Vitamin D in Multiple Sclerosis

Subjects: Pathology

Contributor: Xiaolei Tang

Multiple sclerosis (MS) is a chronic demyelinating disease of central nervous system and is caused by an aberrant immune response to myelin sheath. Disease-modifying medications, which mainly aim to suppress such aberrant immune response, have significantly improved MS treatment. However, the disease severity continues to worsen. In contrast, progressively more data suggest that 1,25-dihydroxyvitamin D or 1,25$(OH)_2$D, i.e., the active vitamin D, suppresses the differentiation of potentially pathogenic T cells associated with MS, enhances the differentiation of regulatory T cells that suppress the pathogenic T cells, and promotes remyelination. These novel 1,25$(OH)_2$D functions have encouraged investigators to develop vitamin D as a potential therapy for MS. However, because of the hypercalcemia that is associated with high 1,25$(OH)_2$D concentrations, supplementation of native vitamin D has been a major focus in clinical trials for the treatment of MS, but such trials have produced mixed data. In this article, we will review current progress in the supplementation of different vitamin D forms for the treatment of experimental autoimmune encephalomyelitis (i.e., an MS animal model) as well as MS.

Keywords: Multiple sclerosis ; Experimental autoimmune encephalomyelitis ; Vitamin D ; 1,25(OH)2D ; Hypecalcemia

1. Role of Vitamin D Supplementation in MS

1.1. Effects of Supplementation of Native Vitamin D on Disease Activity in MS Patients:

Based on previous animal studies, 1,25$(OH)_2$D supplementation can readily cause hypercalcemia, whereas supplementation of native vitamin D is relatively safe. Therefore, while assessing native vitamin D supplementation in MS animal models, investigators also studied supplementation of native vitamin D in MS patients. In as early as 1986, such vitamin D supplementation, in combination with calcium and magnesium, was initially evaluated for the treatment of MS [1]. Because of the findings that 1,25$(OH)_2$D has at least three biological functions that are potentially beneficial to MS patients, recent clinical investigations began to focus on randomized, placebo-controlled studies to assess the role of supplementation of native vitamin D in the treatment of MS. In one study, using the data from a 96-week randomized and placebo-controlled trial that was initially designed to assess the effect of oral vitamin D3 supplementation (20,000 IU/week) on bone mineral density in MS patients, Kampman et al. reported a modified analysis. This modified post hoc analysis included 35 patients in the vitamin D3 group and 33 in the placebo group. The goal of this analysis was to evaluate potential therapeutic effect of the vitamin D3 supplementation on MS [2]. The results showed that there was no significant difference between groups in annualized relapse rate, expanded disability status scale, MS functional composite components, grip strength, or fatigue [2]. In another study, Soilu-Hanninen et al. performed a one-year study of vitamin D3 supplementation as an add-on treatment to IFN-b1b in MS patients [3]. The study compared 34 MS patients who received an oral supplementation of vitamin D3 (20,000 IU once a week) with 32 MS patients who received a placebo. Their data showed that the vitamin D supplementation reduced the numbers of T2 lesions (p = 0.286) and T1 enhancing lesions (p = 0.004). In addition, the vitamin D supplementation reduced disability accumulation (p = 0.071) and improved timed tandem walk (p = 0.076). However, there were no significant differences in adverse events and annual relapse rate. The authors concluded that vitamin D3 supplementation, as an add-on treatment to IFN-b1b, reduced magnetic resonance imaging (MRI) disease activity in MS patients [3]. In the third study, Stein et al. performed a six-month clinical trial [4]. This trial included 11 MS patients who received oral supplementation of vitamin D2 (an initial dose of 6000 IU twice daily and subsequently adjusted to maintain 25[OH]D levels at 130-175 nM) and 12 MS patients who received a placebo. In addition, all patients received 1000 IU vitamin D daily. Their data showed that no significant treatment differences were detected in the primary MRI endpoints [4]. In the fourth study, Mosayebi et al. performed a six-month trial in which 26 MS patients received 300,000 IU/month of vitamin D3 via intramuscular injection and 33 MS patients received a placebo. This trial showed no significant treatment differences in terms of expanded disability status scale and number of gadolinium-enhancing lesions [5]. Hence, these earlier randomized , placebo-controlled clinical trials do not provide reproducible data in support of the use of vitamin D supplementation for the treatment of MS.

