

Update in vitamin D and multiple sclerosis

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ABSTRACT

التصلب اللويحي المتعدد (MS) يعتبر مرض عصبي شائع، ولا يزال سبب هذا المرض غير معروف. في الأعوام الماضية تزايد الاهتمام بعلاقة نقص فيتامين د بمرض التصلب اللويحي المتعدد. نستعرض في هذا المقال مراجعة لأحدث المواضيع التي تطرقت لهذه العلاقة والتي بينت أن نقص فيتامين د عامل مساعد في حدوث المرض، ولكن الأدلة غير كافية عن علاقة نقص فيتامين د بتفاقم المرض.

Multiple sclerosis (MS) is a common neurological disease, and its etiology remains unknown. In recent years, increasing attention has been paid to the possible association between MS and vitamin D deficiency. Here, we review the current literature between MS and vitamin D, showing clear evidence that vitamin D deficiency is a risk factor for MS despite the lack of direct evidence for the effects of vitamin D in MS progression.

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Multiple sclerosis (MS) is a chronic, inflammatory, and demyelinating disorder that affects the CNS.¹ Multiple sclerosis is a common neurologic disorder; the estimated number of individuals with MS has increased from 2.1 million in 2008 to 2.3 million in 2013.² Based on the 1975 Kurtzke classification,³ the Arabian Gulf Region is located in a low-risk zone for MS; however, recent studies suggest a moderate-to-high prevalence of MS in this region (31-55 MS patients per 100,000 individuals).⁴ The risk of MS is determined by genetic and environmental factors. One of the latter is vitamin D deficiency, which has attracted increased attention in the last decade.⁵ Entering the words “multiple sclerosis” and

“vitamin D” as a PubMed search yields approximately 790 results in the last 10 years alone. Approximately one billion people worldwide have vitamin D deficiency or insufficiency.⁶ In a retrospective observational study of 10,709 patients in tertiary hospital clinics in Saudi Arabia, the prevalence of vitamin D deficiency was found to be 83.6%, which is high.⁷ The present article reviews the correlation between MS and vitamin D, considering updated studies from the literature.

Vitamin D metabolism. Vitamin D is a fat-soluble vitamin; its 2 main forms are ergocalciferol (vitamin D₂), which is of plant origin, and cholecalciferol (vitamin D₃), which is of animal origin. Vitamin D₂ is considered less bioactive than vitamin D₃. Vitamin D can be obtained from food, such as fatty fish, fortified foods, and vitamin supplements. However, the diet provides only a small percentage of human vitamin D intake, and the main source is skin exposure to sunlight. Total-body sun exposure easily provides the equivalent of 250 µg (10000 IU) vitamin D/d.⁸ In the skin, 7-dehydrocholesterol is photolyzed by ultraviolet radiation (UVR) from the sun and converted to pre-vitamin D₃, which is isomerized to vitamin D₃.⁹ The vitamin D binding protein transports vitamin D₃ through the blood to the liver, where vitamin D is hydroxylated by one or more cytochrome P450 vitamin D 25-hydroxylases, resulting in the formation of 25-hydroxyvitamin D₃ (25(OH)D₃).¹⁰ Vitamin D status is reflected by serum levels of 25(OH)D₃, which is the longest-living vitamin D metabolite (in terms of half-life).¹¹ The 25(OH)D₃ metabolite is further hydroxylated by renal CYP27B1 to 1,25-dihydroxyvitamin D [1,25(OH)₂D; calcitriol], which is the most bioactive vitamin D metabolite.

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Vitamin D signaling is mediated by calcitriol binding to the vitamin D receptor, which forms a nuclear heterodimer with the retinoid X receptor. This complex is capable of binding to genomic vitamin D response elements, modulating the expression of a variety of genes.¹² Also, like many other hormones, vitamin D can exert rapid actions at a cellular level (non-genomic effects), these actions are mediated within seconds to minutes.¹³ It is through these pathways that vitamin D modulates calcium hemostasis and performs its immunomodulatory functions.

