state and presence of environmental signals.

4. VITAMIN D AND ITS EFFECTS ON IMMUNE CELLS

4.1. Immune cell phenotype and function

Immune cells express VDR and the enzymatic machinery to metabolise vitamin D to varying degrees. As such, vitamin D can modulate the functions of both innate and adaptive immune cells. Exposure to vitamin D, *in vitro,* enhances the innate immune system's capacity to eliminate pathogens (Xu et al., 1993; Liu et al., 2007) and promotes tolerance induction through its interactions with adaptive immune cells (Penna and Adorini, 2000; Piemonti et al., 2000; Unger et al., 2009). In adaptive immune cells, vitamin D promotes differentiation towards regulatory B and T cells, increases anti-inflammatory cytokine production such as of IL-10 and TGF-β and decreases pro-inflammatory cytokines (Heine et al., 2008; Jeffery et al., 2009; Lysandropoulos et al., 2011) (the *in vitro* effects of vitamin D are summarised in

Supporting Table 1). Overall, these effects facilitate pathogen clearance and minimise excessive inflammation and consequent tissue damage.

By contrast, relatively few studies have assessed the effects of vitamin D supplementation on peripheral blood immune cells of healthy adults *in vivo* (Table 1). The limited evidence however, also supports an anti-inflammatory effect of vitamin D supplementation, with an increase in regulatory T cell (Treg) proportions and IL-10-producing cells together with a decrease in IFN-γ- and IL-17-secreting $CD4^+$ T cells (Drozdenko et al., 2014; Prietl et al., 2014). Another consideration is that whilst peripheral blood is accessible, change may be occurring at other sites. Bak et al. (2018) observed a decrease in $CD103^+$ DC, which are known to induce Treg differentiation, in colonic mucosa after vitamin D supplementation. This was interpreted as tolerogenic, the DC migrating to mesenteric lymph nodes where they interact with adaptive immune cells. Further study of other tissue may yield novel understanding of vitamin D-immune system interactions.

4.2. Changes to the immune transcriptome

The sites at which VDR binds to the genome are enriched at regions of strong enhancer and active promoter elements, which are important for gene regulation (Ramagopalan et al., 2010; Tuoresmäki et al., 2014). Hence, the changes to gene expression after vitamin D treatment have been the focus of investigation in an attempt to gain insight into its mechanisms. Investigation of the transcriptome, the set of all RNA transcripts of a cell or group of cells, has identified up to 3650 differentially expressed genes following vitamin D treatment in a single immune cell line (Neme et al., 2017). Several primary vitamin D target genes encode for transcription factors which can in turn modulate transcription of secondary target genes of vitamin D (Nurminen et al., 2015). Pathway analysis of differentially expressed genes identify enrichment in immune and metabolic functions, though these differ between cell types. The mechanisms that lead to these changes have also been examined, such as the alterations to chromatin accessibility by vitamin D (the molecular modulations by vitamin D are summarised in Supporting Table 2).

While there have been extensive *in vitro* studies of vitamin D-induced effects in immune cells, it is unclear whether similar changes are seen *in vivo*. As *in vitro*

studies use supraphysiological concentrations of calcitriol they may not reflect *in vivo* effects in tissues. A small number of vitamin D supplementation studies in healthy adults included assessment of immune cell gene expression *in vivo*, with mixed findings (Supporting Table 3). Two studies treated participants for up to six months with cholecalciferol doses between 400-10,000 IU daily (Hossein-nezhad et al., 2013; Shirvani et al., 2019). These identified up to 1289 differentially expressed genes and suggested a dose-dependent response with more genes modulated by a higher dose. The longest and largest study included 305 participants supplemented with either 2000 or 4000 IU daily for 12 months (Berlanga-Taylor et al., 2018). This did not identify any differentially expressed genes after supplementation. These mixed findings could suggest that the modulatory effect of vitamin D supplementation has a time-dependent course with diminishing effect over time due to negative regulatory and other effects. However, methodological differences between these few studies limit any firm conclusions. These differences include varying doses, peripheral blood cells assessed and statistical approaches.

5. THE ROLE OF VITAMIN D IN MULTIPLE SCLEROSIS RISK AND DISEASE ACTIVITY

Latitudinal variation of MS prevalence and incidence implicates environmental factors. The most probable candidates are UVB radiation exposure and vitamin D status. Their potential contribution to MS risk and disease activity is an area of intensive study as vitamin D supplementation could be a safe and effective pharmacological intervention for primary prevention and treatment of MS. Evidence linking vitamin D to MS causation and disease activity will be reviewed in this section.

5.1. Vitamin D deficiency is a risk factor for MS

Low serum vitamin D status has been linked with increased MS risk. Munger et al. (2006) conducted a prospective nested case-control study from a sample of over seven million United States military personnel. They assessed serum vitamin D status prior to MS diagnosis and observed a 41% reduction in subsequent MS diagnosis for every 50 nmol/L increase in serum $25(OH)D_3$ in whites. Among blacks, a smaller reduction of 34% was seen. In the Ausimmune Study, a multicentre Australian incidence study of cases with a first CNS demyelinating event, a 50 nmol/L increase of serum

25(OH)D_3 reduced the risk of a first demyelinating event (FDE) by 31% , independent of prior sun exposure (Lucas et al., 2011). A prospective study of women in the Finnish Maternity Cohort showed that a 50 nmol/L higher level in serum 25(OH)D_3 sampled during first trimester of pregnancy reduced their later MS risk by 39% (Munger et al., 2017). Interestingly, there is also some evidence that lower vitamin D levels in the mother during gestation and of the neonate increases MS risk in the offspring (Mirzaei et al., 2011; Munger et al., 2016; Nielsen et al., 2017). A Swedish study used databases of individuals with prospectively collected blood samples tested for serum 25(OH)D_3 and showed that levels of at least 75 nmol/L were associated with lower MS risk with an odds ratio of 0.39 (Salzer et al., 2012). Based on these findings, higher vitamin D levels in many prospective cohort studies are strongly associated with lower subsequent MS risk.

The Nurses' Health Study and Nurses' Health Study II studied the association between vitamin D intake and MS risk. They prospectively followed two large cohorts of female nurses in the United States of America and found that total vitamin D intake and supplementation were inversely associated with risk of later developing MS (Munger et al., 2004). The relative risks were 0.67 for total intake (highest versus lowest quintile) and 0.59 for supplementation of ≥ 400 IU daily versus no supplementation. Fatty fish and cod liver oil are dietary sources relatively high in vitamin D. Their consumption has also been associated with reduced risk of MS (Bäärnhielm et al., 2014). These studies suggest a role for vitamin D consumption and supplementation in reducing MS risk.

Application of Mendelian randomisation has further strengthened the evidence base for a causal relationship between vitamin D and MS risk. Mendelian randomisation uses genetic variants associated with a proposed risk factor as an instrumental variable to assess their relationship with a given outcome. A significant benefit of this technique over observational studies is its ability to overcome confounding by unknown factors. Using genetic variants or single nucleotide polymorphisms associated with 25(OH)D_3 level, four studies have shown a protective effect against developing MS in the presence of variants associated with higher vitamin D levels (Mokry et al., 2015; Rhead et al., 2016; Gianfrancesco et al., 2017; Jacobs et al., 2020).