The above-described earlier randomized, placebo-controlled trials may have several drawbacks such as relatively small sample size, insufficient dose, or short length. In this regard, recently published clinical trials tried to overcome these insufficiencies. For instance, Camu et al. studied 63 relapse-remitting MS (RRMS) patients who received an oral vitamin D supplementation (100,000 IU every other week) for 96 weeks and 66 RRMS patients who received a placebo (CHOLINE) [6]. This intervention did not meet the primary outcome, i.e. mean annualized relapse rate. However, for the patients who completed the 2-year follow-up (45 with vitamin D and 45 with placebo), the vitamin D treatment led to significant reductions in annualized relapse rate (p = 0.012), new hypointense T1-weighted lesions (p = 0.025), volume of hypointense T1-weighted lesions (p = 0.031), and progression of expanded disability status scale (p = 0.026) [6]. In a multicenter randomized controlled clinical trial (EVIDIMS) [7], Dorr et al. compared high dose vitamin D supplementation (20,400 IU, every other day) with those of low dose vitamin D (400 IU, every other day) as an add-on treatment to IFN-b1b in patients with RRMS or clinically isolated syndrome. Fifty-three patients were randomized (28 in high dose group and 25 in low dose group) and 41 patients completed the 18-month study. The data showed that there were no differences between the two groups regarding relapse rates, disability progression, T2-weighted lesion development, contrast-enhancing lesion development, and brain atrophy [7]. In another study (SOLAR) [8], Hupperts et al. investigated an oral supplementation of high dose vitamin D as an add-on treatment to IFN-b1a in RRMS patients for 48 weeks. This study randomized 229 patients in which 113 patients received a daily supplementation of 14,007 IU vitamin D and 116 patients received a placebo. The data showed that there were no significant differences between the two groups in “no evidence of disease activity” (primary outcome). However, patients in the vitamin D group had better MRI outcomes for combined unique active lesions (p = 0.0045) and change from baseline in total volume of T2 lesions (p = 0.035) [8]. Notwithstanding the MRI results, data from previous comprehensive randomized, placebo-controlled clinical trials fail to show positive effects on the symptomatology of MS, which is the required endpoint for a successful therapy.

1.2. Role of Supplementation of Native Vitamin D in Potentially Pathogenic T cells in MS Patients

While being evaluated for its potential role in MS disease activity, vitamin D supplementation has also been investigated for its effects on the control of potentially pathogenic T cells. Since IL-17$^+$CD4$^+$ T cells have been shown to be the major pathogenic cell subset in EAE, IL-17 has been intensively studied in MS patients. In a study of 94 RRMS patients in which 47 patients received a supplementation of 50,000 IU vitamin D3 every five days for 12 weeks and 47 patients received a placebo, Toghianifar et al. reported that, the vitamin D3 supplementation appeared to abrogate the non-significant increase of serum IL-17 levels observed in the placebo group [9]. Similar results were seen in another study reported by Golan et al. in which 21 MS patients received 800 IU/day of vitamin D3 (low dose) and 24 MS patients received 4,370 IU/day (high dose). At 3 months after the intervention, a significant increase in serum IL-17 levels was observed in the low dose group, which was not seen in the high dose group [10]. In addition, this study did not observe a significant difference in the serum levels of IFN-g between the low dose and the high dose vitamin D groups. In this respect, Th1 cells, which secrete IFN-g, are also an important pathogenic T cell subset in MS patients [11]. In contrast, in a study in which 30 RRMS patients received 20,000 IU/week of vitamin D supplementation and 29 received a placebo for 12 months. Aivo et al. reported that there was an increase in the serum IL-17A levels compared to baseline in the vitamin D group (p = 0.0666) while the serum IL-17 levels remain similar in the placebo group (p = 0.5243) [12]. In another study, 19 MS patients received 10,400 IU/day of vitamin D supplementation (high dose) and 21 received 800 IU/day (low dose) for 6 months [13]. From this study, Sotirchos et al. reported that the high dose vitamin D supplementation significantly reduced the percentages of IL-17$^+$CD4$^+$ T cells (p = 0.016), CD161$^+$CD4$^+$ T cells (p = 0.03), and effector memory CD4$^+$ T cells (p = 0.021), but increased the percentages of central memory CD4$^+$ T cells (p = 0.018) and naïve CD4$^+$ T cells (p = 0.04). These effects were not seen in the low-dose group [13]. In the SOLAR trial in which 30 RRMS patients received 7000 IU/day of vitamin D for 4 weeks followed by 14,000 IU/day up to week 48 and 23 received a placebo, Muris et al. reported no differences in the percentages of IL-17$^+$CD4$^+$ T cells between the vitamin D3 (p=0.59) and placebo (p = 0.96) groups [14]. In an earlier study in which 15 RRMS patient were supplemented with 20,000 IU/day vitamin D3 for 12 weeks [15], Smolders et al. did not observe significant differences in the percentages of CD4$^+$IL-17$^+$ and CD4$^+$IFN-g$^+$ cells before and after the treatment. These results, while not dramatic, are sufficiently positive to justify further exploration into the effects of vitamin D supplementation on potentially pathogenic T cells at local levels such as in the immune system and CNS of MS patients.