Vitamin D as an immunomodulator. Vitamin D receptor expression has been reported in most immune cells,¹⁴ as well as in CNS tissues.¹⁵ Additionally, the rate-limiting enzyme for vitamin D synthesis, 25(OH)D3-1 α -hydroxylase (CYP27B1), is expressed in immune cells. These cells are therefore, able to synthesize and secrete active vitamin D in both an autocrine and paracrine fashion,¹⁶ indicating that vitamin D plays a role in the immune system. The in vitro addition of 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) to antigen-presenting cells (namely, monocytes, macrophages, and dendritic cells) inhibits the surface expression of major histocompatibility complex II-complexed antigens, and of costimulatory molecules, leading to reduced T cell stimulatory capacity. Additionally, 1,25(OH)2D3 directly exerts its immunomodulatory effects on T lymphocytes by inhibiting the production of Type 1 helper T cell cytokines (considered to be the key mediators in graft rejection and autoimmune diseases) and stimulating the production of Type 2 helper T cell cytokines, which have immunoregulatory functions.¹⁷ Furthermore, 1,25(OH)2D3 inhibits T cell and B cell proliferation and blocks B cell differentiation and immunoglobulin secretion. This compound also affects T cell maturation, inducing a shift away from the inflammatory T-helper 17 cells phenotype and facilitating the production of regulatory T cells.¹⁸ All of these immunomodulatory effects of 1,25(OH)2D3 can lead to the protection of target tissue in autoimmune diseases and transplantation. However, experimental studies have reported that the observed immunomodulatory effects of vitamin D only occur at hyper-physiologic concentrations, which causes hypercalcemia in humans.¹⁷ Therefore, the development of novel vitamin D analogs that have immunosuppressive effects, but do not cause significant hypercalcemia is required. Many clinical trials have been, or are currently being conducted to test the therapeutic application of vitamin D or its analogs in inflammatory processes.¹⁹

Vitamin D and MS risk. It is well known that MS is more prevalent in higher latitudes, where sunlight

is of lower intensity than in lower latitudes. Recent meta-analyses²⁰ supported a latitude gradient in MS prevalence. Several recent studies found that increased body exposure to sunlight, and hence a decreased susceptibility to vitamin D deficiency, is also associated with a decreased risk of MS,²¹⁻²⁶ especially if the sun exposure occurred during childhood and adolescence. Studies have also shown that the birth month is correlated with MS risk; individuals born in the fall (namely, whose mothers were exposed to summer sunlight) have a low MS risk, whereas individuals born in the spring have a higher risk of MS. This observation indicates the presence of an association among maternal sunlight exposure during pregnancy, vitamin D status, and the risk of MS. In 2013, a systematic review²⁷ analyzed published data on the effects of birth month for 151,978 MS patients born in the Northern Hemisphere. The results of this analysis demonstrated a significant increase of MS risk among individuals who were born in April and a reduction in risk in people who were born in October and November. However, sunlight also has an immunosuppressive effect,²⁸ and UVR was recently found to suppress experimental autoimmune encephalomyelitis.^{29,30} Therefore, the effects of sunlight on MS risk could be related to sunlight itself; instead of vitamin D. Studies that evaluate either serum vitamin D levels or vitamin D intake, are needed to determine whether vitamin D deficiency is a risk factor for MS independent of sun exposure. The strongest evidence in this regard came from a large prospective case-control study³¹ of more than 7 million US military personnel from whom serum samples were obtained before any appearance of MS symptoms. This study concluded that, among Caucasians, there was a 41% decrease in MS risk for every 50-nmol/L increase in 25-hydroxyvitamin D; the effect was stronger for samples taken before the age of 20. Another case-control study³² prospectively collected blood samples from 192 MS patients and showed that serum 25-hydroxyvitamin D (25(OH)D) levels ≥ 75 (versus < 75) nmol/L in the blood were associated with a 61% decrease in the risk of MS. A recent study concluded that sun exposure and vitamin D status independently affect the risk of MS.²⁴ Vitamin D intake and the risk of developing MS were assessed in a large prospective cohort that included approximately 200,000 women.³³ In this study, the incidence of MS was 41% lower among women with a vitamin D intake of ≥ 400 IU/day, compared with women who did not take supplements.³³ Another 2 studies concluded that the intake of fatty fish was associated with a decreased risk of MS, even at higher latitudes.^{34,35} Mirzaei et al³⁶ studied a large cohort and analyzed the association

