Vitamin D as a Risk Factor for Multiple Sclerosis: Immunoregulatory or Neuroprotective?

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Vitamin D insufficiency during childhood has been linked to the development of multiple sclerosis (MS), typically an adult-onset inflammatory demyelinating disease of the central nervous system (CNS). Since vitamin D was known to have immunoregulatory properties on both innate and adaptive immunity, it was hypothesized that low vitamin D resulted in aberrant immune responses and the development of MS. However, vitamin D receptors are present on many cell types, including neurons, oligodendrocytes, astrocytes and microglia, and vitamin D has profound effects on development and function of the CNS. This leads to the possibility that low vitamin D may alter the CNS in a manner that makes it vulnerable to inflammation and the development of MS. This review analyses the role of vitamin D in the immune and nervous system, and how vitamin D insufficiency in children may contribute to the development of MS.

Keywords: multiple sclerosis, vitamin D, neuroprotection, immune regulation, oxidative stress

INTRODUCTION

The importance of vitamin D (VitD) in health was formally recognized in 1922 when it was determined that cod liver oil and sunlight cured rickets (1, 2). Our understanding of the role of VitD has expanded well beyond bone health with the observation that VitD receptors (VDR) are widely expressed on many cell types in many tissues. VitD is a fat-soluble vitamin with limited availability in foods. Thus, the predominant source of VitD is synthesis in the skin after sun exposure. VitD is a steroid hormone that regulates numerous genes important in cell differentiation, proliferation and homeostasis. Unfortunately, VitD deficiency is prevalent worldwide, with infants, pregnant women, the elderly, and dark-skinned individuals being the most affected (3). There is substantial literature describing the importance of VitD as an immune regulator with a growing body of evidence on the importance of VitD on the development and function of the nervous system. While VitD deficiency has been correlated with a variety of human diseases, there is substantial evidence that VitD deficiency, particularly in children, may be a major risk factor for the development of multiple sclerosis (MS), which is typically diagnosed in young adults. Historically, it has been postulated that low VitD may be promoting a dysregulated and/or hyperactivated immune system that leads to CNS inflammation. However, the fact that low VitD in children appears to be more closely associated with MS than other autoimmune diseases suggests that VitD insufficiency may be playing an important role in the central nervous system (CNS), making it more vulnerable to inflammation. Thus, VitD deficiency in children may be contributing to the risk of developing MS as an adult by dysregulation of genes in both the immune system and CNS.
MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is an inflammatory, demyelinating disease affecting an estimated 2.8 million people worldwide (4). Clinically, MS is characterized by relapsing and progressive neurological dysfunction. In most patients, the disease begins with episodes of neurological dysfunction followed by complete or partial remission—the relapsing/remitting form of the disease (RRMS). In some RRMS patients, the disease is later transformed into uninterrupted progression of neurological deficits—the secondary progressive phase of the disease (SPMS). Other patients’ disease initiates with a slow, progressive accumulation of neurological dysfunction—primary progressive multiple sclerosis (PPMS) (5). Pathologically, MS is characterized by focal plaques of demyelination with activated microglia and abundant peripheral inflammatory cells in the CNS. The cause of the disease is unknown and therapies are limited to disease modifying medications that reduce the number inflammatory lesions and slow disease progression.

Geographical Distribution of MS

Many decades ago it was found that MS has the lowest frequency along the equator, and increases prevalence with increasing latitude (6). The relationship between latitude and MS risks has been observed in several studies. MS frequency among French farmers displayed a north-south gradient and was inversely correlated with exposure to sunlight, though the gene pools and life styles of individuals were broadly comparable (7). UK migrants who live in Tasmania in the south had greater MS frequency than those that migrated to tropical Queensland (8). Although MS risks seem to decrease with migration from high to low latitudes (9), the timing of migration has critical effects on this change. Migration studies have shown that people who are younger than 15 years at the time of migration tend to adopt MS risks of the country to which they migrate, whereas those who are older than 15 years retain similar incidence as their country of origin. A recent study in New Zealand confirmed the latitude gradient, but also found that the prevalence gradient was strongest at birth (10). A comprehensive meta-analysis of 94 studies published through 2018 confirmed the latitude gradient in MS (11). Analysis of sun exposure based on age found that living in an area with high UV-B before MS onset was associated with a 45% lower risk of MS, and a 51% reduction in risk when living in a medium to high UV-B area from 5-15 year of age (12). Overall, the risk of developing MS is largely determined before the age of 15 years (13–17) or at least within the first two decades (18), suggesting a role for the environment in modifying MS risks during childhood and adolescence.

Genetic and Environmental Risk Factors in MS

The cause of MS is unknown. It remains unclear what triggers the immune system to attack the myelin sheath. Twin studies reveal that genetic factors have important roles in MS risk. The rate of concordance for MS among monozygotic twins is 25–40%, which is much higher than the 5% concordance rate among dizygotic twins (19). Genome-wide sequencing studies have further identified human leukocyte antigen (HLA) class II exerting the strongest association with MS risks (20, 21). On the other hand, the concordance rate among monozygotic twins is not 100%, which means MS risks are not fully determined by genetics.

Unquestionably, the environment is also influential on disease susceptibility. Although the identity of environmental factors involved in MS is not yet unequivocally known, accumulating evidence lends strong supports to several candidates: Epstein-Barr virus (EBV) infection, cigarette smoking and VitD. The relationship between EBV seropositivity and MS risks is now firmly established (p < 10–23). Virtually all (99.5%) patients with MS are seropositive for antibodies directed against EBV (22). A recent study analyzed multiple environmental factors that may contribute to MS risk, including VitD levels and EBV antibody titers (23). EBV antibody titers were significantly higher in MS patients and there was an inverse relationship between VitD levels and EDSS, yet no correlation between VitD levels and EBV antibody titers. The prevalence of EBV infection is high (94%) in age/gender-matched controls, so the vast majority of infected individuals do not develop MS, which suggests that EBV infection may be a necessary contributing factor to MS risk but not a cause of MS. For cigarette smoking, a positive association between smoking before age of onset and MS risks is found in some case-control studies (24, 25). Individuals with RRMS have an increased risk of developing SPMS if they have ever smoked, compared with non-smokers (26). These factors—genetics, EBV infection and smoking—may work interactively to determine MS susceptibility, but none of them can fully explain the geographic variations in MS frequency and the changes in risk that occur with migration.

Mouse Model of MS

Much of our fundamental understanding of MS is based on observations in rodent models of MS such as experimental autoimmune encephalomyelitis (EAE). EAE resembles MS in both clinical and pathological aspects (27, 28). Susceptibility to EAE and clinical course vary among strains of mice. For instance, B10.PL and SJL/J mouse strains are two of the more commonly used susceptible strains, whereas BALB/c is much less susceptible (29). SJL/J mice develop a relapsing-remitting disease that can transition into a progressive disease over time, closely resembling RRMS and the transition to the SPMS form (28). C57B/6 mice have become the most utilized EAE model due to the availability of genetically modified mice on the C57B/6 background that allows for defining the role of specific molecules in CNS autoimmunity. The downside of using the C57B/6 mouse model is that disease course and inflammatory components of the lesion have distinct differences from the human condition. Instead, C57B/6 mice develop a rapid-onset, chronic neurological disease without relapses. Furthermore, antibodies and neutrophils contribute significantly to lesion formation which is not typical of MS (30, 31). Some have speculated that C57B/6 EAE may actually be a better model for neuromyelitis optica (NMO), a rare autoimmune neurodegenerative disease very similar in phenotype to MS in which antibodies to aquaporin 4 and neutrophils are known to
contribute to the formation of demyelinating lesions (32). These
different EAE models have contributed to our understanding of
CNS autoimmunity, yet we should be cognizant of how they may
or may not reflect MS.

EAE can be induced by several methods. Active induction of
EAE is done by direct immunization with myelin proteins or
peptides emulsified in complete Freund's adjuvant. The myelin
protein and/or peptide used differs because of variations in MHC
between strains of mice. Passive induction of EAE is
done by transfer of activated myelin-specific CD4 T cells into a
naive mouse. The myelin-specific CD4 T cells can be generated
by immunization with a myelin protein/peptide, followed by
removal of the draining lymph nodes, reactivation of the myelin-
specific T cells in vitro, and injection of the myelin-specific
T cells into naive mice resulting in EAE. Alternatively, T cell
receptor transgenic T cells specific for a myelin peptide can be
used. For example, CD4 T cells from a T cell receptor transgenic
B10.PL mouse in which all the CD4 T cells recognize myelin
basic protein (MBP) Ac1-11 peptide can be activated in vitro and
inhaled into naive B10.PL mice, resulting in classical EAE (33–
35). In EAE, both myelin-specific Th1 and Th17 cells contribute
to pathogenesis, and both cell types have been implicated in
MS (35, 36). While myelin-specific T cells can be found in
both healthy individuals and MS patients, myelin-reactive
CD4 T cells from MS patients have an activated and/or memory
phenotype, whereas those cells are naive in healthy individuals
(37–41), supporting the idea that myelin-specific CD4 T cells are
contributing to disease pathology in MS.

PHYSIOLOGY OF VITAMIN D

For most people, exposure to sunlight is their major source of
VitD. Ultraviolet B (UVB) photolyses 7-dehydrocholesterol
to pre-vitamin D3 in the epidermis, which then isomerizes to
vitamin D3 (Figure 1). VitD can be also obtained from
diet through ingestion of vitamin D2 (ergocalciferol) derived
from plants, colecalciferol supplements/fortified foods and oily
fish. Vitamin D3 in the body then undergoes a series of
hydroxylations, first to 25-hydroxyvitamin D3 (25(OH)D3) in
the liver, the main circulating form of the vitD with relatively
long half-life, and then to biologically active hormone 1,25-
dihydroxyvitamin D3 (1,25(OH)2D3, also known as calcitriol)
in the kidney (42). 1,25-Dihydroxyvitamin D3 is the ligand for
VitD receptor (VDR), a member of the nuclear receptor family of
transcription factors which activates or represses the expression
of many genes (43), and exerts rapid non-genomic effects via the
membrane VDR (44).