Collectively, these studies suggest that vitamin D deficiency significantly increases the risk of MS and that repletion of vitamin D status, such as through dietary intake, could minimise this risk. These observations also pose the question of whether vitamin D supplementation could be used as a therapeutic to prevent MS.

5.2. Vitamin D deficiency and MS disease activity

Studies have also examined the relationship between vitamin D status and various measures of MS disease activity such as risk of relapse after an initial attack, annualised relapse rates, disability progression and change to MRI lesion load.

In an Italian retrospective study of 100 clinically isolated syndrome (CIS; first demyelinating attack) patients, low serum vitamin D was associated with conversion to clinically definite MS (CDMS), most marked among subjects in the lowest 10^{th} percentile of vitamin D level at CIS (serum $25(OH)D_3$ of < 59.3 nmol/L) (Martinelli et al., 2014). Ascherio et al. (2014) reported on the BENEFIT study, a randomised-controlled trial of IFN beta-1b in CIS patients. Participants had vitamin D status assessed at baseline and over up to 24 months. Using the average serum $25(OH)D_3$ level in the first 12 months of CIS, a 50 nmol/L increment was associated with a 56% reduction in hazard of converting to MS over the subsequent four years. Higher serum vitamin D level was associated with fewer new active MRI lesions, less T2 lesion volume accumulation and brain volume loss, fewer relapses and less change in disability.

Simpson et al. (2010) performed a prospective cohort study of 145 relapsing-remitting MS patients who had biannual review and $25(OH)D_3$ measurements, with mean follow-up duration of 2.3 years. They found a reduction in relapse risk by 12% for every 10 nmol/L increase in serum $25(OH)D_3$. A similar association was identified in another prospective study of 73 relapsing-remitting MS patients (Runia et al., 2012), and in patients with paediatric-onset MS (Mowry et al., 2010). Recent Mendelian randomisation studies are also supportive of an association between vitamin D status and relapse rate (Graves et al., 2019). Collectively, these studies suggest an association between reduced acute inflammatory activity (relapses) in MS patients and higher serum $25(OH)D_3$ levels. Further support for this association was observed

in two prospective studies showing that higher vitamin D levels were associated with fewer new MRI lesions (Mowry et al., 2012; Fitzgerald et al., 2015).

In summary, MS risk and disease activity are strongly associated with lower vitamin D levels. Exposures throughout life, from time in the intrauterine environment through to birth, and into early adulthood, appear to influence disease susceptibility. Vitamin D is recognised to have immunomodulatory effects. However, the specific mechanisms by which vitamin D could affect MS risk and disease activity are not well understood.

6. MOLECULAR EVIDENCE FOR A ROLE OF VITAMIN D IN MS RISK

The epidemiologic body-of-work linking vitamin D deficiency and MS risk, and the *in vitro* actions of vitamin D on immune cells, strongly suggest a role for vitamin D in MS pathogenesis. However, it is currently unclear what specific mechanisms or interactions between vitamin D and the immune system result in this elevated risk. A number of molecular observations support the relationship between vitamin D and MS risk (summarised in Supporting Table 4, and briefly described below).

Firstly, a number of MS risk genes are known to be modulated by vitamin D. From genome wide association studies, over 200 MS risk single nucleotide variants have been identified from which more than 500 susceptibility genes have been proposed (International Multiple Sclerosis Genetics Consortium, 2019). The majority of these risk variants are located in non-coding regions of the genome which may have regulatory effects on gene expression. Expression quantitative trait locus analyses of these candidate MS risk variants have implicated both adaptive (T and B cells) and innate immune (Natural Killer, DC, monocyte, microglia) cells in MS pathogenesis (International Multiple Sclerosis Genetics Consortium, 2019; Gresle et al., 2020). Several genes have been shown to be modulated by vitamin D treatment in various immune cells, and a number have also been shown to be altered at the protein level. One example is the HLA-DRB1 gene whose variant, DRB1*15:01, is the strongest genetic risk factor for MS with odds ratio 3 (Ramagopalan et al., 2009; International Multiple Sclerosis Genetics Consortium, 2019). This variant has a functional vitamin D response element in its promoter. Calcitriol treatment of lymphoblastoid cells homozygous for HLA-DRB1*15 showed an increase in HLA-DRB1 cell surface

expression (Ramagopalan et al., 2009). This interaction between two significant MS risk factors is hypothesis-generating. There could be an effect of vitamin D on antigen presentation and thus adaptive immune system activation, with potential impacts on $CD4^+$ T cell maturation and central tolerance. The combination of a state of vitamin D deficiency and downregulated expression of HLA-DRB1*15 could allow myelin-specific autoreactive T cells to escape negative selection which then culminates in later development of MS. A number of other MS risk genes have also been shown to be vitamin D target genes, such as IL2RA which encodes CD25, a subunit of the high-affinity IL-2 receptor expressed on Treg cells & effector T cells (Berge et al., 2016). Of interest, CD25 has been the target of daclizumab, a monoclonal antibody previously approved for MS treatment, but now withdrawn from the market due to safety concerns (Cohan et al., 2019).

Secondly, VDR binding is enriched near MS risk loci indicating that the genes associated with these loci are potentially regulated by vitamin D (Ramagopalan et al., 2010; Booth et al., 2016). These MS risk variants may also alter vitamin D responsiveness of respective genes. Thirdly, several enzymes involved in vitamin D metabolism have genetic variants in or near their genes associated with MS susceptibility, in particular CYP27B1, CYP24A1 and CYP2R1 (International Multiple Sclerosis Genetics Consortium, 2019). Changes to enzyme function or expression may then lead to altered vitamin D metabolism with downstream effects. MS patients have been reported to have a lower increase in serum 25(OH)D level following supplementation than controls (Bhargava et al., 2016). Altered vitamin D metabolism provides a potential explanation for this observation.

Unfortunately, *in vivo* confirmation for most of the above observations is lacking, and hence the biological relevance of these observations remain unclear.

7. VITAMIN D SUPPLEMENTATION AND POTENTIAL AS AN MS THERAPEUTIC

7.1. Immunologic and molecular changes in MS following supplementation with Vitamin D

Interventional studies, including randomised-controlled trials, have investigated immunological alterations following vitamin D supplementation in people with MS

(Table 2). Safety was excellent at all vitamin D dosages, even greater than 10,000 IU daily (Burton et al., 2010; Smolders et al., 2010; Golan et al., 2013); safety is discussed in more detail in Section 7.3. Several studies found a reduced T cell proliferative response following supplementation (Burton et al., 2010; Kimball et al., 2011; Mosayebi et al., 2011). The studies showed reduced Th1 and Th17 cells, increased IL-10-producing cells, as well as reduction in effector memory T cells (Smolders et al., 2010; Sotirchos et al., 2016). However this is not a consistent finding. Other studies did not identify significant T cell subset changes (Mrad et al., 2017; O'Connell et al., 2017). The SOLAR study was a randomised-controlled trial of vitamin D_3 supplementation (6670 IU daily for 4 weeks, then 14,007 IU daily for 44 weeks) in relapsing-remitting MS patients treated with IFN beta (Muris et al., 2016; Rolf et al., 2018b). 53 participants from this study had peripheral blood mononuclear cells collected at baseline and 48 weeks. Reduced IL-4^+ Th cell proportion and CD25 expression on Treg cells were observed in the placebo group. The investigators hypothesised that vitamin D may have a role in maintenance of immune homeostasis.