1.3. Effects of Supplementation of Native Vitamin D on Potentially Immune Regulatory Mechanisms in MS Patients

Treg cells and their associated cytokines (e.g. TGF-b and IL-10) are critical in the control of MS [16][17][18][19]. In one study mentioned above, Golan et al. showed that there were no differences in the serum levels of IL-10 between the high dose (4,370 IU/day) and the low dose (800 IU/day) vitamin D groups [10]. In another study mentioned above, Avio et al. reported a significant increase in the serum levels of latency activated peptide of TGF-b (p = 0.0249) in MS patients who were

treated with vitamin D but not those who were treated with placebo (TGF-b is a cytokine used by Treg cells to execute immune regulatory functions) [12]. In addition, this study also showed a mild increase in serum IL-10 levels in the vitamin D-treated patients, which was however not significant (p = 0.1466). In contrast, serum IL-10 levels were non-significantly decreased in the placebo-treated patients (p = 0.2503) [12]. In the SOLAR trial mentioned above, Muris et al evaluated the effects of the high vitamin D supplementation on various regulatory cell subsets [14]. In both vitamin D and placebo groups, the authors did not see significant increase in the percentages of various regulatory cell subsets including $CD4^+CD25^+CD127^-$ natural Treg (nTreg), $CD4^+CD25^+FoxP3^+$ nTreg, $CD4^+CD25^+CD127^-FoxP3^+$ nTreg, $CD4^+CD25^+CD127^-CD39^+$ nTreg, $CD4^+CD25^+CD127^-CD45RA^-$ memory nTreg, $CD4^+IL\text{-}10^+$ induced Treg (iTreg), $CD19^+IL\text{-}10^+$ regulatory B cells (Breg) [14]. Finally, in the earlier study mentioned above in which 15 RRMS patients were supplemented with 20,000 IU/day vitamin D3 for 12 weeks [15], Smolders et al. reported that the vitamin D supplementation led to a significant increase in the percentage of $CD4^+IL\text{-}10^+$ cells. These results are again sufficiently interesting to warrant the pursuit of the effects of vitamin D supplementation on immune cells at local levels such as in the immune system and CNS of MS patients.