VitD primarily acts as a hormone that regulates gene
transcription. VitD enters cells using carrier proteins or diffusion
where it can bind VDR in the cytoplasm (Figure 2). VitD/VDR
complexes are translocated to the nucleus where VDR dimerizes
with retinoid X receptors (RXR). VDR/RXR complexes bind
to VitD response elements which are present in nearly 1,000
genes, thus playing a major transcriptional role in many cell
types. There are non-genomic roles for VitD which occur within
minutes, far too soon to be mediated by altered gene expression.

The most noteworthy non-genomic effect of VitD is calcium
regulation. VitD binds to protein disulfide isomerase family
A, member 3 (PDI/A), resulting in the upregulation of PKA,
p38K and p38MAPK which contribute to the intracellular flux of
calcium (Figure 2). While changes in intracellular calcium
may be independent of VDR engagement, calcium homeostasis
is affected by VDR signaling as seen in people with type
II genetic rickets and VDR-deficient mice which have severe
hypocalcemia (45–48).

Although the best-known function of VitD is to regulate
calcium physiology, it also has important effects on the
development and function of CNS. Neurons and microglia
express VDR. In addition, they can directly metabolize
25(OH)D3 because they express 1-α hydroxylase (49). 1,
25(OH)2D3 has been shown to regulate glial cell line-derived
neurotrophic factor (GDNF) (50) and nerve growth factor
(NGF) (51) expression. The ability of 1,25(OH)2D3 to regulate
certain neurotrophic factors and influence inflammation has led
to the hypothesis that 1,25(OH)2D3 is neuroprotective (52). In
fact, it has shown a reduction in ROS induced cell death and
increased anti-oxidant species in glia cell by 1,25(OH)2D3 (53).
VitD insufficiency is associated with several other neurological
disorders beside MS, including Parkinson disease, schizophrenia,
depression and cognitive decline (54), suggesting its essential
role in maintaining normal CNS function.

How much VitD is optimal is somewhat controversial. The
most commonly published normal range for blood VitD levels
is 20–40 ng/mL (50–100 nmol/L) with levels below 20 ng/mL
(50 nmol/L) considered VitD deficient. The Endocrine Society
considers VitD levels of 20–29 ng/mL (50–74 nmol/L) to indicate
VitD insufficiency, and that VitD levels should be 30–100 ng/mL
(75–250 nmol/L) for optimal health benefit (55). Based on these
guidelines, it is estimated that 30–50% of Americans may be
VitD deficient. A New England Journal of Medicine article
suggested that VitD deficiency may be overstated, and perhaps
our current metrics for VitD-deficiency are incorrect, because of
misinterpretation of the Institute of Medicine's reference values
(56). It should be noted that most of the studies evaluating VitD
levels in health are based on intestinal uptake of calcium and bone
health, so it remains unclear what the optimal dose may be for
optimal overall health.

VITAMIN D AND MS

One of the strongest correlates of latitude is the duration and
intensity of sunlight, and the synthesis of VitD is subsequently
affected by ultraviolet B (UVB) radiation. The incidence gradient
according to latitude and the effect of migration within
genetically uniform groups can be explained by VitD— as the link
between latitude and MS risk. The VitD hypothesis is supported
by studies of sunlight exposure history. The seasonal fluctuations
in VitD levels resulted in decreased VitD concentrations in utero,
which may contribute the month-of birth effect in MS (57). While
not all studies are in agreement, a large meta-analysis found
that individuals born in the Spring have a significantly higher
risks of developing MS compared to individuals born in the fall
FIGURE 1 | Vitamin D production pathway. Vitamin D3 is produced in the skin by UVB irradiation. Typically, the liver and kidney generate intermediates that ultimately generate calcitriol, the active form of VitD. The brain and immune cells also express the enzyme that allows for the generation of calcitriol.

FIGURE 2 | Intracellular function of vitamin D. VitD typically acts as a transcription factor in association with retinoid-X-receptors (RXR) to mediate gene transcription at VitD response elements in promoter regions of genes. However, VitD can have immediate effects on cell function (non-genomic) via interaction with PDIA3 that leads to changes in calcium transport.

(58–60). Insufficient maternal 25-hydroxyvitamin D during early pregnancy is associated with a 2-fold increased risk of MS in offspring (61). Similarly, a Danish study used dried blood spot samples collected near the time of birth to measure VitD in individuals who later developed MS and matched controls (62). Neonatal VitD levels were inversely associated risk of developing MS, supporting the notion that maternal VitD levels may be important to prevent MS in children. Higher sun exposure during childhood (age of 6–15 years) was shown associated with reduced MS risks (63). Time spent on outdoor activities during childhood and adolescence (significant for age of 6–20 years) in the summer was inversely related to the risks, whereas there was no such effect in the winter (64). A study of monozygotic twins who were discordant with MS has shown that twins with MS reported significantly lower levels of childhood sun exposure than their healthy sibling (65). However, sunlight may have benefits to prevent CNS autoimmunity beyond VitD. A study in EAE found that UV light suppressed EAE independent of VitD and VDR (66).

Further evidence for the VitD hypothesis comes from the studies of dietary VitD intake. At high latitudes, prevalence of MS was lower than expected in populations with high
consumption of VitD-rich oily fish (67). A 40% reduction in MS risks was found among women who used supplemental VitD, compared with women who did not use supplements (68). Lastly, a study directly measured the circulating 25(OH)D3 (the circulating form of VitD) concentrations in individuals who served in the US military, and concluded that serum level of 25(OH)D3 in healthy young white adults is an important predictor of their risk of developing MS (69). These epidemiology studies (latitude, migration, history of sunlight exposure, VitD intake and serum concentration of VitD) give credibility to the hypothesis that VitD, especially in early life, has protective effect against MS development. Nevertheless, due to often confounding variables in epidemiology studies, prospective experimental studies are needed to validate the effect of VitD in determining MS risks. A mendelian randomization study in which single nucleotide polymorphisms associated with 25-hydroxyvitamin D were identified and analyzed in the International Multiple Sclerosis Genetics Consortium found that there was a significant increased susceptibility to MS in individuals with a genetically lowered level of 25-hydroxyvitamin D (70). This genetic study supports the epidemiology data that optimal VitD levels are protective against the development of MS. Interestingly, a study of polymorphisms in the VitD-binding protein found an association with MS risk in whites, but not blacks or Hispanics, indicating that VitD may not be a significant risk factor in all ethnicities (71).

After disease onset, VitD also acts in modulating MS clinical course. Serum concentrations of 25-hydroxyvitamin D3 in MS patients were lower during relapses than during remissions (72), and correlated inversely with disease severity (73) and frequency of relapse (74, 75). Although these results might indicate lower sun exposure in patients with severe MS rather than a beneficial effect of VitD, convincing studies with EAE have demonstrated the immunomodulatory effect of VitD in inflammatory CNS disease. Expression of VDR has been described in immune cells, including dendritic cells, macrophages and activated T and B cells (76). VitD supplementation clearly suppressed EAE preventively (77, 78) and therapeutically (79). Moreover, the therapeutic effects of VitD required VDR function in T cells (80), and were through promoting IL-4, TGF-β (81) and IL-10 (82) production, and inhibiting TH1 cells differentiation (83, 84). With these results established from EAE, experimental basis supports the beneficial role of VitD in modulating disease progression. Yet, there are numerous studies that indicate that VitD supplementation in MS patients has little, if any benefit to reducing symptoms (85). The SOLAR trial found that VitD supplementation (14,000 IU/d) in MS patients on interferon beta-1a was beneficial in reducing new lesions, but no change in progression of disability or annualized relapse rate was observed (86). In the 2-year CHOLINE trial, MS patients on interferon beta-1a were given 100,000 IU of oral cholecalciferol or placebo biweekly. The end point (changed in annualized relapse rate) was
not met, yet there were positive benefits observed on imaging and the average EDSS score was significantly lower in the treatment group (87). Analysis of 12 random controlled trials evaluating VitD supplementation concluded that VitD supplementation had no significant benefit on relapse rates, progression of disability or MRI lesions (88). Thus, the benefit of VitD supplementation in MS patients is unclear, but given that most MS patients are VitD deficient, supplementation is prudent and likely benefits that overall health of the patients.

**VITAMIN D AND THE IMMUNE SYSTEM**

The first evidence that VitD may affect the immune system came from a study in 1983 in which VitD promoted the fusion of macrophages (89). In 1986, it was shown that VitD inhibited IL-2 production and proliferation by T cells (90), providing solid evidence that VitD had the capacity to regulate T cells. There is now substantial evidence that VitD is a major regulator of both innate and adaptive immune cells and influences the outcomes of infections, cancer and autoimmunity.

**Innate Immunity**

Activated macrophages and monocytes upregulate expression of CYP27B1, the gene that encodes 1α-hydroxylase, the enzyme that converts 25-hydroxyvitamin D₃ to 1,25-dihydroxyvitamin D₃ (calcitriol), the active form of VitD (Figure 1), indicating that macrophages/monocytes have the capacity to increase VitD at site of inflammation (91). The local expression of VitD by activated macrophages/monocytes sets up an autocrine pathway since macrophages/monocytes express VDR, resulting in the production of anti-microbial products such as cathelicidin and defensins. Cathelicidin is particularly important against infections by destabilizing microbial membranes and disrupting viral envelopes (92–94). Equally important, VitD regulates the maturation and activation of macrophages and dendritic cells, compromising their ability to be effective antigen presenting cells (Figure 3). Upon TLR engagement, macrophages and dendritic cells upregulate MHCII, CD80/CD86, CD40 and cytokines which are critical to antigen presentation to T cells. VitD suppresses these molecules, promoting macrophages and dendritic cells that are immature and somewhat tolerogenic (95, 96). This suppression of macrophages and dendritic cells may be due to the suppression of toll-like receptors (97–99) or inhibition of IL-12 via NF-κB (100) mediated by VitD.

VitD affects the function of microglia which are known to secrete inflammatory mediators that contribute to myelin damage during CNS autoimmunity. Mice with EAE treated with calcitriol immediately following EAE induction have reduced microglia activation and oxidative stress, and less blood brain barrier permeability (101). Partial deletion of VDR in young mice attenuated microglia activation and reduced the incidence and severity of EAE (102). In various models of CNS diseases and injury, VitD has been shown to regulate microglia phenotype and oxidative stress (103–106). Thus, VitD appears to effectively skew microglia from a pro-inflammatory M1 phenotype to a reparative M2 phenotype, reducing inflammation and limiting demyelination.