Some studies demonstrated increases in anti-inflammatory cytokines IL-10 and TGF-β post-vitamin D supplementation, but again this is not consistent across studies (Mahon et al., 2003; Mosayebi et al., 2011; Åivo et al., 2015; Sotirchos et al., 2016). Reasons for these varied findings may relate to differences in design, including vitamin D dosage, study duration and sample size. Participants' baseline vitamin D status could influence the ability to elicit change following supplementation. A hypothesis is that people who are vitamin D deficient (< 50 nmol/L) could be more likely to have immunologic changes after supplementation. Use of MS disease-modifying therapies, which are immunomodulatory in nature, could also alter immunologic response to vitamin D. Another possibility is that changes could principally occur in immune system compartments not sampled. Despite the inconsistent findings across studies, overall they are suggestive of an anti-inflammatory and regulatory response induced by vitamin D in MS.

Several studies of vitamin D supplementation in people with MS have also found a decrease in Epstein-Barr virus antibodies (ie. anti-EBNA IgG levels) post-supplementation (Disanto et al., 2013; Røsjø et al., 2017; Rolf et al., 2018a). These observations are of interest as they link two significant MS environmental risk factors.

The mechanisms by which vitamin D mediates this effect and their importance in MS pathogenesis are unclear and remain open to further study.

A few studies have examined the effect of vitamin D supplementation on selected gene expression levels using quantitative PCR. Four studies from the same group focussed on mRNA expression of genes encoding pro- and anti-inflammatory cytokines (Supporting Table 5) (Farsani et al., 2015; Naghavi Gargari et al., 2015; Shirvani-Farsani et al., 2015, 2017). However a number of limitations include use of only one housekeeping gene for normalisation and the paucity of details provided in regards to their MS cohort, such as MS therapies used. Control group characteristics and outcomes were not reported in much detail and comparisons of responses between MS and non-MS groups are difficult to evaluate. A study by another group assessed expression of IL-6, IL-17A and IL-10, but again suffers the same drawbacks (Hashemi et al., 2018). A randomised-controlled trial of vitamin D supplementation included relapsing-remitting MS patients on IFN beta and did not identify any significant change in IL2RA mRNA expression in PBMCs after 48 weeks (Rolf et al., 2018b). As yet, studies assessing how vitamin D supplementation modulates the transcriptome in MS patients using next-generating sequencing techniques or in immune cell subsets are lacking. Potential differential response between cell types, and between MS and healthy controls, remains to be determined.

Metabolomics, a method for profiling metabolites in body tissue, was used to assess plasma obtained from MS or healthy participants before and after 5000 IU daily vitamin D supplementation for 90 days (Bhargava et al., 2017). Following supplementation, there was a reduction in metabolites involved in oxidative stress and lipid metabolism. Importantly these changes were attenuated in MS patients compared to controls, suggesting that there could be an impaired response to vitamin D among MS patients. If confirmed, another consideration is whether higher supplementation doses in MS patients could overcome this impaired response.

7.2. Vitamin D supplementation and clinical outcomes in MS

In humans, vitamin D levels are usually an excellent surrogate for UVB exposure in the prior two months. Due to the relationship between UVB and vitamin D, a direct

pharmacological effect of vitamin D can ultimately only be confirmed or refuted by randomised-controlled trials.

The epidemiologic, immunologic and molecular evidence for a beneficial role of vitamin D in MS, are further supported by studies in animal models of neuroinflammatory disease. In the experimental autoimmune encephalomyelitis mouse model, vitamin D supplementation prevents disability and inflammatory demyelination (Cantorna et al., 1996; Sloka et al., 2015). Vitamin D also appears to enhance remyelination through promotion of oligodendrocyte progenitor cell differentiation in rat and mouse models of demyelination (Shirazi et al., 2017; Gomez-Pinedo et al., 2020). As such, a number of human randomised-controlled trials aimed to evaluate the therapeutic role of vitamin D in MS. Unfortunately the majority of these studies used a design of randomisation to vitamin D or placebo on a background of use of IFN beta or other MS therapy in all trial participants, severely limiting their power (Table 3).

Nonetheless, three such studies have reported beneficial effects on MRI measures of disease activity (Soilu-Hänninen et al., 2012; Camu et al., 2019; Hupperts et al., 2019). The largest of these included 229 relapsing-remitting MS participants and randomised them to either placebo or vitamin D supplementation (14,007 IU daily after initial four-week uptitration) for at least 48 weeks (Hupperts et al., 2019). The investigators found a significant reduction in inflammatory gadolinium-enhancing or new T2 lesions on MRI in their treatment arm as well as a smaller mean percentage change in total T2 lesion volume at 48 weeks compared to baseline. Their primary outcome of proportion of patients with no relapses, disability progression or new MRI lesions was not met. However three other studies did not identify any amelioration of MRI disease activity, though a limitation of these, as well as other randomised-controlled trials, is their small sample size and low statistical power (Mosayebi et al., 2011; Stein et al., 2011; Dörr et al., 2020).

In a recently published study, Camu and colleagues recruited 129 relapsing-remitting MS patients on IFN beta-1a and randomised them to either vitamin D_3 100,000 IU or placebo every two weeks and monitored them over a two-year period (Camu et al., 2019). Their primary end-point of significant change in annualised relapse rate was

not met in the intention-to-treat population. In those that completed the 96-week study period, a significant reduction in annualised relapse rate was found in the treatment arm, in addition to reduction in new T1 hypointense lesion formation and disability progression as measured with the Expanded Disability Status Scale (EDSS). Approximately 30% of subjects dropped out of each arm, the most common reason being a switch in MS treatment (Camu et al., 2019).

Two recent meta-analyses have examined vitamin D supplementation and its impact on clinical outcomes of MS (McLaughlin et al., 2018; Zheng et al., 2018). Both concluded that there was no significant evidence of a clinical benefit from vitamin D supplementation. The relatively small sample sizes of available studies limit the power to detect significant differences between treatment arms. The add-on design to MS therapies results in marked amelioration of disease activity in all subjects and thus greatly reduces the power of these studies. Study duration of one year may be insufficient to assess changes in relapse rate and, particularly, confirmed disability progression. An additional consideration is that beneficial clinical effects could be greatest in patients who are vitamin D deficient (< 50 nmol/L) at baseline. A recent Cochrane Review did not identify any benefit of vitamin D but cautioned that their assessment was based on studies which they graded as providing very low-quality evidence (Jagannath et al., 2018).