1.4. Role of Metabolic Disorders in the Supplementation of Native Vitamin D for the Treatment of MS

Increasing amount of evidence suggests that disordered lipid metabolism in both peripheral tissues and CNS is associated with MS pathogenesis [20][21]. One report showed that serum levels of low-density lipoprotein and total cholesterol inversely correlated with cognitive function of MS patients [22]. Although we did not find any studies that investigated the effects of vitamin D supplementation on lipid metabolism in MS patients, it is worth discussing this type of studies in other settings such as diabetes. We reason that findings of these studies in other settings may shed light on future similar studies in MS patients. One study investigated blood lipid levels in type 2 diabetes patients at baseline, 3 months, and 6 months following vitamin D supplementations at a daily dose of either 4,000 IU or 6,000 IU. This study reported significant decrease in total cholesterol and triglycerides in the patients who received 6,000 IU vitamin D for 6 months [23]. However, when adjusted for the confounders, the observations were not significant anymore. In another study, Ponda et al. performed a randomized, placebo-controlled trial in which 151 vitamin D insufficient adults (defined as serum 25[OH]D levels < 20 ng/mL) received either 50,000 IUs of vitamin D3 weekly or placebo for 8 weeks. Data from this study showed that the vitamin D supplementation did not improve lipid profile [24]. In the third study, Kane et al performed an eighteen-week randomized, double-blind, placebo-controlled clinical trial among 26 individuals who had insufficient serum 25(OH)D levels (<25 ng/mL). All the individuals, when entering the study, received daily supplementation of 1,000 IU vitamin D. Subsequently, the vitamin D doses was first adjusted at week 6 to 2,000 IU/day if serum 25(OH)D levels were not greater than 25 ng/mL for those individuals whose baseline serum 25(OH)D levels were less than 20 ng/mL or if serum 25(OH)D levels were not increased by at least 25% for those individuals whose baseline serum 25(OH)D levels were 21-25 ng/mL. The dose was further adjusted at week 12 if the above-mentioned 25(OH)D levels were still not met. As a result, vitamin D3 was titrated to 1000 IU/day in 15/26 (58%), 2000 IU/day in 10/26, and 3000 IU/day in 1/26 individuals. Data from the above study showed that serum levels of free but not total 25(OH)D levels inversely correlated with serum levels of triglycerides and low density lipoproteins cholesterol (LDLC) [25]. In the fourth study, Schwetz et al performed a post hoc analysis of a single-center, randomized double-blind, placebo-controlled clinical trial in which two hundred individuals who had arterial hypertension and serum 25(OH)D levels less than 75 nmol/L were randomized to 2,800 IU/day of vitamin D or placebo for 8 weeks. Among the two hundred patients, one hundred sixty-three patients (79 in vitamin D group and 84 in placebo group) had lipid data and these individuals were included in the analysis. The analysis showed that the vitamin D supplementation significantly increased total cholesterol, triglycerides, very low density lipoproteins (VLDL), low density lipoproteins (LDL), high-density lipoprotein (HDL), triglycerides, apolipoprotein B (ApoB), LDL-ApoB, ApoCII, ApoCIII, phospholipids, and ApoE [26]. Considering the different impacts of vitamin D supplementation on lipid metabolism that were observed under different pathological settings, impact of vitamin D supplementation on lipid metabolism in MS patients should be carefully evaluated.

1.5. Role of HLA in the Supplementation of Native Vitamin D for the Treatment of MS

In the past, genome-wide association studies (GWAS) have revealed over 200 genetic loci that are firmly associated with MS susceptibility [27]. Among all these association studies, the major histocompatibility (MHC) gene, HLA-DRB1, has been consistently observed across all populations studied [27]. Additional findings suggest that the genetic association is affected by environmental factors [27]. To understand the influence of vitamin D on the genetic association of MS, Ramgopalan et al analyzed the entire genomic sequence of the HLA-DRB1, HLA-DQA1, and HLA-DQB1 genes as well as 5 Kb upstream from the transcriptional start sites of these genes that contained promoter regions. Their analysis revealed only one potential vitamin D-responsive element (VDRE) that was located in the proximal promoter region immediately 5' to the transcriptional start site of HLA-DRB1. Further experiments confirmed that the identified VDRE element was functional and that addition of $1,25(OH)_2D$ led to a significant increase in the cell surface expression of HLA-DRB1

specifically and only in HLA-DRB1*15-bearing cells [28]. This data provides strong evidence that 1,25(OH)$_2$D can directly modify the expression of HLA-DRB1*15 molecule that has linkage to MS [29]. However, the implication of 1,25(OH)$_2$D-mediated modification of HLA-DRB1*15 expression requires further investigation.