**Adaptive Immunity**

Both T cells and B cells can express VDR and respond to VitD (Figure 3). There is actually very little, if any, VDR expression on resting human T and B cells; however, VDR is rapidly upregulated upon activation (107–109). Similar to innate immune cells, activated T cells express CYP27B1 and can make active VitD. VitD suppresses T cell proliferation via reduced IL-2. In addition, VitD alters the differentiation of CD4+ T cells by skewing CD4+ T cells toward Th2 and away from Th1 and Th17, the phenotypes associated with MS (110, 111). Also of particular relevance to MS, VitD promotes the differentiation of Tregs (112), which are known to be defective in MS patients (113–118). The mechanism by which VitD promotes Treg development appears to be via altered APCs, since VitD added to human dendritic cells alters glucose metabolism favoring the differentiation of Tregs over Teff cells (119). VitD status in MS patients positively correlates with Treg function, supporting the observation the VitD promotes Treg development (120). VitD in association with CD46 was shown to promote Type I regulatory T cells (Tr1) cells in MS patients (121), indicating that optimal VitD may be an important component of immune regulation.

B cell differentiation and maturation into plasma cells is also regulated by VDR, thus affecting antibody production. In addition, VitD downregulates co-stimulatory molecules on B cells, similar to what is observed for macrophages and dendritic cells (122). Thus, optimal VitD may compromise the ability of B cells to act as antigen presenting cells. B cell –depletion therapies have been very beneficial in the treatment of MS. Given that the beneficial affects appear to be independent of antibody production, there is speculation and evidence that B cells are critical antigen-presenting cells in MS (123–125). An immune profile study on MS patients on B cell depletion therapy indicated that the T cell profile showed a favorable change, reflected by a reduction in memory T cells and an increase in Tregs (124, 125), consistent with the role of B cells as antigen-presenting cells. Thus, low VitD may enhance the ability of B cells to drive the activation and differentiation of T cells, increasing the probability developing MS.

**VITAMIN D AND THE CENTRAL NERVOUS SYSTEM**

Substantial evidence indicates that VitD acts as a neurosteroid. VDR is expressed throughout the developing and mature brain, including the hippocampus, amygdala, hypothalamus, cortex and cerebellum (49, 126, 127), implicating VitD as an important modulator of gene expression throughout the CNS. Furthermore, 1α-hydroxylase and 25-hydroxylase are both expressed in the brain providing the critical enzymes to generate VitD locally (49, 128). While a major role of VitD is gene regulation, it also has non-genomic functions, particularly regulation of calcium signaling which is critical in normal cellular function.

**Vitamin D and Neurogenesis**

VitD promotes cell differentiation and apoptosis which are critical for embryonic development. When VitD is removed
during gestation in model systems, multiple regions of the brain have increased cell proliferation and decreased apoptosis, as well as enhance cell proliferation, leading to CNS anomalies (129–131). The increased proliferation was mediated by increased expression of cyclin genes which were regulated by VDR signaling (132), while the reduction in apoptosis may have been due to increased levels of BAX and Bcl-2 (131). Low VitD also leads to more neural stem cells which may be due to a loss of regulation of cell proliferation or a failure in neural stems to efficiently differentiate into neural cell progenitors (133). Changes in brain morphology have been observed in rodents with VitD deficiency (129, 134). In humans, VitD deficiency is associated with decreased brain volume and enlarged ventricles in older adults (135). Ex vivo studies illustrated that VitD inhibits the proliferation of hippocampal neurons, while promoting neurite outgrowth (130). Analysis of dopaminergic neurons found that VitD-deficiency reduced Nurr1 and P57kip2 gene expression during embryogenesis which are critical to the development and homeostasis of dopaminergic neurons (136). In addition, VitD has been found to regulate the expression of genes essential in the normal function of dopaminergic neurons (137, 138). Interestingly, while it appears that VitD promotes the differentiation of neurons, astrocyte differentiation appears to be impaired by VitD when using adult neural stem cells (139). VitD also promotes the differentiation of neural stem cells into oligodendrocytes (139), the myelinating cells of the CNS. Oligodendrocyte precursor cells fail to differentiate into oligodendrocytes when VDR signaling is blocked (140). These studies implicate VitD as an essential regulator of neuron and oligodendrocyte development. The signaling between neurons and oligodendrocytes is essential to the development of a properly myelinated CNS during early life, as well as remyelination that occurs following CNS damage.

Functional Consequences of Low VitD in the CNS

In addition to the development and differentiation of CNS cells, VitD plays a role in their ability to function properly. The release of several neurotransmitters is affected by VitD. In dopaminergic neurons, VitD promotes the release of dopamine (141). VitD appears to regulate neurotransmitter synthesis, for example, VitD regulates the inhibitory neurotransmitter GABA, via upregulation of GAD65 and GAD67 (142–144). Neurotrophic factors, which are essential for CNS homeostasis and communication between cells in the CNS, are also regulated by VitD. VitD appears to induce nerve growth factor (NGF) expression in neurons (129, 135). Neural stem cells upregulate brain-derived growth factor (BDNF), Glia cell line-derived nerve factor (GDNF), and ciliary neurotropic factor (CNTF) in the presence of VitD (139). In astrocytes, VitD appears to regulate the expression of neurotrophin receptors, as well GDNF, NT-3 and NT-4 (145–147).

VitD also plays a role in neuronal plasticity. In cultured cortical neurons, VitD increased the expression of microtubule associated protein-2, growth-associated protein-43, and synapsin 1 which are important in synaptic vesicle transport and axonal growth (148). Low VitD during embryogenesis resulted in altered expression of proteins important in cytoskeletal integrity, organelle transport, and synaptic plasticity (149, 150), suggesting that VitD is critical to the normal development and function of the CNS.

Calcium Regulation

VitD plays a vital role in regulating calcium levels in the CNS which is particularly important given that high levels of calcium are neurotoxic. In neurons, VitD modulates L-type voltage-gated calcium channels by downregulation of A1C subunits (151). Mice lacking VitD have upregulated L-type voltage-gated calcium channels and elevated calcium influx in neurons (152) (Figure 2). In vitro treatment of neurons with VitD downregulated L-type voltage-gated calcium channels and protected neurons from excitotoxicity (153). VitD was shown to very rapidly increase the uptake of extracellular calcium via L-type voltage gated calcium channels (154). Since this occurred within a few minutes, it was clear that the effect was independent of gene transcription. The increase in calcium influx was dependent on the PKA, p3K, and p38MAPK. The non-genomic effects of VitD have largely been attributed to VitD interaction with PDIa3 (also known as 1,25D3-Marrs) on the plasma membrane (155, 156). In addition, VitD regulates the expression of numerous genes associated with calcium homeostasis vital to the proper regulation of calcium in the CNS and other tissues (150, 154, 157).

Vitamin D and Neuroprotection

Epidemiological data indicate that VitD has neuroprotective properties. Optimal VitD levels during early life appear to be important to minimize the risk of several psychiatric and neurodegenerative diseases. Schizophrenia, depression and autism spectrum disorders have all been associated with low VitD, particularly during embryogenesis and infancy (158–162). There is an inverse correlation between Parkinson’s disease risk and VitD levels (163, 164). Given that VitD protects dopaminergic neurons by upregulating genes numerous genes associated with the function of dopaminergic neurons (137, 138), it is logical that VitD may be a critical factor in minimizing the risk of developing Parkinson’s disease. Similarly, Alzheimer’s disease patients tend to have low serum VitD levels compared to matched healthy controls (165). The risk of dementia and symptoms of neurodegenerative diseases, such as cognitive and memory impairments and impaired motor function, increases with low serum VitD levels (166–169). Serum VitD deficiency is linked to greater infarct volumes, increased overall stroke severity, and worse long-term outcomes in stroke patients (170–172). Impacts on the risks and outcomes in these neurological conditions are clearly multifactorial, but it stands to reason that VitD plays a role in susceptibility and outcome.

The neuroprotective properties of VitD take effect though several mechanisms. Direct neuroprotective action of VitD is associated with the regulation of neurotrophic factors and reduction in oxidative stress. Neurotrophic factors are critical for the differentiation, survival and maintenance of neural and glial cells. VitD stimulates expression of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial cell
line-derived neurotrophic factor (GDNF) and neurotrophin-3 (NT3) (173). Neurotrophic factors downregulate their expression into adulthood, therefore remaining levels have critical cell maintenance functions. Reduction in VitD-assisted neurotrophic factor expression due to deficiency may leave neurons more vulnerable to insult.

Neurons are particularly susceptible to oxidative damage because of increased oxygen consumption, bi-products of neurotransmitter production, excitotoxicity and high overall lipid content (174). Additional neuroinflammation will increase the reactive oxygen species (ROS) load. Adequate VitD levels downregulate intracellular oxidative-stress related activities, while suboptimal levels result in increased oxidative damage and neuronal apoptosis (175). Increased reactive oxygen production has been implicated in the pathogenesis of multiple neurodegenerative conditions, including Parkinson’s disease (176), Alzheimer’s disease (177), Huntington’s disease (178), stroke (179) and Multiple Sclerosis (180), and suboptimal serum VitD levels have been linked to these conditions. VitD is a potent regulator of the nuclear factor erythroid-2-related factor 2 (Nrf2) antioxidant pathway in neurons and glial cells, and intracellular Nrf2 levels are inversely correlated with the accumulation of mitochondrial ROS. Within the CNS, upregulation of Nrf2 target genes superoxide dismutase (SOD), catalase (CAT) and heme oxygenase-1 (HMOX1) to make neurons more resistant to oxidative insults (181). Furthermore, neuroprotective signaling pathways, such as the BDNF-TrkB pathway that is essential for mature neuron survival and normal function, also signal Nrf2 activation (182, 183). VitD then has double the influence on neuronal survival – first in neurotrophic action by increasing levels of BDNF, and second in oxidative defense by direct activation of Nrf2. The neuroprotective properties of VitD center around its antioxidant function, and in conjunction with neurotrophic factor expression, likely enhances neural defense and repair mechanisms.