A limited number of studies have assessed whether vitamin D can prevent conversion from CIS to MS. Derakhshandi et al. randomised 30 patients with optic neuritis to take either placebo or 50,000 IU vitamin D_3 weekly for 12 months (Derakhshandi et al., 2013). Their primary endpoint was conversion to MS and their secondary endpoints were changes to measures of MRI lesion load. They reported a 68.4% risk reduction in conversion to MS in their vitamin D treatment group as well as reductions of new MRI lesions. O'Connell and colleagues studied the immunological effects of vitamin D supplementation in 29 patients with CIS and 38 healthy controls (O'Connell et al., 2017). They randomised participants to take placebo, 5000 IU or 10,000 IU vitamin D_3 daily. The primary endpoint of difference in T cell subset frequencies was not met. There was also no benefit in clinical and radiological disease activity with vitamin D supplementation, though their study was limited by short duration of 24 weeks.

Despite trends of benefit on clinical or paraclinical measures of MS disease activity in vitamin D randomised-controlled studies, firm conclusions cannot yet be drawn and further study is warranted. Well-designed and sufficiently powered trials are ongoing. These include the VIDAMS study (NCT01490502). Another important question is whether vitamin D supplementation can prevent MS. The Australia and New Zealand-based PREVANZ study (ACTRN12612001160820) and the French D-LAY-MS study (NCT01817166) of vitamin D supplementation in CIS will hopefully shed additional light. Both PREVANZ and D-LAY-MS are recruiting patients with a recent attack consistent with CIS and randomising them to placebo or vitamin D supplementation at various doses (1000 IU, 5000 IU or 10,000 IU daily in PREVANZ, and 100,000 IU fortnightly in D-LAY-MS). Primary outcomes are risk of recurrent disease activity in PREVANZ and conversion to MS in D-LAY-MS, respectively. Most pertinently, these trials use their intervention as monotherapy, with participants not on any immunomodulatory therapies before or during the trial. These therefore avoid the confounding which occurs when vitamin D is supplemented as an add-on with concurrent MS-specific therapy, and increases the power of these studies to detect a treatment benefit.

7.3. Vitamin D supplementation and safety

Safety signals were excellent from the vitamin D randomised-controlled trials in MS. Patients with contraindications for high-dose vitamin D supplementation, such as primary hyperparathyroidism, renal dysfunction and granulomatous disease, were generally excluded. In the largest of these which supplemented their vitamin D arm with 14,007 IU daily for 44 out of the 48 week study duration, median serum 25(OH)D level increased to 215 nmol/L at the end of the study (Hupperts et al., 2019). Vitamin D supplementation was well-tolerated and no patients developed hypercalcaemia.

Several observational studies have reported an increased risk of mortality at both low and high serum vitamin D levels to suggest a J-curve relationship between serum vitamin D level and mortality, although this is not a consistent finding (Melamed et al., 2008; Michaëlsson et al., 2010). However a harmful effect on mortality has not been borne out by vitamin D supplementation studies. A recent large randomised-

controlled trial of vitamin D supplementation (2000 IU daily) with 25,871 participants and median intervention period of 5.3 years did not find any significant association with all-cause mortality but did suggest a protective effect against cancer mortality (Manson et al., 2019). There were no significant safety concerns detected in regards to supplementation. A reduced risk of cancer mortality was also echoed by two meta-analyses (Keum et al., 2019; Zhang et al., 2019). Based on the available evidence, vitamin D supplementation is overall safe in the absence of any disturbed calcium-vitamin D metabolism. Given vitamin D toxicity can occur at extremely high doses (often >50,000 IU/day) over prolonged duration, it is prudent to aim for vitamin D repletion (between 75-120 nmol/L) among MS patients, pending further evidence of benefits at higher levels.

8. CONCLUSION

Since its discovery and use as treatment for rickets, vitamin D is now recognised to have extra-skeletal effects, including immunomodulatory effects. Overall, vitamin D induces a tolerogenic immune phenotype, with promotion of Treg phenotype differentiation and increased production of the anti-inflammatory cytokine IL-10. The strongest evidence for this comes from *in vitro* studies, but is also supported by *in vivo* experiments. Vitamin D deficiency is associated with increased risk for of a number of autoimmune diseases including MS, with these observations being compatible with vitamin D's immune effects. The involvement of autoimmune disease risk genes in vitamin D metabolism and enrichment of VDR binding in proximity to these genes further suggest a role for vitamin D in disease pathogenesis.

However, the specific mechanism by which vitamin D alters MS and other autoimmune disease risk remains elusive. Vitamin D's immunoregulatory capacity as a therapeutic to potentially prevent, or ameliorate, MS is not yet proven. To better understand the *in vivo* actions of vitamin D, examination of immune cell subsets rather than whole blood particularly with transcriptomic analysis may yield novel cell-specific insights. Whether patients with MS have significant differences in vitamin D processing and response when compared to healthy individuals is still unanswered. The use of established and well-accepted bioinformatic pipelines will allow for improved comparability of results between transcriptomic studies. In regards to its therapeutic potential, pre-existing supplementation studies for MS have failed to

demonstrate a definitive benefit in disease activity measures. These studies were underpowered, though the larger studies suggest a beneficial effect on MRI measures of MS disease activity. Two randomised, placebo-controlled monotherapy trials are studying prevention of MS (recurrent disease activity) in patients after a first attack. Results are eagerly awaited.

NOMENCLATURE OF TARGETS AND LIGANDS

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander et al., 2019).

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TABLES

Table 1. Vitamin D supplementation studies in relatively healthy adults and effects on the immune system

Study	Study design	Participants	Interventions	Intervention duration	Baseline serum 25(OH)D, mean (nmol/L)	Post-intervention serum 25(OH)D, mean (nmol/L)	Findings			
							Monocytes & dendritic cells	B cells	T cells	Cytokines
Prietl et al., 2010	Interventional study	Healthy adults. N = 50	D_3 140,000 IU at baseline and at 4 weeks	8 weeks	59.6	145			↑ %Treg	
Bock et al., 2011 Prietl et al., 2014	Double-blind RCT	Healthy adults. N = 59	D_3 140,000 IU monthly (N = 30). Placebo (N= 29).	12 weeks	Vitamin D group: 63.6 Placebo: 64.5	Vitamin D group: 138 Placebo: 52.8	No change in %DC (peripheral) and absolute monocyte and myeloid DC counts.	No change in %$CD19^+$ B cell.	↑ %Treg only in vit D group. No change to Treg suppressive capacity.	
Allen et al., 2012	Interventional study	Healthy adults. N = 4	D_3 5000 IU daily for 10 weeks, then either 10,000 IU or 5000 IU daily for 5 weeks	15 weeks	38	180			↓ %Th17	↑ IL-10 level (culture supernatant)
Drozdenko	Interventional	Healthy	D_3	12 weeks	Vitamin	Vitamin D		↑ %$CD38^+$ B	↓ %IFN-γ^+	

et al., 2014	study	adults. N = 43	escalating dose (2000-8000 IU daily) (N = 25). Control (N= 18).		D: 40.1 Placebo: 49.1	group: 159 Placebo: 30		cells in vit D group. No change to serum Ig concentrations.	and IL-17$^+$ CD4$^+$ T cells.	
Konijeti et al., 2016	Double-blind RCT	Pre- or stage 1 hypertensive participants. N = 38	D_3 low (400 IU daily; N = 18) or high (4000 IU daily; N = 20) dose	2 months	Low dose: 39.3 High dose: 41.8	Low dose: 53.7 High dose: 66.2			↓ CD4$^+$ T cell activation in high dose group.	
Bak et al., 2018	Interventional study	Healthy adults. N = 10	D_3 200,000 IU loading dose, then 20,000 IU daily	15 days	52[a]	200[a]	↓ %CD103$^+$ DCs from colonic biopsies			↑ IL-10, TGF-β, TNF-α mRNA expression