1.6. Role of Microbiota in the Supplementation of Native Vitamin D for the Treatment of MS

The role of microbiota in the pathogenesis and treatment of paralytic disease has been investigated in animals. In one study, microbiota was modulated through antibiotic treatment in mice that spontaneously developed EAE. The data showed that microbiota modulation before disease onset prevented the disease development. However, microbiota modulation after disease onset did not affect the ongoing disease [30]. In another study, Cignarella et al reported that animals with intermittent fasting, when compared to those with a normal diet, showed ameliorated paralytic disease following EAE induction [31]. In addition, in the fasting animals, there was an enrichment of Lactobacillaceae, Bacterioidaceae, and Prevotellaceae families in gut microbiota. Subsequently, the author transplanted fecal microbiome from intermittent fasting mice or normal diet mice into recipient mice that were depleted of microbiota. The mice were then induced for EAE. Their data showed that the fecal microbiome from intermittent fasting mice, but not that from normal diet mice, significantly meliorated paralytic disease in the recipient mice [31]. In summary, the above-mentioned data suggest that the alteration of microbiota changes the susceptibility to EAE induction but has minimal effect on ongoing paralytic disease in animals.

In humans, a recent systemic review suggests that there is no significant difference in microbiota diversity between MS patients and normal healthy controls [32]. However, taxonomic differences in microbiota were noticed. These taxonomic differences indicate a potential role of gut bacteria in MS pathogenesis. In addition, the potential impact of vitamin D supplementation on microbiota in humans was also investigated. In one randomized, placebo-controlled study, 26 vitamin D-insufficient (defined as serum 25[OH] levels < 50 nmol/L), overweight or obese (BMI ≥ 25 kg/m^2) otherwise healthy adults were recruited [33]. Among the 26 adults, fourteen adults received vitamin D (100,000 IU of loading dose followed by 4000 IU/day for 16 weeks) and 12 adults received placebo. Fecal microbiota at baseline and 16 week were collected for analysis. The analysis did not see significance in microbiome a-diversity between the two groups at baseline and 16 week. However, adults in the vitamin D group had a higher abundance of genus Lachnospira and lower abundance of genus Blautia. Furthermore, adults with 25(OH)D > 75 nmol/L, when compared to those with 25(OH)D < 50 nmol/L, had a higher abundance of genus Coprococcus and had a lower abundance of genus Ruminococcus [33]. In another study in which 20 adults with vitamin D insufficiency (defined as serum 25[OH]D levels < 30 ng/ml) were provided with 600, 4,000, or 10,000 IU/day of oral vitamin D3. Stool samples at baseline and week 8 were collected for the analysis of gut microbiota. The data showed that the vitamin D supplementation led to dose-dependent increase in bacteria associated with amelioration of inflammatory bowel disease activity [34][34]. In addition to healthy subjects, the impact of vitamin D supplementation (5,000 IU/day for 90 days) on microbiota in MS patients was also studied [35]. Data from this study showed a lower abundance of otherwise operational bacterial unit Faecalibacterium in MS patients. The vitamin D treated MS patients had an increase in the Akkermansia, Faecalibacterium, and Coprococcus genera. The authors hence concluded that vitamin D supplementation was associated with differences or changes in the microbiota [35]. In summary, recent studies show that vitamin D supplementation has effects on the composition of microbiota. However, future studies are warranted to understand how the microbiota changes affect the therapeutic outcome of vitamin D supplementation in MS patients.