**Vitamin D as a Neuroprotective Agent in MS Through Antioxidant Pathways**

Oxidative stress and mitochondrial dysfunction are prominent features of MS. T cells can produce ROS and T cell activity and proliferation are influenced by ROS, adding an increased level of complexity to the impact of ROS in MS (184). Activated microglia and macrophages are the major contributors to the elevated ROS observed in the disease. These cells produce oxidizing radicals such as superoxide, hydrogen peroxide and nitric oxide with the help of ROS-generating enzymes, such as myeloperoxidase, NADPH oxidase and nitric oxide synthase. ROS have shown to mediate demyelination in both MS and its animal models (184, 185). Studies in postmortem brains of MS patients have identified myeloperoxidase expression in activated macrophages and microglia near lesions. Elevated expression of myeloperoxidase was detected in demyelinated regions of postmortem MS brain homogenates when compared to unaffected regions from the same individual (186, 187). Other markers of oxidative damage, such as 4-hydroxy-2-nonenal (4-HNE), produced by lipid peroxidation of cell membranes, and nitrotyrosine, the product of nitric oxide and superoxide reactions, increase and accumulate in macrophages and astrocytes in MS lesions (188–190).

ROS in later stages of MS stems from mitochondrial dysfunction within neurons themselves. Mitochondrial dysfunction and associated ROS have been implicated in non-inflammatory mediated axonal degeneration that occurs with chronic demyelination. It is believed that mitochondria become taxed after sodium channel redistribution in response to demyelination. Sodium channel redistribution causes large influxes of sodium, taxing the ATP dependent sodium-potassium pump (191). Increased ATP needs trigger mitochondria production and proliferation, resulting in increased ROS (192, 193). Notably, increased mitochondrial heat shock protein 70, a marker of mitochondrial stress, has been observed in astrocytes and axons within MS lesions (192). There is some controversy regarding whether increased ROS from mitochondria exists primarily due to mitochondrial proliferation and ATP production after demyelination (chronic injury) or if mitochondria actually acquire oxidative damage during the inflammatory stage of the disease (acute injury) (194).

Antioxidant enzymes are the endogenous ROS defense system in the CNS. ROS exposure activates Nrf2, which then translocates to the nucleus and activates antioxidant response element promoters (ARE) for antioxidant enzyme production. Expression of hundreds Nrf2 responsive antioxidant genes have already been identified (195). Numerous studies have suggested a role for Nrf2 inactivity in the pathogenesis of MS. Nrf2 knock-out EAE mice experience more rapid disease onset, a more severe clinical course, increased gial activation, increased pro-inflammatory cytokine expression and increased axonal degeneration compared to Nrf2 inclusive controls (196–198). Conversely, increasing the activity of Nrf2 in the CNS of EAE mice lessened the clinical severity (199). In postmortem brain tissue of MS patients, NRF2 is strongly upregulated in active MS lesions and expression is most pronounced in degenerating neurons and glial cells, including oligodendrocytes (200, 201).

NRF2 activity is already relevant in MS clinical treatment. Both VitD and dimethyl fumerate (DMF or Tecfidera™) are NRF2 activators. DMF treatment is an approved oral RRMS therapy known to reduce disease activity and progression, and accomplishes these outcomes via immunomodulatory and neuroprotective mechanisms (202–207). VitD and DMF both signal through the NRF2/KEAP1 pathway to generate antioxidant action and specifically increase glutathione signaling for neuroprotection. DMF and VitD can also exert protective effects by reducing proinflammatory cytokine expression and increasing neurotrophic factor expression (208). DMF and VitD derivatives have demonstrated a cooperative effect on increased VDR expression and Nrf2 activity that limit leukemia progression (209). Although mechanisms of action overlap, DMF is a safer therapeutic option for avoiding calcemic toxicity that can occur with long-term use of high levels of VitD. In fact, excess VitD can exacerbate EAE, emphasizing the point that a measured approach to VitD-based therapies is critical (210). It should also be noted that melatonin which is produced during the dark has similar anti-oxidant properties as VitD in
EAE (211, 212). There is contradicting data on the relationship between melatonin and VitD, yet both appear to be beneficial in reducing CNS inflammation. Thus, the interplay between appropriate sunlight to optimize VitD levels and appropriate darkness to optimize melatonin to maintain circadian rhythm should be considered in strategies to prevent and/or treat MS. It is unlikely that DMF mimics every function of VitD, but similarities in function between DMF and VitD suggest that VitD has a critical role as an endogenous neuroprotective mediator in MS.

An important consideration is the temporal influence of VitD, NRF2 activity, and the endogenous antioxidant system during the course of MS. Upregulated expression of NRF2 in MS brain lesions suggests that the NRF2 pathway is already highly active in distressed cells (200, 201) and it appears that endogenous antioxidant mechanisms are not enough to halt demyelination and axonal degeneration at end stages of disease. However early in the disease, VitD and NRF2 signaling may have increased potential for neuroprotection because less neuroinflammatory-induced oxidative damage has occurred. Understanding the neuroprotective potential of VitD in early stages of MS is complicated by the striking frequency of VitD deficiency in patients at the time of diagnosis. Therefore, whether preventing VitD deficiency prior to disease onset can enhance neuroprotection and alter disease progression warrants further investigation.

**IMMUNOREGULATION VS. NEUROPROTECTION**

MS is a complex disease in which immune and nervous system components interact to form and sometimes, resolve CNS inflammatory, demyelinating lesions. Genetic studies have largely implicated immune genes as susceptibility factors, supporting the hypothesis that MS is an immune-mediated disease and that the CNS is the unfortunate target of the aberrant immune response. There is significant data to support that VitD has a profound immunoregulatory role on both innate and adaptive immune cells, indicating that low VitD may alter normal immune regulation leading to autoimmunity. Given that the CNS is the sole target of the aberrant immune response in MS, it is important to consider if the CNS is somehow more vulnerable to inflammation in some individuals that may make them at an increased risk of developing MS. While the answer is still unknown, the literature clearly indicates that VitD impacts CNS development and function.

The epidemiology studies indicate that optimal VitD is particularly important during embryogenesis and childhood in determining risk of developing MS. During childhood, our immune system is repeatedly being challenged by pathogens, most of which are cleared with minimal clinical consequences. Some childhood viruses, such as Epstein-Barr, varicella zoster, and some strains of influenza, can infect neurons, yet they typically do not cause clinical CNS manifestations. Even in the absence of CNS clinical signs of illness, do these viruses affect the CNS differently in children with low VitD? Our recent study in which partial VDR deletion was induced in young mice specifically in neurons resulted in an enhanced susceptibility to EAE in adult mice, suggesting that low VitD signaling in neurons in early life may increase the vulnerability of the CNS to inflammation (102). There is substantial evidence that viral infections are affected by VitD levels. Of particular interest in MS is EBV which has been long speculated to be a vital risk factor for the onset of disease and this has been confirmed in a new study of >10 million young adults (213). While many theories have been explored, a recent study identified EBV infection as a precipitating factor that may be driving molecular mimicry (214–217). EBV antibody titers are negatively correlated with VitD levels in MS patients (218) suggesting that these two environmental factors may be synergistic. EBV is also associated with activating endogenous retroviruses (ERVs) and ERV levels in MS plaques correlates with disease activity (219). These ERVs may act as novel antigens that drive CNS inflammation. VitD supplementation mitigates EBV reactivation (220), which may in turn limit ERV activation and the associated inflammation. In a humanized mouse model, it was shown that HLA-DR15-restricted T cells fail to control EBV infection, suggesting that there is potential relationship between the strongest genetic factor (HLA-DR15) and EBV with respect to MS risk (221). The relationship between ERVs, VitD and MS has become of increasing interest and some speculate that EBV may be the missing link between ERVs and VitD that trigger the development of MS (222). While many autoimmune diseases have been associated with low VitD to some extent, the epidemiology data in MS is far more convincing suggesting that low VitD is likely increasing the risk of developing MS due to the negative impact on immune regulation and CNS homeostasis.

A recent study of >1,900 subjects demonstrated that sun exposure negatively correlated with development of MS, and high VitD levels (>30.31 ng/mL) in MS patients reduced the risk of relapses and accumulation of disability (223). The evidence that VitD level is important in risk of developing MS and disease severity appears well established, yet questions still remain as to whether VitD supplementation is beneficial to patients with MS. Since most MS patients have low VitD levels, supplementation is now common practice. Perhaps the more important question is how do we prevent low VitD? Although VitD is currently a common food supplement in many countries, it is unclear whether we are doing enough to ensure that children are getting sufficient VitD. Rickets is rare in many countries, it is unclear whether we are doing enough to ensure that children are getting sufficient VitD. Rickets is rare in countries in which food is supplemented with VitD, indicating that the levels of VitD provided via food supplementation is sufficient for bone health. However, it is unclear whether these levels of VitD are adequate for optimal neuroprotection, given that countries like the United States still have a high incidence of MS. Additional VitD supplementation may be essential for children in higher latitudes where sunlight is very limited for several months each year, and perhaps in children with a family history of MS in which genetic risks are highest. We should also balance pros and cons of sunscreen which...
reduces UVB induced VitD synthesis by 95% and may negatively impact health of our immune and nervous systems. Sunlight remains the best source of VitD so ensuring that children play outside daily may be the best solution to the epidemic of low VitD.