DC, dendritic cell; RCT, randomised-controlled trial; TGF-β, transforming growth factor β; Th, T helper cell; Treg, regulatory T cell.
[a]medians provided in article rather than means

Table 2. Vitamin D supplementation studies in multiple sclerosis and clinically isolated syndrome, and immunomodulatory effects

Study	Study design	Participants	Interventions	Intervention duration	Baseline serum 25(OH)D, mean (nmol/L)	Post-intervention serum 25(OH)D, mean (nmol/L)	Findings		
							B cells	T cells	Cytokines
Mahon et al., 2003	Double-blind RCT	MS (N = 39). DMT status not specified.	D_3 1000 IU daily (N = 17). Placebo (N = 22). All received calcium 800mg daily.	6 months	Vitamin D: 43 Placebo: ~38	Vitamin D: 70 Placebo: ~43			↑ serum TGF-β1 in Vitamin D group. No significant change in PBMC TNF-α, IL-2, IFN-γ, IL-13 mRNA expression.
Burton et al., 2010 Kimball et al., 2011	Open-label RCT	MS (N = 49). DMT: IFN beta (24), glatiramer (4), none (21).	D_3 escalating doses up to 40,000 IU daily for 28 weeks, then 10,000 IU daily for 12 weeks, then downtitration (N = 25). Control (N= 24). All	12 months	Vitamin D: 73 Control: 83	Vitamin D: 179 Control: 83		↓ T cell proliferative responses in Vitamin D group.	Cytokine levels below detection sensitivities of assays.

			participants also took calcium 1200mg daily. Controls could take up to cholecalciferol 4000 IU daily.						
Smolders et al., 2010 Knippenberg et al., 2011 Peelen et al., 2013	Interventional study	RRMS (N = 14-15). All on IFN beta.	D_3 20,000 IU daily	12 weeks	50[a]	380[a]	No significant change in B cell numbers or subsets, and plasma Ig.	↑ % $CD4^+IL\text{-}10^+$ T cells. ↓ IFN-γ^+:$IL4^+$ $CD4^+$ T cell ratio. No change in $CD8^+$ T cell cytokine subsets. Non-significant ↑ in Treg function.	Non-significant trend of decrease in BAFF levels.
Mosayebi et al., 2011	RCT	RRMS (N = 62). All on IFN beta-1a.	D_3 300,000 IU monthly (N = 28). Placebo (N = 34).	6 months	Vitamin D: ~25 Placebo: ~25	Vitamin D group: ~140 Placebo: ~25		↓ T cell proliferation response.	↑ IL-10 and TGF-β in culture supernatant.
Golan et al., 2013	Double-blind randomised trial	RRMS (N = 45). All on IFN beta.	D_3 4370 IU (N = 24) or 800 IU (N = 21) daily.	12 months	High dose: 48.2 Low dose: 48	High dose: 122.6 Low dose: 68			↑ serum IL-17 in low dose group.
Røsjø et al., 2015	Double-blind RCT	RRMS (N = 68).	D_3 20,000 IU weekly (N =	96 weeks	Vitamin D: 56	Vitamin D 123			No significant differences in

		DMT: IFN beta (32), glatiramer (2), natalizumab (1), none (33).	36). Placebo (N = 32).		Placebo: 57	Placebo: 63			change to 11 serum markers of inflammation.
Åivo et al., 2015	RCT	RRMS (N = 59). All on IFN beta-1b.	D_3 20,000 IU weekly (N = 30). Placebo (N = 29).	12 months	Vitamin D: 54 Placebo: 55	Vitamin D: 109 Placebo: 51			↑ serum TGF-β/LAP levels. No significant change of other measured cytokines, including IL-10, IFN-γ.
Ashtari et al., 2015 Toghianifar et al., 2015	Double-blind RCT	RRMS (N = 94). All on IFN beta.	D_3 50,000 IU every 5 days (N = 47). Placebo (N = 47).	3 months	Vitamin D: 70.7[a] Placebo: 99[a]	Vitamin D: 211[a] Placebo: 71.7[a]			Significant positive correlations of vitamin D treatment with log of IL-10 & IL-17, but changes in serum IL-10 & IL-17 not significant.
Sotirchos et al., 2016	Double-blind randomised trial	RRMS (N = 40). DMT: IFN	D_3 10,400 IU (N = 19) or 800 IU (N =	6 months	High dose: 67.8	High dose: 155 Low dose:		↓ % $IL\text{-}17^{+}CD4^{+}$, $CD161^{+}CD4^{+}$, effector memory	No change in 51 measured serum cytokine levels.

		beta (12), glatiramer (10), natalizumab (11), fingolimod (4), none (2), other (1).	21) daily.		Low dose: 69.8	87		$CD4^+$, & $CD85j^+CD8^+$ T cells, and ↑ % central memory & naïve $CD4^+$ T cells in high dose group.	
Muris et al., 2016 Rolf et al., 2018b	RCT	RRMS (N = 53). All on IFN beta-1a.	D_3 6670 IU daily for 4 weeks, then 14,007 IU daily (N = 30). Placebo (N = 23).	48 weeks	Vitamin D: 60 Placebo: 54	Vitamin D: 231 Placebo: 60	No change in % regulatory B cell.	↓ $\%IL4^+CD3^+CD8^-$ T cells in placebo group. ↓ CD25 expression on T reg in placebo group. No change in % Treg.	↑ IL-5 & TGF-β (culture supernatant) in placebo group.
Mrad et al., 2017	Interventional study	RRMS (N = 46). All on IFN beta.	D_3 10,000 IU weekly if serum $25(OH)D_3$ < 62.5 nmol/L at baseline (N = 21).	3 months	Low vitamin D status group: 39.8 High vitamin D status group: 146	Low vitamin D status group: 129 High vitamin D status group: 155		No change in T cell subsets.	IFN-γ level (culture supernatant) significantly higher in low vitamin D group.
Rolf et al., 2019	Double-blind RCT	RRMS (N = 27).	D_3 4000 IU daily (N =	16 weeks	Vitamin D: 76[a]	Vitamin D: 135[a]			↓ TNF-α levels (culture

		DMT: IFN beta (15), glatiramer (2), dimethyl fumarate (5), teriflunomide (2), none (3)	12). Placebo (N = 15).		Placebo: 78[a]	Placebo: 81[a]			supernatant) following supplementation.
O'Connell et al., 2017	Double-blind RCT	CIS (N = 29). No DMT. Healthy controls (N = 38).	D_3 10,000 IU (N = 25) or 5000 IU (N = 23) daily. Placebo (N = 19).	24 weeks	CIS: 53 Control: 52	CIS 10,000 IU: 168 CIS 5000 IU: 129 CIS placebo: 71 Control 10,000 IU: 188 Control 5000 IU: 144 Control placebo: 54		No change in % IFN-γ^+ or IL-17^+ $CD4^+$ T cells.	No change in IL-10, IL-17, IFN-γ (culture supernatant).