References

1. P. Goldberg; M.C. Fleming; E.H. Picard; Multiple sclerosis: Decreased relapse rate through dietary supplementation with calcium, magnesium and vitamin D. *Medical Hypotheses* **1986**, *21*, 193-200, 10.1016/0306-9877(86)90010-1.
2. Margitta T. Kampman; Linn H Steffensen; Svein I Mellgren; Lone Jørgensen; Effect of vitamin D3 supplementation on relapses, disease progression, and measures of function in persons with multiple sclerosis: exploratory outcomes from a double-blind randomised controlled trial. *Multiple Sclerosis Journal* **2012**, *18*, 1144-1151, 10.1177/1352458511434607.
3. Soilu-Hanninen, M.; Aivo, J.; Lindstrom, B. M.; Elovaara, I.; Sumelahti, M. L.; Farkkila, M.; Tienari, P.; Atula, S.; Sarasoja, T.; Herrala, L.; Keskinarkaus, I.; Kruger, J.; Kallio, T.; Rocca, M. A.; Filippi, M., A randomised, double blind, placebo controlled trial with vitamin D3 as an add on treatment to interferon beta-1b in patients with multiple sclerosis. J Neurol Neurosurg Psychiatry 2012, 83, 565-571.
4. Stein, M. S.; Liu, Y.; Gray, O. M.; Baker, J. E.; Kolbe, S. C.; Ditchfield, M. R.; Egan, G. F.; Mitchell, P. J.; Harrison, L. C.; Butzkueven, H.; et al. A randomized trial of high-dose vitamin D2 in relapsing-remitting multiple sclerosis. *Neurology* **2011**, *77*, 1611-1618, .

5. Ghasem Mosayebi; Ali Ghazavi; Keyvan Ghasami; Yahya Jand; Parviz Kokhaei; Therapeutic Effect of Vitamin D3 in Multiple Sclerosis Patients. *Immunological Investigations* **2011**, *40*, 627-639, 10.3109/08820139.2011.573041.

6. Camu, W.; Lehert, P.; Pierrot-Deseilligny, C.; Hautecoeur, P.; Besserve, A.; Jean Deleglise, A. S.; Payet, M.; Thouvenot, E.; Souberbielle, J. C., Cholecalciferol in relapsing-remitting MS: A randomized clinical trial (CHOLINE). Neurol Neuroimmunol Neuroinflamm 2019, 6, (5).

7. Dorr, J.; Backer-Koduah, P.; Wernecke, K. D.; Becker, E.; Hoffmann, F.; Faiss, J.; Brockmeier, B.; Hoffmann, O.; Anvari, K.; Wuerfel, J.; Piper, S. K.; Bellmann-Strobl, J.; Brandt, A. U.; Paul, F., High-dose vitamin D supplementation in multiple sclerosis - results from the randomized EVIDIMS (efficacy of vitamin D supplementation in multiple sclerosis) trial. Mult Scler J Exp Transl Clin 2020, 6, (1), 2055217320903474.

8. Hupperts, R.; Smolders, J.; Vieth, R.; Holmoy, T.; Marhardt, K.; Schluep, M.; Killestein, J.; Barkhof, F.; Beelke, M.; Grimaldi, L. M. E.; Group, S. S., Randomized trial of daily high-dose vitamin D3 in patients with RRMS receiving subcutaneous interferon beta-1a. Neurology 2019, 93, (20), e1906-e1916.

9. Nafiseh Toghianifar; Fereshteh Ashtari; Sayyed Hamid Zarkesh Esfahani; Marjan Mansourian; Effect of high dose vitamin D intake on interleukin-17 levels in multiple sclerosis: A randomized, double-blind, placebo-controlled clinical trial. *Journal of Neuroimmunology* **2015**, *285*, 125-128, 10.1016/j.jneuroim.2015.05.022.

10. Daniel Golan; Basheer Halhal; Lea Glass-Marmor; Elsebeth Staun-Ram; Orit Rozenberg; Idit Lavie; Sara Dishon; Mira Barak; Sofia Ish-Shalom; Ariel Miller; et al. Vitamin D supplementation for patients with multiple sclerosis treated with interferon-beta: a randomized controlled trial assessing the effect on flu-like symptoms and immunomodulatory properties. *BMC Neurology* **2013**, *13*, 60, 10.1186/1471-2377-13-60.

11. Dale G. Ando; Julie Clayton; Dwight Kono; James L. Urban; Eli E. Sercarz; Encephalitogenic T cells in the B10.PL model of experimental allergic encephalomyelitis (EAE) are of the Th-1 lymphokine subtype. *Cellular Immunology* **1989**, *124*, 132-143, 10.1016/0008-8749(89)90117-2.