REFERENCES

1. Chick H, Dalyell EJ, Hume EM, Mackay HMM, Henderson-Smith H. The aetiology of rickets in infants: prophylactic and curative observations at the Vienna University Kinderklinik. Lancet. (1922) ii:7–11. doi: 10.1016/S0140-6736(01)08835-2

2. McCollum EV, Simmonds N, Becker JE, Shipley PG. Studies on experimental rickets. XXI An experimental demonstration of the existence of a vitamin which promotes calcium deposition. J Biol Chem. (1922) 53:293–312. doi: 10.1016/S0021-9258(18)85783-0

3. Lips P. Worldwide status of vitamin D nutrition. J Steroid Biochem Mol Biol. (2010) 124:197–300. doi: 10.1016/j.jsbmb.2010.02.021

4. Walton C, King R, Rechtman L, Kaye W, Leray E, Marrie RA, et al. Rising prevalence of multiple sclerosis worldwide: Insights from the atlas of MS, third edition. Multi Scler. (2020) 26:1816–21. doi: 10.1177/13524585209870841

5. Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: a redefinition. Neurology. (1996) 46:907–11. doi: 10.1212/WNL.46.4.907

6. Archeson ED, Bachrach CA. The distribution of multiple sclerosis in US veterans by birthplace. Am J Hyg. (1960) 72:88–99. doi: 10.1093/oxfordjournals.aajh.a120137

7. Vukusic S, Van Bockstael V, Gosselin S, Confavreux C. Regional variations in the prevalence of multiple sclerosis in French farmers. J Neurol Neurosurg Psychiatry. (2007) 78:707–9. doi: 10.1136/jnnp.2006.101196

8. Hammond SR, de Wytt C, Maxwell IC, Landy PJ, English E, McLeod G, et al. The epidemiology of multiple sclerosis in Queensland, Australia. J Neurol. (1987) 280:185–204. doi: 10.1007/BF00215014

9. Gale CR, Martyn CN. Migrant studies in multiple sclerosis. Prog Neurobiol. (1995) 47:425–48. doi: 10.1016/S0079-6180(95)80008-V

10. Sabel CE, Pearson JF, Mason DE, Willoughby E, Abernathy DA, Taylor BV. The latitude gradient for multiple sclerosis prevalence is established in the early life course. Brain. (2021) 144:2038–46. doi: 10.1093/brain/awab104

11. Simpson S, Wang W, Otahal P, Blizard L, van der Mei IAF, Taylor BV. Latitude continues to be significantly associated with the prevalence of multiple sclerosis: an updated meta-analysis. J Neurol Neurosurg Psychiatry. (2019) 90:1193–200. doi: 10.1136/jnnp-2018-320189

12. Tremlett H, Zhu F, Ascherio A, Munger KL. Sun exposure over the life course and associations with multiple sclerosis. Neurology. (2018) 90:e1191–9. doi: 10.1212/WNL.0000000000005257

13. Alter M, Leibowitz U, Spere J. Risk of multiple sclerosis related to age at immigration to Israel. Arch Neurol. (1966) 15:234–7. doi: 10.1001/archneur.1966.00400150012002

14. Dean G, Elian M. Age at immigration to England of Asian and Caribbean immigrants and the risk of developing multiple sclerosis. J Neurol Neurosurg Psychiatry. (1997) 63:565–8. doi: 10.1136/jnnp.63.5.565

15. Hammond SR, English DR, McLeod GJ. The age range of risk of developing multiple sclerosis: evidence from a migrant population in Australia. Brain. (2000) 123:968–74. doi: 10.1093/brain/123.5.968

16. McLeod JG, Hammond SR, Kurtzke JF. Migration and multiple sclerosis in immigrants to Australia from United Kingdom and Ireland: a reassessment. I Risk of MS by age at immigration. J Neurol. (2011) 258:1140–9. doi: 10.1007/s00410-010-5898-4

17. McLeod JG, Hammond SR, Kurtzke JF. Migration and multiple sclerosis in United Kingdom and Ireland immigrants to Australia: a reassessment. II. Characteristics of early (pre-1947) compared to later migrants. J Neurol. (2012) 259:684–93. doi: 10.1007/s00410-011-6244-1

18. Kurtzke JF, Beebe GW, Norman JE. Epidemiology of multiple sclerosis in US veterans III. Migration and the risk of MS. Neurology. (1985) 35:672. doi: 10.1212/WNL.35.5.672

19. Willer CJ, Dyment DA, Risch NJ, Sadovnick AD, Ebers GC. Canadian Collaborative Study Group. Proc Natl Acad Sci USA. (2003) 100:12877–82. doi: 10.1073/pnas.1932604100

20. International Multiple Sclerosis Genetics Consortium, Hafler DA, Compston A, Sawyer S, Landr R, Daly MJ, et al. Risk alleles for multiple sclerosis identified by a genomewide study. N Engl J Med. (2007) 357:851–61. doi: 10.1056/NEJMoa073493

21. Australia and New Zealand Multiple Sclerosis Genetics Consortium, Bahlo M, Booth DR, Broadley SA, Brown MA, Foote SJ, et al. Genome-wide association study identifies new multiple sclerosis susceptibility loci on chromosomes 12 and 20. Nat Genet. (2009) 41:824–8. doi: 10.1038/ng.396

22. Goodin DS. The Causal Cascade to Multiple Sclerosis: a model for MS pathogenesis. PLoS ONE. (2009) 4:e4565. doi: 10.1371/journal.pone.0004565

23. Dominguez-Mozo N, Perez-Perez S, Villarrubia N, Costa-Frassard L, Fernandez-Velasco JL, Ortega-Madrueno I, et al. Herpesviruses antibodies, vitamin d and short-chain fatty acids: their correlation with cell subsets in multiple sclerosis patients and healthy controls. Cells. (2021) 10:119. doi: 10.3390/cells1010119

24. Riise T, Nortvedt MW, Ascherio A. Smoking is a risk factor for multiple sclerosis. Neurology. (2003) 61:1122–4. doi: 10.1212/01.WNL.0000081305.66687.D2

25. Hedstrom AK, Baarnhuijsen M, Linson T, Alfredsson L. Tobacco smoking, but not Swedish snuff use, increases the risk of multiple sclerosis. Neurology. (2009) 73:696–701. doi: 10.1212/WNL.0b013e3181b59c40

26. Hernan MA, Jick SS, Logroscino G, Olek MJ, Ascherio A, Jick H. Cigarette smoking and the progression of multiple sclerosis. Brain. (2005) 128:1461–5. doi: 10.1093/brain/awb471

27. Bjelobaba I, Begovic-Kupresanin V, Pekovic S, Lavrnja I. Animal models of multiple sclerosis: focus on experimental autoimmune encephalomyelitis. Neurosci Res. (2018) 96:1021–42. doi: 10.1002/jnr.24224

28. Kipp M, Nyamoya S, Hochstrasser T, Amor S. Multiple sclerosis models: a clinical and histopathological perspective. Brain Pathol. (2016) 27:123–37. doi: 10.1111/bpa.12454

29. Lindsey JW. Characteristics of initial and reinduced experimental autoimmune encephalomyelitis. Immunogenetics. (1996) 46:292–7. doi: 10.1007/BF02625599

30. Wu F, Cao W, Yang Y, Liu A. Extensive infiltration of neutrophils in the acute phase of experimental autoimmune encephalomyelitis in C57BL/6 mice. Histochem Cell Biol. (2010) 133:313–22. doi: 10.1007/s00418-009-0673-2

AUTHOR CONTRIBUTIONS

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36. Lovett-Racke AE, Yang Y, Racke MK. Th1 versus Th17: are T cell cytokines relevant in multiple sclerosis? Biochim Biophys Acta. (2011) 1812:246–51. doi: 10.1016/j.bbadis.2010.05.012

37. Allegretta M, Nicklas JA, Sirram S, Albertini RJ. T cell responsive to myelin basic protein in patients with multiple sclerosis. Science. (1990) 247:718–21.

38. Lovett-Racke AE, Trotter JL, Lauber J, Perrin PJ, June CH, Racke MK. Decreased dependence of myelin basic protein-reactive T cells on CD28-mediated costimulation in multiple sclerosis patients. A marker of activated/memory T cells. J Clin Invest. (1998) 101:725–30. doi: 10.1016/S0022-5211(98)91630-7

39. Perrin PJ, Lovett-Racke A, Phillip SM, Racke MK. Differential requirements of naïve and memory T cells for CD28 costimulation in autoimmune pathogenesis. Histol Histopathol. (1999) 14:1269–76. doi: 10.14670/HH.14.1269

40. Burns J, Bartholomew B, Lobo S. Isolation of myelin basic protein-specific T cells predominantly from the memory T-cell compartment in multiple sclerosis. Ann Neurol. (1999) 45:33–9.

41. Racke MK, Ratts RB, Arredondo L, Perrin PJ, Lovett-Racke A. The role of costimulation in autoimmune demyelination. J Neuroimmunol. (2000) 107:205–15. doi: 10.1016/S0165-5728(00)00230-7

42. Holick MF. The vitamin D epidemic and its health consequences. J Nutr. (2005) 135:2739S–45S. doi: 10.1093/jn/135.11.2739S

43. Wang TT. Large-scale in silico and microarray-based identification of direct 1,25-dihydroxyvitamin D3 target genes. Mol Endocrinol. (2005) 19:2685–95. doi: 10.1210/me.2005-0106

44. Norman AW. Minireview: vitamin D receptor: new assignments and hints about vitamin D functions in the nervous system. J Chem Neuroanat. (1994) 20:70–8.