CIS, clinically isolated syndrome; DMT, disease-modifying therapy for multiple sclerosis; MS, multiple sclerosis; RCT, randomised-controlled trial; RRMS, relapsing-remitting multiple sclerosis.

[a]medians provided in article rather than means

Table 3. Vitamin D supplementation randomised-controlled trials in multiple sclerosis and clinically isolated syndrome, and clinical outcomes

Study	Participants	Interventions	Intervention duration	Baseline serum 25(OH)D, mean (nmol/L)	Post-intervention serum 25(OH)D, mean (nmol/L)	Findings		
						Relapse	Progression	MRI
Burton et al., 2010	MS (N = 49): RRMS (45), SPMS (4). DMT: IFN beta (24), glatiramer (4), none (21).	D_3 escalating doses up to 40,000 IU daily for 28 weeks, then 10,000 IU daily for 12 weeks, then downtitration (N = 25). Control (N= 24). All participants also took calcium 1200mg daily. Controls could take up to D_3 4000 IU daily.	12 months	Vitamin D: 73 Control: 83	Vitamin D: 179 Control: 83	Treatment group ARR 0.44 pre to 0.26 post (↓ 41%), NS.	Treatment group EDSS 1.46 pre to 1.15 post, NS.	
Stein et al.,	RRMS (N =	D_2 high dose	6 months	High	High dose:	36.5% of high-dose	Higher	No significant

2011	23). DMT: IFN beta (14), glatiramer (5), none (4)	(titrate to serum 25(OH)D 130-175 nM; N = 11) or low dose (1000 IU daily; N = 12).		dose: 59[a] Low dose: 53.5[a]	120[a] High dose: 69[a]	group had relapse vs 0% of low dose group.*	median EDSS post-treatment in high dose group (3 vs 2)*	differences in changes in no. gadolinium-enhancing or T2 lesions.
Kampman et al., 2012	RRMS (N = 68). DMT: IFN beta (31), glatiramer (2), natalizumab (1), none (34).	D_3 20,000 IU weekly (N = 35). Placebo (N = 33).	96 weeks	Vitamin D: 56 Placebo: 57	Vitamin D 123 Placebo: 62	Treatment group ARR 0.11 pre to 0.14 post, NS.	Treatment group EDSS 2.61 pre to 2.77 post. No significant differences in changes in EDSS, MSFC composites, grip strength, fatigue.	
Soilu-Hänninen et al., 2012	RRMS (N = 66). All on IFN beta-1b	D_3 20,000 IU weekly (N = 34). Placebo (N = 32).	12 months	Vitamin D: 54 Placebo: 56	Vitamin D: 110 Placebo: 50	Treatment group ARR 0.49 pre to 0.26 post, NS.	Treatment group EDSS 2 pre to 1.8 post. No significant differences in changes in EDSS, time 10 foot tandem walk, timed 25 foot	↓ T1 enhancing lesions in treatment group at 12 months, 0.1 vs 0.7 in placebo group.* Change in T2 burden of disease, 83 mm^3 treatment group vs 287 mm^3

							walk.	placebo group; NS.
Mosayebi et al., 2011	RRMS (N = 62). All on IFN beta-1a.	D_3 300,000 IU monthly (N = 28). Placebo (N = 34).	6 months	Vitamin D: ~25 Placebo: ~25	Vitamin D group: ~140 Placebo: ~25		Treatment group EDSS 2.1 pre to 2.31 post, NS.	No significant difference in no. of gadolinium-enhancing lesions.
Shaygannejad et al., 2012	RRMS (N = 50). DMT: IFN beta (43), other (2), none (5)	Calcitriol 0.25 µg for 2 weeks, then 0.5 µg daily (N = 25). Placebo (N = 25).	12 months	Not reported	Not reported	↓ ARR in both groups, NS.	EDSS stable in Vitamin D group, ↑ in Placebo group.* EDSS at 12 months between groups, NS.	
Golan et al., 2013	RRMS (N = 45). All on IFN beta.	D_3 4370 IU (N = 24) or 800 IU (N = 21) daily.	12 months	High dose: 48.2 Low dose: 48	High dose: 122.6 Low dose: 68	No significant change in ARR.	No significant change in EDSS.	
Achiron et al., 2015	MS (N = 158). 107 (67.7%) on DMT.	Alfacalcidol 1 µg daily (N = 80). Placebo (N = 78).	6 months	Not reported	Not reported	Greater ↓ in no. of relapses in Treatment (52 to 8) vs Placebo (48 to 25) arm.* Relapse-free 89.5% Treatment vs 67.1% Placebo.*	No significant difference in EDSS.	
Camu et al.,	RRMS (N =	D_3 100,000 IU	96 weeks	Vitamin	Vitamin D:	ARR not	Of	Of completers:

2019	129). All on IFN beta-1a.	2-weekly (N = 63). Placebo (N = 66).		D: 49.19 Placebo: 48.25	156.92 Placebo: 57.23	significantly different. Of subjects completing follow up: 60.5% ↓ in relapse risk.*	completers: Lower progression of EDSS in treatment group (-0.06 vs 0.32).*	50.6% ↓ in new T1 lesions in treatment group.* ↓ volume of T1 hypointense lesions.* No significant changes to no. enhancing or T2 lesions.
Hupperts et al., 2019	RRMS (N = 229). All on IFN beta-1a.	D_3 6670 IU daily for 4 weeks, then 14,007 IU daily (N = 113). Placebo (N = 116).	48 weeks	Vitamin D: 53[a] Placebo: 54[a]	Vitamin D: 215[a] Placebo: 49[a]	ARR 0.28 vs 0.41 in treatment vs placebo; NS.	No difference in risk of EDSS progression.	32% ↓ in no. of enhancing or new/enlarging T2 lesions in treatment group.* ↓ mean percentage change from baseline in total T2 lesion volume (3.57% vitamin D vs 6.07% placebo group)*
Dörr et al., 2020	RRMS or CIS (N = 53).	D_3 20,400 IU (N = 28) or 400 IU (N =	18 months	High dose: 47 Low dose:	High dose: 163 Low dose:	No difference in cumulative number of relapses.	No difference in disability progression.	No significant difference in new T2 lesions