12. Julia Åivo; Arno Hänninen; Jorma Ilonen; Merja Soilu-Hänninen; Vitamin D3 administration to MS patients leads to increased serum levels of latency activated peptide (LAP) of TGF-beta. *Journal of Neuroimmunology* **2015**, *280*, 12-15, 10.1016/j.jneuroim.2015.01.005.

13. Daniel Kantor; Elias S. Sotirchos; Peter A. Calabresi; Safety and immunologic effects of high- vs low-dose cholecalciferol in multiple sclerosis.. *Neurology* **2016**, *87*, 1424-1424, 10.1212/01.wnl.0000502811.31151.c9.

14. Muris, A. H.; Smolders, J.; Rolf, L.; Thewissen, M.; Hupperts, R.; Damoiseaux, J.; group, S. s., Immune regulatory effects of high dose vitamin D3 supplementation in a randomized controlled trial in relapsing remitting multiple sclerosis patients receiving IFNbeta; the SOLARIUM study. J Neuroimmunol 2016, 300, 47-56.

15. Joost Smolders; Evelyn Peelen; Marielle Thewissen; Jan Willem Cohen Tervaert; Paul Menheere; Raymond Hupperts; Jan Damoiseaux; Safety and T Cell Modulating Effects of High Dose Vitamin D3 Supplementation in Multiple Sclerosis. *PLOS ONE* **2010**, *5*, e15235, 10.1371/journal.pone.0015235.

16. Anne L. Astier; David A. Hafler; Abnormal Tr1 differentiation in multiple sclerosis. *Journal of Neuroimmunology* **2007**, *191*, 70-8, 10.1016/j.jneuroim.2007.09.018.

17. Xiaohua Wang; Jintao Zhang; David J. Baylink; Chih-Huang Li; Uglas M. Watts; Yi Xu; Xuezhong Qin; Michael H. Walter; Xiaolei Tang; Targeting Non-classical Myelin Epitopes to Treat Experimental Autoimmune Encephalomyelitis. *Scientific Reports* **2016**, *6*, 36064, 10.1038/srep36064.

18. Li, C. H.; Zhang, J.; Baylink, D. J.; Wang, X.; Goparaju, N. B.; Xu, Y.; Wasnik, S.; Cheng, Y.; Berumen, E. C.; Qin, X.; Lau, K. W.; Tang, X., Dendritic cells, engineered to overexpress 25-hydroxyvitamin D 1alpha-hydroxylase and pulsed with a myelin antigen, provide myelin-specific suppression of ongoing experimental allergic encephalomyelitis. FASEB J 2017, 31, 2996-3006.

19. K. M. Danikowski; S. Jayaraman; Bellur S. Prabhakar; Regulatory T cells in multiple sclerosis and myasthenia gravis. *Journal of Neuroinflammation* **2017**, *14*, 117, 10.1186/s12974-017-0892-8.

20. Ankanee Chanakul; Martin Y. H. Zhang; Andrew Louw; Harvey J. Armbrecht; Walter L. Miller; Anthony A. Portale; Farzana Perwad; FGF-23 Regulates CYP27B1 Transcription in the Kidney and in Extra-Renal Tissues. *PLOS ONE* **2013**, *8*, e72816, 10.1371/journal.pone.0072816.

21. Akiko Murayama; Ken-Ichi Takeyama; Sachiko Kitanaka; Yasuo Kodera; Yoshindo Kawaguchi; Tatsuo Hosoya; Shigeaki Kato; Positive and Negative Regulations of the Renal 25-Hydroxyvitamin D3 1α-Hydroxylase Gene by Parathyroid Hormone, Calcitonin, and 1α,25(OH)2D3 in Intact Animals*. *Endocrinology* **1999**, *140*, 2224-2231, 10.1210/endo.140.5.6691.

22. R. T. Turner; J. E. Puzas; M. D. Forte; G. E. Lester; T. K. Gray; G. A. Howard; D. J. Baylink; In vitro synthesis of 1 alpha,25-dihydroxycholecalciferol and 24,25-dihydroxycholecalciferol by isolated calvarial cells.. *Proceedings of the National Academy of Sciences* **1980**, *77*, 5720-5724, 10.1073/pnas.77.10.5720.