45. Li YC, Pirro AE, Amling M, Delling G, Baron R, Bronson R, et al. Targeted deletion of vitamin D receptor and 1α-hydroxylase results in phenotypic and kinetic, bone density, and bone structure in patients with hereditary vitamin D-resistant rickets. J Bone Miner Res. (1997) 12:3701–9. doi: 10.1359/jbmr.1997.12.11.3701

46. Yoshizawa T, Handa Y, Uematsu Y, Takeda S, Sekine K, Yoshihara Y, et al. Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. Nat Genet. (1997) 16:391–6. doi: 10.1038/ng0897-391

47. Van Cromphaut SJ, Dewerchin M, Hoenderop JG, Stockmans I, Vanhems P. Expression of inducible nitric oxide synthase during rat brain inflammation: its geographic and occupational incidence in relation to nutrition. J Nutr. (2007) 137:312–6. doi: 10.1093/jn/137.2.312

48. Tiosano D, Hada S, Chen Z, Nemirovsky A, Gepstein V, Militianu D, et al. Month of birth-effect in multiple sclerosis in Austria. J Neurol. (2011) 258:1870–7. doi: 10.1007/s00415-011-6432-8

49. Mokry LE, Ross S, Ahmad OS, Forgetta V, Smith GD, Goltzman D, et al. Vitamin D status during pregnancy and risk of multiple sclerosis in offspring of women in the Finnish Maternity Cohort. JAMA Neurol. (2016) 73:513–5. doi: 10.1001/jamaneurol.2015.4800

50. Nielsen NM, Munker KL, Koch-Henriksen N, Hougaard DM, Magyari M, Jorgensen KT, et al. Neonatal vitamin D status and risk of multiple sclerosis: a population-based case-control study. Neurol. (2017) 88:44–51. doi: 10.1212/WNL.0000000000004354

51. Van der Mei LA, Ponsonby AL, Dwyer T, Blizzard L, Simmons R, Taylor BV, et al. Past exposure to sun, skin phenotype, and risk of multiple sclerosis: case-control study. BMJ. (2003) 327:316. doi: 10.1136/bmj.327.7410.3136

52. Kampman MT, Wilsgaard T, Mellgren SJ. Outdoor activities and diet in childhood and adolescence relate to MS risk above the arctic circle. J Neurol. (2007) 254:471–7. doi: 10.1007/s00415-006-0395-5

53. Islam T, Gauderman WJ, Cozen W, Mack TM. Childhood sun exposure influences risk of multiple sclerosis in monoyzygotic twins. Neurology. (2007) 69:381–8. doi: 10.1212/01.wnl.0000268266.50850.48

54. Irving AA, Marling SJ, Seeman J, Plum LA, DeLuca HF. Ultraviolet suppression of EAE (a mouse model of multiple sclerosis) is independent of vitamin D and its receptor. Proc Natl Acad Sci U S A. (2019) 116:22552–5. doi: 10.1073/pnas.1913294116

55. Swank RL, Erlandson J, Ader J. Multiple Sclerosis in Rural Norway – its geographical and occupational incidence in relation to nutrition. N Engl J Med. (1952) 246:721–8. doi: 10.1056/NEJM195205082461901

56. Munker KL, Zhang SM, O'Reilly E, Hernán MA, Olek MJ, Willett WC, et al. Vitamin D intake and incidence of multiple sclerosis. Neurology. (2004) 62:605–10. doi: 10.1212/01.wnl.0000101723.79681.38

57. Munker KL, Levin LL, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA Neurol. (2006) 62:2832–8. doi: 10.1001/jama.296.23.2832

58. Mokry LE, Ross S, Ahmad OS, Forgetta V, Smith GD, Goltzman D, et al. Vitamin D and risk of multiple sclerosis: a Mendelian randomization study. PLoS Med. (2015) 12:e1001866. doi: 10.1371/journal.pmed.1001866

59. Langer-Gould A, Lucas RM, Xiang AH, Wu J, Chen LH, Gonzales E, et al. Vitamin D-binding protein polymorphisms, 25-hydroxyvitamin D, sunshine and multiple sclerosis. Nutrients. (2018) 10:1814. doi: 10.3390/nu10020184

60. Korlach J, Syraeit MC, Gaitan MI. Immunomodulatory effects of vitamin D in multiple sclerosis. Brain. (2009) 132:1146–60. doi: 10.1093/brain/awp033

61. van der Mei IAF, Ponsonby AL, Dwyer T, Blizzard L, Taylor BV, Kilpatrick T, et al. Vitamin D levels in people with multiple sclerosis and community controls in Tasmania, Australia. J Neurol. (2007) 254:581–90. doi: 10.1007/s00415-006-0315-8

62. Simpson S, Taylor B, Blizzard L, Ponsonby AL, Pitts F, Tremlett H, et al. Higher 25-hydroxyvitamin D is associated with a lower relapse risk in multiple sclerosis. Ann Neurol. (2010) 68:193–203. doi: 10.1002/ana.22043

63. Runia TF, Hop WCJ, de Rijke YB, Bulbeck D, Huitgen RQ. Lower serum vitamin D levels are associated with a higher relapse risk in multiple sclerosis. Neurology. (2012) 79:261–6. doi: 10.1212/WNL.0b013e31825dfc7e
76. Provvedeni D, Tsoukas C, Deftos L, Manolagas S. 1,25-dihydroxyvitamin D3 receptors in human leukocytes. *Science.* (1983) 221:1181–3. doi: 10.1126/science.6310748

77. Lemire JM, Archer DC. 1,25-dihydroxyvitamin D3 prevents the in vivo induction of murine experimental autoimmune encephalomyelitis. *J Clin Invest.* (1991) 87:1103–7. doi: 10.1172/JCI15072

78. Cantorna MT, Hayes CE. 1 alpha,25-dihydroxyvitamin D3 reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. *Proc Natl Acad Sci USA.* (1996) 93:7861–4. doi: 10.1073/pnas.93.15.7861

79. Nashold FE, Miller DJ, Hayes CE. 1,25-dihydroxyvitamin D3 treatment decreases macrophage accumulation in the CNS of mice with experimental autoimmune encephalomyelitis. *J Neuroimmunol.* (2000) 103:171–9. doi: 10.1016/S0165-5728(99)00247-7

80. Mayne CG, Spanier JA, Rolland LM, Williams CB, Hayes CE. 1,25-Dihydroxyvitamin D3 acts directly on the T lymphocyte vitamin D receptor to inhibit experimental autoimmune encephalomyelitis. *Eur J Immunol.* (2011) 41:822–32. doi: 10.1002/eji.201040632

81. Cantorna MT, Woodward WD, Hayes CE, DeLuca HF. 1,25-dihydroxyvitamin D3 is a positive regulator for the two anti-encephalitogenic cytokines TGF-beta 1 and IL-4. *J Immunol.* (1998) 160:3314–9.

82. Spach KM, Nashold FE, Dittel BN, Hayes CE. IL-10 Signaling Is Essential for 1,25-Dihydroxyvitamin-D3-Mediated Inhibition of Experimental Autoimmune Encephalomyelitis. *J Immunol.* (2006) 177:6030–7. doi: 10.4049/jimmunol.177.9.6030

83. Mattner F, Smiroldo S, Galiatti F. Inhibition of Th1 development and treatment of chronic-relapsing experimental allergic encephalomyelitis by a non-hypercalcemic analogue of 1,25-dihydroxyvitamin D3. *Eur J Immunol.* (2000) 30:498–508. doi: 10.1002/1521-4147(200003)30:2<498::AID-IMMU498>3.0.CO;2-Q

84. Muthian G, Raikwar HP, Rajasingh J, Bright JJ. 1,25 dihydroxyvitamin-D3 decreases macrophage accumulation in the CNS of mice with experimental autoimmune encephalomyelitis. *Eur J Immunol.* (1998) 28:1299–309. doi: 10.1002/eji.20826

85. Iglewski BH, Leibovich SJ, Reisine T, Murrell JD. 1alpha,25-dihydroxyvitamin D3 has a direct effect on naïve CD4+ T cells. *J Immunol.* (1996) 157:4434–41. doi: 10.4049/jimmunol.157.9.4434

86. Hupperts R, Smolders R, Delgatty E, Delelois ASJ, et al. Cholecalciferol in relapsing-remitting MS: a randomized clinical trial (CHOLINE). *Neurol Neuroimmunol Neuroinflamm.* (2019) 6:e597. doi: 10.1212/NXI.0000000000000597

87. Chalutz G, Goldstaub-Auriel S, Ishchian NA, Wolf J, et al. Vitamin D receptor triggering of a vitamin D-mediated human antimicrobial response. *Science.* (2006) 311:1770–3. doi: 10.1126/science.1123933

88. Li P, Xu X, Cao E, Yu B, Li W, Fan M, et al. Vitamin D deficiency causes defective resistant to Aspergillus fumigatus in mice via aggravated and sustained inflammation. *PLoS ONE.* (2014) 9:e99805. doi: 10.1371/journal.pone.0099805

89. Martinez-Moreno J, Hernandez JC, Urcuequi-Inchima S. Effect of high doses of vitamin D supplementation on dengue virus replication, toll-like receptor expression, and cytokine profiles on dendritic cells. *Mol Cell Biochem.* (2009) 326:109–18. doi: 10.1007/s11010-009-0363-0

90. D’Ambrosio D, Cappelli M, Cocciolo MG, Mazzeo D, Di Lucia P, Lang R, et al. Inhibition of IL-12 production by 1,25-dihydroxyvitamin D3. Involvement of NF-κB p50 in downregulation in transcriptional repression of the p40 gene. *J Clin Invest.* (1998) 102:252–62. doi: 10.1172/JCI1050

91. de Oliveira RC, Mimura LAN, Fraga-Silva TFC, Ishikawa LLW, Fernandes AAH, Zorzella-Pezavento SFG, et al. Calcitriol prevents neuroinflammation and reduces blood-brain barrier disruption and local macrophage/microglia activation. *Front Pharmacol.* (2020) 11:161. doi: 10.3389/fphar.2020.00161

92. Lee PW, Selhorst A, Lampé SG, Liu Y, Yang Y, Lovett-Racke AE. Neuron-specific vitamin D signaling attenuates microglia activation and CNS autoimmunity. *Front Neurol.* (2020) 11:19. doi: 10.3389/fneur.2020.00019

93. Evans MA, Kim HA, Ling YH, Uong S, Vinh A, De Silva TM, et al. Vitamin D(3) supplementation reduces subsequent brain injury and inflammation with ischemic stroke. *Neuroimmunol Med.* (2018) 20:147–59. doi: 10.1002/nim.2018-08-484-x

94. Calvello R, Cianciulli A, Nicolardi G, De Nuccio F, Giannotti L, Salvatore R, et al. Vitamin D treatment attenuates neuroinflammation and dopaminergic neurodegeneration in an animal model of Parkinson’s disease, shifting M1 to M2 microglia. *J Neuroimmunol Pharmacol.* (2019) 12:327–39. doi: 10.1002/jnph.11970-7

95. Cui C, Xu P, Li G, Qiao Y, Han W, Geng C, et al. Vitamin D receptor activation regulates microglia polarization and oxidative stress in spontaneously hypertensive rats and angiotensin II-exposed microglial cells: role of renin-angiotensin system. *Redox Biol.* (2019) 26:101295. doi: 10.1016/j.redox.2019.101295

96. He MC, Shi Z, Sha NN, Chen N, Peng SY, Liao DF, et al. Paricalcitol alleviates lipopolysaccharide-induced depressive-like behavior by suppressing hypothalamic microglia activation and neuroinflammation. *Biochem Pharmacol.* (2019) 163:1–8. doi: 10.1016/j.bcp.2019.01.021