	All on IFN beta-1b.	25) every alternate day.		44.5	55.8			or lesion volume, no. of new enhancing lesions or percentage brain volume change.
Derakhshandi et al., 2013	CIS with optic neuritis (N = 30). None on DMT.	D_3 50,000 IU weekly (N = 15). Placebo (N = 15).	12 months	Vitamin D: 34.3 Placebo: 41.1	Not reported. In the vitamin D group, target level was 250 nmol/L. Dose adjusted once this was reached.	68.4% relative risk reduction of relapse/conversion to MS.*		Lower incidence rates of black holes, new enhancing and new T2 lesions in Vitamin D group.*
O'Connell et al., 2017	CIS (N = 29). No DMT.	D_3 10,000 IU (N = 12) or 5000 IU (N = 10) daily. Placebo (N = 7).	24 weeks	53	10,000 IU: 168 5000 IU: 129 Placebo: 71	Only 1 patient experienced relapse during study (5000 IU group).	No significant difference in EDSS.	No significant differences in new T2 or enhancing lesions.
Etemadifar and Janghorbani, 2015	Pregnant women with MS (N = 15).	D_3 50,000 IU weekly (N = 6). Control (N = 9).	~24-28 weeks; from 12-16 weeks gestation until delivery	Vitamin D: 38.3 Control: 45.8	Vitamin D: 84.3[b] Control: 36.5[b]	Vitamin D group had 0 relapses while control group had 5 and 4 relapses in pregnancy and post-partum	Control group mean EDSS 1.7 vs Vitamin D group 1.1 at 6 months after	

						respectively.	delivery.*	

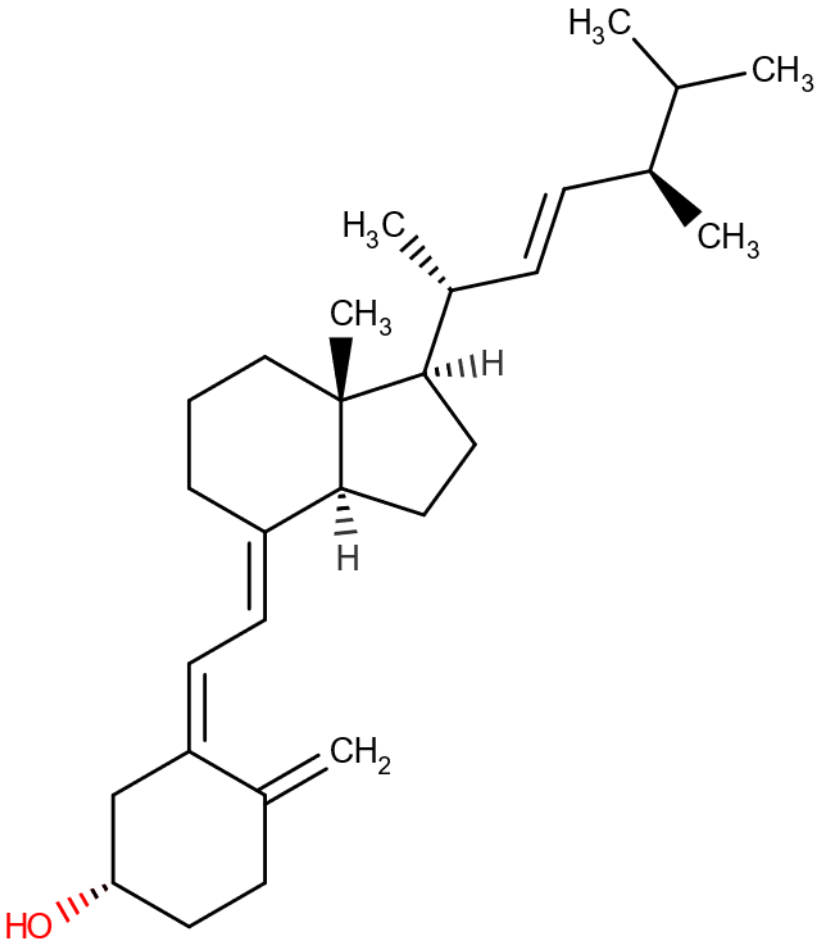
25(OH)D, 25-hydroxyvitamin D; ARR, annualised relapse rate; CIS, clinically isolated syndrome; DMT, disease-modifying therapy for multiple sclerosis; EDSS, Expanded Disability Status Scale; MS, multiple sclerosis; MSFC, multiple sclerosis functional composite; NS, non-significant; RCT, randomised-controlled trial; RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis.
[a]medians provided in article rather than means. [b]serum vitamin D results reported for six-months post-delivery. *$p < 0.05$.

FIGURE LEGENDS

Figure 1. Structural formulae of vitamin D_2 (ergocalciferol) (a) and vitamin D_3 (cholecalciferol) (b), respectively (Wishart et al., 2018).

Figure 2. Metabolism and mechanism of action of vitamin D.
Vitamin D is either synthesised in the skin during UVB exposure or obtained through intake from supplementation or dietary sources. Vitamin D is then metabolised first to 25(OH)D by the liver and then to $1,25(OH)_2D$, which is its active form, by the kidney. The kidney can also metabolise $1,25(OH)_2D$ to the inactive calcitroic acid by the action of CYP24A1. Many target cells have the metabolic enzymes required to convert vitamin D to its active form. In the target cell, $1,25(OH)_2D$ binds to vitamin D receptor (VDR) and retinoid X receptor (RXR). This complex then binds to the genome at vitamin D response elements to modulate gene expression.

FIGURE 1
1a)



b)