23. John Adams; Brandon Rafison; Sten Witzel; Rachel E. Reyes; Albert Shieh; Rene Chun; Kathryn Zavala; Martin Hewison; Philip Liu; Regulation of the extrarenal CYP27B1-hydroxylase. *The Journal of Steroid Biochemistry and Molecular Biology* **2014**, *144*, 22-27, 10.1016/j.jsbmb.2013.12.009.
24. Zehnder, D.; Bland, R.; Williams, M.C.; McNinch, R.W.; Howie, A.J.; Stewart, P.M.; Hewison, M. Extrarenal expression of 25-hydroxyvitamin d(3)-1 alpha-hydroxylase. J. Clin. Endocrinol. Metab. 2001, 86, 888–894.
25. St-Arnaud, R.; Dardenne, O.; Prud'homme, J.; Hacking, S.A.; Glorieux, F.H. Conventional and tissue-specific inactivation of the 25-hydroxyvitamin D-1alpha-hydroxylase (CYP27B1). J. Cell Biochem. 2003, 88, 245–251.
26. Roy Pascal Naja; Olivier Dardenne; Alice Arabian; René St-Arnaud; Chondrocyte-Specific Modulation of Cyp27b1 Expression Supports a Role for Local Synthesis of 1,25-Dihydroxyvitamin D 3 in Growth Plate Development. *Endocrinology* **2009**, *150*, 4024-4032, 10.1210/en.2008-1410.
27. Karl P. Schlingmann; Martin Kaufmann; Stefanie Weber; Andrew Irwin; Caroline Goos; Ulrike John; Joachim Misselwitz; Günter Klaus; Eberhard Kuwertz-Bröking; Henry Fehrenbach; et al. Mutations inCYP24A1and Idiopathic Infantile Hypercalcemia. *New England Journal of Medicine* **2011**, *365*, 410-421, 10.1056/nejmoa1103864.
28. Goldberg, P. Multiple Sclerosis–Vitamin D and Calcium as Environmental Determinants of Prevalence (a Viewpoint). 1. Sunlight, Dietary Factors and Epidemiology. Int. J. Environ. Stud. 1974, 6, 19–27.
29. Sintzel, M.B.; Rametta, M.; Reder, A.T; Vitamin D and Multiple Sclerosis: A Comprehensive Review. *Neurol. Ther.* **2018**, *7*, 59–85, 10.6084/m9.figshare.6015938.v1.
30. Lemire, J.M.; Archer, D.C. 1,25-dihydroxyvitamin D3 prevents the in vivo induction of murine experimental autoimmune encephalomyelitis. J. Clin. Investig. 1991, 87, 1103–1107.
31. M. T. Cantorna; C. E. Hayes; H. F. DeLuca; 1,25-Dihydroxyvitamin D3 reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. *Proceedings of the National Academy of Sciences* **1996**, *93*, 7861-7864, 10.1073/pnas.93.15.7861.
32. M T Cantorna; W D Woodward; C E Hayes; H F DeLuca; 1,25-dihydroxyvitamin D3 is a positive regulator for the two anti-encephalitogenic cytokines TGF-beta 1 and IL-4. *The Journal of Immunology* **1998**, *160*, 5314–5319, .
33. Terrence F. Meehan; Hector F DeLuca; The vitamin D receptor is necessary for 1alpha,25-dihydroxyvitamin D(3) to suppress experimental autoimmune encephalomyelitis in mice. *Archives of Biochemistry and Biophysics* **2002**, *408*, 200–204, .
34. Margherita T. Cantorna; Jean Humpal-Winter; Hector F. DeLuca; Dietary calcium is a major factor in 1,25-dihydroxycholecalciferol suppression of experimental autoimmune encephalomyelitis in mice. *The Journal of Nutrition* **1999**, *129*, 1966-1971, 10.1093/jn/129.11.1966.
35. Hector F. DeLuca; L. Plum; UVB radiation, vitamin D and multiple sclerosis. *Photochemical & Photobiological Sciences* **2017**, *16*, 411-415, 10.1039/c6pp00308g.