97. Bhatta AD, Amento EP, Clemens TL, Holick MF, Krane SM. Specific high-affinity receptors for 1,25-dihydroxyvitamin D3 in human peripheral blood mononuclear cells: presence in monocytes and induction in T lymphocytes following activation. *J Clin Endocrinol Metab.* (1983) 57:1308–16. doi: 10.1210/jcem-57-6-1308

98. Amento EP, Bhatta AK, Kurnick JT, Kradin RL, Clemens TL, Holick SA, et al. alpha,25-dihydroxyvitamin D3 induces maturation of the human monocyte cell line U937, and, in association with a factor from human T lymphocytes, augments production of the monokine, mononuclear cell factor. *J Clin Invest.* (1984) 73:731–9. doi: 10.1172/JCI11266

99. Chen S, Sims GP, Chen XX, Yu YY, Chen S, Lipsky PE. Modulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differentiation. *J Immunol.* (2007) 179:1634–47. doi: 10.4049/jimmunol.179.9.1634

100. Boonstra A, Barrat FJ, Ciulfani C, Heath VL, Savelkoul HF, O’Garra A. Essential for 1,25-Dihydroxyvitamin D3-Mediated Inhibition of murine thymocyte proliferation. *J Immunol.* (2006) 164:923–44. doi: 10.4049/jimmunol.164.9.944

101. Shahmiri M, Enciso M, Adda CG, Smith BJ, Savellkou H, O’Garra A. 1alpha,25-dihydroxyvitamin D3 has a direct effect on naïve CD4(+) T cells
to enhance the development of Th2 cells. J Immunol. (2001) 167:9474–80. doi: 10.4049/jimmunol.167.9.9474

111. Tang J, Zhou R, Luger D, Zhu W, Silver PB, Grajewski RS, et al. Calcitriol suppresses antiretroviral autoimmunity through inhibitory effects on the Th17 effector response. J Immunol. (2009) 182:4624–32. doi: 10.4049/jimmunol.200801543

112. Gregori S, Giarratana N, Smiroldo S, Uskokovic M, Adorini L, A alpha,25-dihydroxyvitamin D(3) analog enhances regulatory T cells and arrests autoimmune diabetes in NOD mice. Diabetes. (2002) 51:1367–74. doi: 10.2337/diabetes.51.5.1367

113. Viglietta V, Baecher-Allan C, Weiner HL. Loss of functional CD4+CD25+Foxp3+ T lymphocytes fail to suppress myelin basic protein-induced proliferation in patients with multiple sclerosis. J Neuroimmunol. (2006) 180:178–84. doi: 10.1016/j.jneuroim.2006.08.003

114. Huan J, Culbertson N, Spencer L, Bartholomew R, Burrows GG, Chou YK et al. Decreased FOXP3 levels in multiple sclerosis patients. J Neurosci Res. (2005) 81:45–52. doi: 10.1002/jnr.20522

115. Kumar M, Putzki N, Limroth V, Remus R, Lindemann M, Knop D et al. Compromised CD4+CD25(high) regulatory T-cell function in patients with relapsing-remitting multiple sclerosis is correlated with a reduced frequency of FOXP3-positive cells and reduced FOXP3 expression at the single-cell level. Immunology. (2008) 123:79–89. doi: 10.1111/j.1365-2567.2007.02690.x

116. Vanherweghen AS, Eelen G, Ferreira GB, Ghesquiere B, Cook DP, Nikolic T, et al. Vitamin D controls the capacity of human dendritic cells to induce regulatory T cells on the T cell immune response against myelin oligodendrocyte glycoprotein in patients with multiple sclerosis. Eur J Immunol. (2005) 35:3343–52. doi: 10.1002/eji.200526060

117. Koomar R, Muthukumar S, Limroth V, Remus R, Lindemann M, Knop D et al. Vitamin D status is positively correlated with regulatory T cell function in patients with multiple sclerosis. J Immunol. (2000) 164:2307–12. doi: 10.4049/jimmunol.164.4.2307

118. Venken K, Hellings N, Thewissen M, Somers V, Hensen K, Rummens JL et al. B cell depletion changes the immune cell profile in multiple sclerosis patients: one-year report. J Neuroimmunol. (2009) 210:136–44. doi: 10.1016/j.jneuroim.2009.04.009

119. Haas J, Fritzsching B, Trübwerter P, Korporal M, Milkova L, Fritz B et al. Prevalence of newly generated naive regulatory T cells (Treg) is critical for Treg suppressive function and determines Treg dysfunction in multiple sclerosis. J Immunol. (2007) 179:1322–30. doi: 10.4049/jimmunol.179.2.1322

120. Smolders J, Thewissen M, Peelen E, Menheere P, Tervaert JW, Kamaoise J et al. Vitamin D status is positively correlated with regulatory T cell function in patients with multiple sclerosis. PLoS ONE. (2009) 4:e6635. doi: 10.1371/journal.pone.0006635

121. Killlick J, Hay J, Morandi E, Vermeren S, Kari S, Angles T, et al. Vitamin D(3)/25OHD cross-talk in human T cells in multiple sclerosis. Front Immunol. (2015) 6:1–10. doi: 10.3389/fimmu.2015.01522

122. Drozdenco G, Scheel T, Heine G, Baumgrass R, Worm M. Impaired T cell activation and cytokine production by calcitriol-primed human B cells. Clin Exp Immunol. (2014) 178:364–72. doi: 10.1111/cei.12406

123. Cross AH, Stark JL, Lauber J, Ramsbottom MJ, Lyons JA. Rituximab reduces B cells and T cells in cerebrospinal fluid of multiple sclerosis patients. J Neuroimmunol. (2006) 180:63–70. doi: 10.1016/j.jneuroim.2006.06.029

124. Lovett-Racke AE, Gormley M, Liu Y, Graham C, Wray S, et al. B cell depletion with ublituximab reshapes the T cell profile in multiple sclerosis patients. J Neuroimmunol. (2019) 332:187–97. doi: 10.1016/j.jneuroim.2019.04.017

125. Lovett-Racke AE, Yang Y, Liu Y, Gormley M, Kraus E, Graham C, et al. B cell depletion changes the immune cell profile in multiple sclerosis patients: one-year report. J Neuroimmunol. (2021) 359:577676. doi: 10.1016/j.jneuroim.2021.577676

126. Stumpf WE, Sar M, Clark SA, DeLuca HF. Brain target sites for 1,25-dihydroxyvitamin D3. Science. (1982) 215:1403–5. doi: 10.1126/science.697846

127. McGrath JJ, Feron FP, Burton TH, Mackay-Sim A, Eyles DW. Vitamin D3: implications for brain development. J Neuroimmunol. (2004) 139:49–57. doi: 10.1016/j.jneuroim.2003.04.007

128. Zehnder D, Bland R, Williams MC, McNinch RW, Howie AJ, Stewart PM, et al. Extraenal expression of 25-hydroxyvitamin d(3)-1 alpha-hydroxylase. J Clin Endocrinol Metab. (2001) 86:888–94. doi: 10.1210/jc.86.2.888
glutamate in association with upregulation of vitamin D receptor mRNA expression in cultured rat cortical neurons. *J Neurosci.* (2006) 83:1179–89. doi: 10.1021/pr208824

149. Almeras L, Eyles D, Benech P, Lafitte D, Villard C, Patatian A, et al. Developmental vitamin D deficiency alters brain protein expression in the adult rat: implications for neuropsychiatric disorders. *Proteomics.* (2007) 7:769–80. doi: 10.1002/pmic.200603932

150. Eyles D, Almeras L, Benech P, Patatian A, Mackay-Sim A, McGrath J, Feron F. Developmental vitamin D deficiency alters the expression of genes encoding mitochondrial, cytoskeletal and synaptic proteins in the adult rat brain. *J Steroid Biochem Mol Biol.* (2007) 103:538–45. doi: 10.1016/j.jsbmb.2006.12.096

151. Gezen-Ak D, Dursun E, Yilmazer S. The effects of vitamin D receptor silencing on the expression of LVS-CC-A1C and LVS-CC-A1D and the release of NGF in cortical neurons. *PLoS ONE.* (2011) 6:e17553. doi: 10.1371/journal.pone.0017553

152. Zhu Y, Zhou R, Yang R, Zhang Z, Bai Y, Chang F, et al. Abnormal neurogenesis in the dentate gyrus of adult mice lacking 1,25-dihydroxy vitamin D3 (1,25(OH)2 D3). *Hippocampus.* (2012) 22:421–33. doi: 10.1002/hipo.20908

153. Brewer LD, Thibault V, Chen KC, Langub MC, Landfield P, et al. 1alpha,25-dihydroxyvitamin D3 regulates rapid responses to 1alpha,25(OH)(2)D(3). *Steroids.* (2013) 78:897–902. doi: 10.1016/j.steroids.2012.04.002

154. Zanatta L, Goulart PB, Goncalves R, Pierozan P, Winkelmann-Souza K, Silwal S, et al. Maternal vitamin D levels during pregnancy alter oxidative stress and pathophysiology of ischemic stroke. *J Stroke Cerebrovasc Dis.* (2015) 24:1555–63. doi: 10.1016/j.jstrokecerebrovasdis.2015.03.051

155. Boyan BD, Sylvia VL, Dean DD, Schwartz Z. Membrane mediated signaling mechanisms are used differentially by metabolites of vitamin D(3) in musculoskeletal cells. *Stereoids.* (2002) 67:421–7. doi: 10.1016/S0039-128X(01)00178-7

156. Chen J, Doroudi M, Cheung H, Grozier AL, Schwartz Z, Trist BG, et al. Oxidative stress and pathophysiology of ischemic stroke: novel therapeutic opportunities. *CNS Neurol Disord Drug Targets.* (2010) 9:34. doi: 10.1186/s40035-020-00016-7

157. Nemere I, Garbi N, Hammerling G, Hintze KJ. Role of the Nrf2-ARE Antioxidant System. *Neuroprotection Against Oxidative Stress: Phytochemicals Targeting TrkB Signaling and the Nrf2-ARE Antioxidant System.* (2020). doi: 10.3389/fnmol.2020.00116