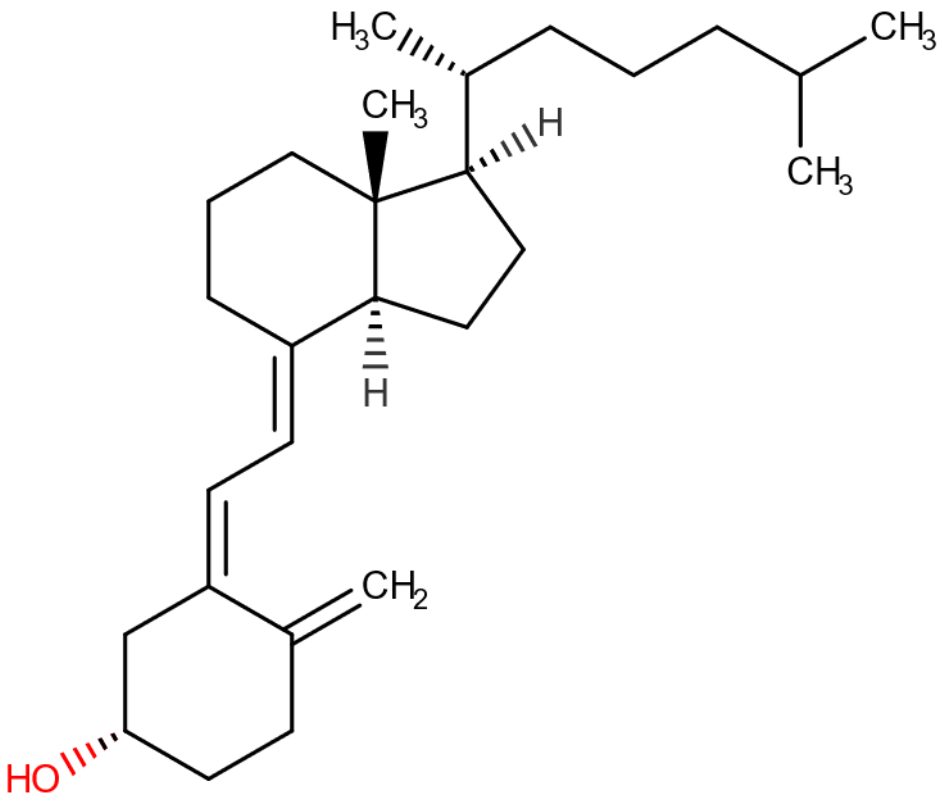
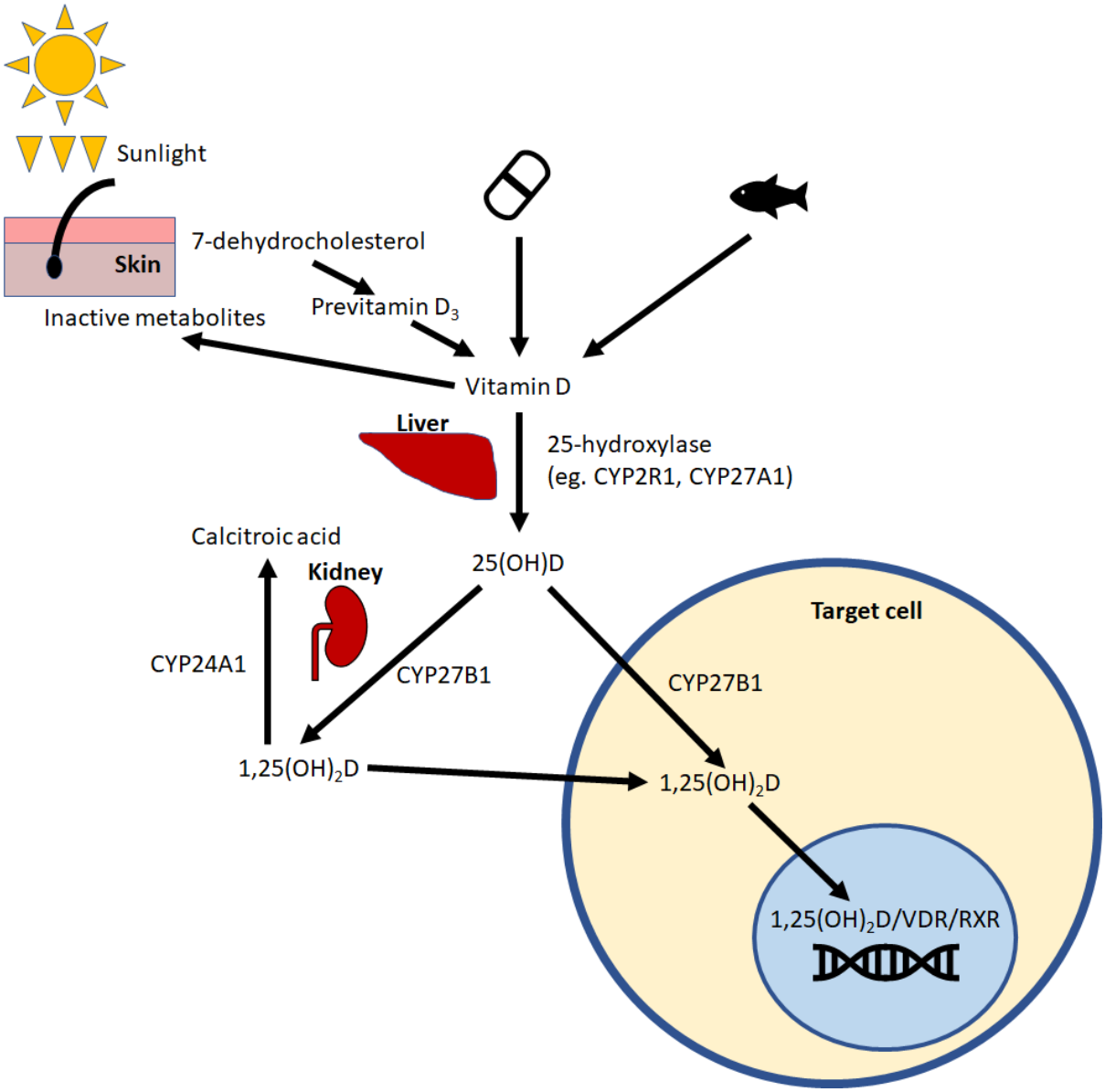
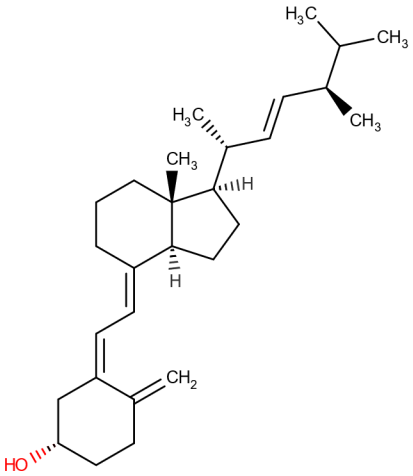


FIGURE 2





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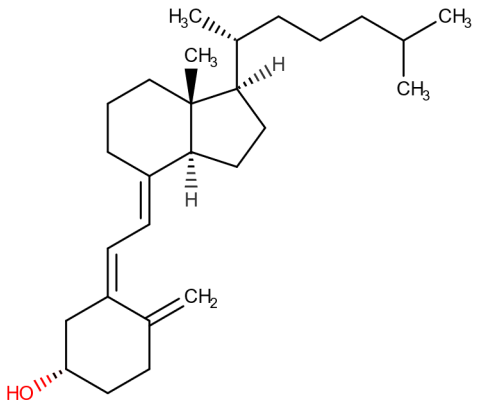

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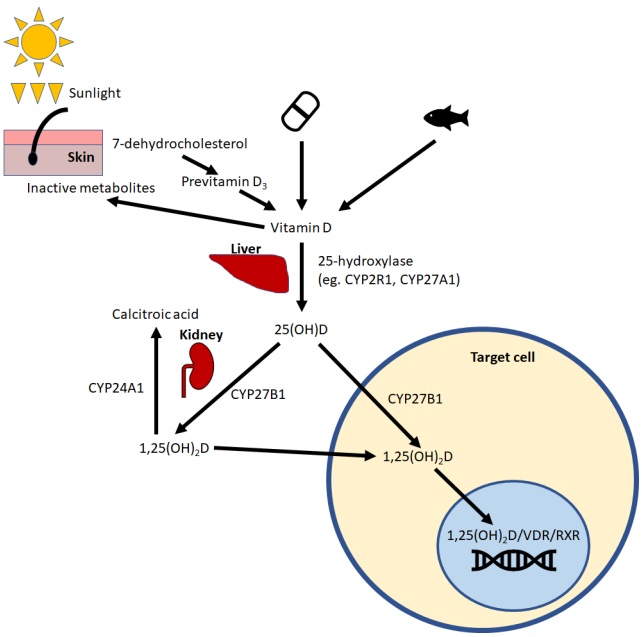

BPH_15201_Figure_2.tif

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