158. Duarte EC, Woehl VM, et al. 1alpha,25-kihydroxyvitamin D(3) signaling and the Nrf2-ARE Antioxidant System. *Neuroprotection Against Oxidative Stress: Phytochemicals Targeting TrkB Signaling and the Nrf2-ARE Antioxidant System.* (2020). doi: 10.3389/fnmol.2020.00116

159. Evatt ML, Delong MR, Khazai N, Rosen A, Triche S, Tangpricha V. Vitamin D supplementation with Parkinson’s disease. *Transl Neurodegener.* (2020) 9:34. doi: 10.1186/s40035-020-00213-2

160. Coskun S, Simsek S, Camkurt MA, Cim A, Celik SB. Association of polymorphisms in the vitamin D receptor gene and serum 25-hydroxyvitamin D levels in children with autism spectrum disorder. *Gene.* (2016) 588:109–14. doi: 10.1016/j.gene.2016.05.004

161. Sourander A, Upadhyaya S, Surcel HM, Hinkka-Yli-Salomaki S, Chelsack-Postava K, Silwal S, et al. Maternal vitamin D levels during pregnancy and offspring autism spectrum disorder. *Biol Psychiatry.* (2021) 90:790–7. doi: 10.1016/j.biopsych.2021.07.012

162. Cui X, McGrath JJ, Bune THJ, Eyles DW. Vitamin D and schizophrenia: 20 years on. *Mol Psychiatry.* (2021) 26:2708–20. doi: 10.1038/s41386-021-01025-0

163. Evatt ML, Delong MR, Khazai N, Rosen A, Triche S, Tangpricha V. Prevalence of vitamin D insufficiency in patients with Parkinson disease and Alzheimer disease. *Arch Neurol.* (2008) 65:1348–52. doi: 10.1001/archneur.65.10.1348

164. Lv L, Tan X, Peng X, Bai R, Xiao Q, Tan J, et al. The relationships of vitamin D, vitamin D receptor gene polymorphisms, and vitamin D supplementation with Parkinson’s disease. *Transl Neurodegener.* (2020) 9:34. doi: 10.1186/s40035-020-00213-2

165. Anwiler C, Llewellyn DF, Beauchet O. Low serum vitamin D concentrations in Alzheimer’s disease: a systemic review and meta-analysis. *J Alzheimers Dis.* (2013) 33:659–74. doi: 10.3233/JAD-2012-121432

166. Ballion C, Griffith LE, Stifler L, Henderson M, Patterson C, Heckman G, et al. Vitamin D, cognition, and dementia: a systematic review and meta-analysis. *Neurology.* (2012) 79:1397–405. doi: 10.1212/WNL.0b013e31826c197f

167. Price AL, Mancini M, Horak FB. The relationship between balance control and vitamin D in Parkinson’s disease—a pilot study. *Mov Disorder.* (2013) 28:1133–7. doi: 10.1002/mds.25405

168. Price AL, Munschion C, Zabetian C, Leverenz JB, Watson GS, Montine T, et al. Memory, mood, and vitamin D in persons with Parkinson’s disease. *J Park Dis.* (2013) 3:547–55. doi: 10.3233/JPD-130206

169. Littlejohns TJ, Henley WE, Lang IA, Anwiler C, Beauchet O, Chaves PHM, et al. Vitamin D and the risk of dementia and Alzheimer disease. *Neurology.* (2014) 83:920–8. doi: 10.1212/WNL.000000000000755

170. Turetsky A, Goddau RP, Henninger N. Low serum vitamin D is independently associated with larger lesion volumes after ischemic stroke. *J Stroke Cerebrovasc Dis.* (2015) 24:1555–63. doi: 10.1016/j.jstrokecerebrovasdis.2015.03.051

171. Huang H, Zheng T, Wang S, Wei L, Wang Q, Sun Z. Serum 25-hydroxyvitamin D predicts early recurrent stroke in ischemic stroke patients. *Nutr Metab Cardiovasc Dis.* (2016) 26:908–14. doi: 10.1016/j.numecd.2016.06.009

172. Nie Z, Ji XC, Wang J, Zhang HX. Serum levels of 25-hydroxyvitamin D predicts infarct volume and mortality in ischemic stroke patients. *J Neuroimmunol.* (2017) 313:41–5. doi: 10.1016/j.jneurneuropsych.2017.10.002

173. Di Somma C, Scarno E, Barra L, Zhukouskaya VV, Savastano S, Mele C, et al. Vitamin D and neurological diseases: an endocrine view. *Int J Mol Sci.* (2017) 18:111. doi: 10.3390/ijms18112482

174. Sivandzade F, Prasad S, Blaherao A, Cucullo L. NRF2 and NF-κB signaling mechanisms are used differentially by metabolites of vitamin D(3) in musculoskeletal cells. *Steroids.* (2012) 78:897–902. doi: 10.1016/j.steroids.2012.04.002

175. Iozefowicz O, Rabe-jablonska J, Wozniacka A, Strzelecki D. Analysis of vitamin D status in major depression. *Neurosci Lett.* (2013) 535:349–53. doi: 10.1016/j.neulet.2013.07.031

176. Rodrigo R, Fernandez-Gajardo R, Guierrez R, Matamala JM, Carrasco R, Miranda-Merchak A, et al. Oxidative stress and pathophysiology of ischemic stroke: novel therapeutic opportunities. *CNS Neurol Disord Drug Targets.* (2010) 9:34. doi: 10.1186/s40035-020-00213-2

177. Niedzielska E, Smaga M, Gawkul M, Moniczewski A, Stankowicz P, Pera J, et al. Oxidative stress in neurodegenerative diseases: molecular mechanisms and possible therapeutic approaches. *Redox Biol.* (2019) 22:101009. doi: 10.1016/j.redox.2018.11.017

178. Gombash et al. Vitamin D in Multiple Sclerosis
185. Genersta M. Oxyl radicals, redox-sensitive signaling cascades and antioxidants. Cell Signal. (2007) 19:1807–19. doi: 10.1016/j.cellsig.2007.04.009

186. Gray E, Thomas TL, Betmouni S, Scolding N, Love S. Elevated nitric oxide synthase and nitrotyrosine in multiple sclerosis lesions. Amer J Pathol. (2001) 158:2057–66. doi: 10.1016/S0002-9440(10)64779-7

187. van Horssen J, Drexhage J, Hazes T, Dijkstra CD, van der Neut M, et al. Loss of Nrf2 exacerbates the visual deficits and optic neuritis in a mouse model of multiple sclerosis: the therapeutic potential of Nrf2 activation. J Neuroimmunol. (2013) 259:296–301. doi: 10.1016/j.jneuroim.2014.01.002

188. Larabee CM, Desai S, Agasim A, Georgescu C, Wren JD, Axtell RC, et al. Loss of Nrf2 exacerbates the visual deficits and optic neuritis elicited by experimental autoimmune encephalomyelitis. Molecular Vision. (2016) 22:1303–13.

189. Morales Pantoja E, Hu CL, Perrone-Bizzozero NI, Zheng J, Bizzozero OA. Nrf2 dysregulation correlates with reduced synthesis and low glutathione levels in experimental autoimmune encephalomyelitis. J Neurochem. (2016) 139:640–50. doi: 10.1111/jn.13837

190. Linker RA, Lee DH, RyanS, van Dam AM, Conrad R, Bista P, et al. Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway. Brain. (2011) 134:678–92. doi: 10.1093/brain/awq586

191. van Horssen J, Drexhage JA, Flor T, Gerritsen W, van der Valk P, de Vries HE. Nrf2 and DJ-1 are consistently upregulated in inflammatory multiple sclerosis lesions. Free Radical Biol Med. (2010) 49:1283–9. doi: 10.1016/j.freeradbiomed.2010.07.013

192. Licht-Mayer S, Wimmer I, Tráfčfn S, Metz I, Brück W, Bauer J, et al. Cell type-specific Nrf2 expression in multiple sclerosis lesions. Acta neuropathologica. (2015) 130:263–77. doi: 10.1007/s00401-015-1452-x

193. Ghoreschi K, Bruck J, Kellerer C, Deng C, Peng H, Rothfuss O, et al. Fumarates improve psoriasis and multiple sclerosis by inducing type II dendritic cells. J Exp Med. (2011) 208:2291–303. doi: 10.1084/jem.20100977

194. Albrecht P, Bouchachia I, Goebels N, Henke N, Hofstetter HH, Issberner A, et al. Effects of dimethyl fumarate on neuroprotection and immunomodulation. J Neuroinflammation. (2012) 9:163. doi: 10.1186/1742-2094-9-163

195. Fox RJ, Miller DH, Phillips JT, Hutchinson M, Havrdova E, Kita M, et al. Placebo-controlled phase 2 study of oral BG-12 or glatiramer in multiple sclerosis. N Engl J Med. (2012) 367:1087–97. doi: 10.1056/NEJMoa1206328

196. Gold R, Kappos L, Arnold DL, Bar-Or A, Giovannoni G, Selmay J, et al. Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis. N Engl J Med. (2012) 367:1098–107. doi: 10.1056/NEJMoa1114287

197. Liu JS, Zhao ML, Brosnan CF, Lee SC. Expression of inducible nitric oxide synthase and nitrotyrosine in multiple sclerosis lesions. Amer J Pathol. (2001) 158:2057–66. doi: 10.1016/S0002-9440(10)64779-7
associated with the multiple sclerosis risk factor HLA-DR15. Eur J Immunol. (2021) 51:64–75. doi: 10.1002/eji.202048655

222. Brutting C, Stangl GI, Staeg MS. Vitamin D, Epstein-Barr virus, and endogenous retroviruses in multiple sclerosis - facts and hypothesis. J Integr Neurosci. (2021) 20:233–8. doi: 10.31083/j.jin.2021.01.392

223. Ostkamp P, Salmen A, Pignolet B, Gorlich D, Andlauer TFM, Schulte-Mecklenbeck A, et al. Sunlight exposure exerts immunomolulatory effects to reduce multiple sclerosis severity. Proc Natl Acad Sci U S A. (2021) 18:e2018457118. doi: 10.1073/pnas.2018457118

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