between maternal milk intake, maternal dietary vitamin D intake, and predicted maternal serum 25(OH)D during pregnancy and their daughters' risk of developing MS. The study showed that the relative risk of MS was significantly lower in women whose mothers had high milk or vitamin D intake during pregnancy than in women born to low-intake mothers.³⁶ However, another study of a large cohort of US women showed that total vitamin D intake during adolescence was not associated with the risk for MS in adulthood.³⁷ In addition, 25(OH)D levels during the neonatal period were not associated with the risk of MS in a large population-based case-control study.³⁸ This data may support the hypothesis that vitamin D consumption throughout an individual's entire lifespan, rather than over a short period of time, may contribute to the risk of MS.

Vitamin D and disease progression in MS. Several studies have shown that vitamin D levels are lower in MS patients than in controls.³⁹⁻⁴⁴ A recent study⁴⁵ showed that in clinically isolated syndrome patients (namely, those suffering a single demyelinating attack that is compatible with MS), vitamin D deficiency was a predictor of developing clinically definite MS. The association of disease activity with vitamin D levels in MS patients has been evaluated in multiple studies that demonstrated a lower MS relapse rate in patients with higher levels of vitamin D.⁴⁶⁻⁴⁹ Additionally, low levels of vitamin D appear to be associated with high levels of disability^{41,47-52} as measured by the Expanded Disability Status Scale (EDSS). The EDSS is a commonly used index of clinical disability in MS, with scores ranging from 0 (corresponding to a normal examination and function) to 10 (for death due to MS). Two recent studies conducted in 2014 support this association. In the first study,⁵³ 181 patients were prospectively followed, and EDSS scores were correlated with plasma vitamin D levels. Patients with vitamin D levels >50-nmol/L were 2.78 times more likely to have an EDSS <4 ($p=0.0011$).⁵³ The second study⁵⁴ was originally designed to evaluate the impact of early versus delayed interferon beta-1b treatment in patients with clinically isolated syndrome. Serum 25(OH)D concentrations were measured at baseline and at 6, 12, and 24 months. Patients were followed for 5 years with clinical assessments and MRI. A 50-nmol/L increase in average serum 25(OH)D levels within the first 12 months predicted a 57% lower rate of new active lesions ($p<0.001$), a 57% lower relapse rate ($p=0.03$), and a 25% lower yearly increase in T2 lesion volume ($p<0.001$) from months 12 to 60. Levels ≥ 50 nmol/L at follow-periods of up to 12 months predicted lower

EDSS scores ($p=0.004$) during the subsequent 4 years.⁵⁴ Observational studies correlating vitamin D levels to MS severity cannot prove that increased sun exposure alleviates the symptoms of MS, especially given that severely disabled patients with MS receive less sun exposure, which can cause vitamin D deficiency. Even MS patients who are fully mobile are theoretically more susceptible to vitamin D deficiency because they avoid sun exposure, worsening their symptoms. Therefore, establishing the effects of vitamin D on disease activity and severity in MS patients requires randomized controlled trials (RCTs). Table 1 summarizes interventional studies in which MS patients received vitamin D supplementation.⁵⁵⁻⁶⁶ As shown in Table 1, none of the RCTs demonstrated a significant reduction in relapse rate or EDSS in response to vitamin D supplementation. In one RCT, a significant decrease in T1-enhancing lesions was seen in the treatment group. Multiple studies have demonstrated favorable immunological changes in the serum of MS patients in the treatment group. However, none of the RCTs listed in Table 1 were sufficiently powered to observe a treatment effect. Therefore, we cannot conclude that vitamin D is a clinically effective treatment for MS patients; however, we can conclude that a high dose of vitamin D is safe in the short term. Approximately 6 large RCTs are ongoing; the results of these analyses will provide solid evidence regarding the benefits of vitamin D supplementation.

Vitamin D, genetics, and MS. The increase in the concordance ratio for MS risk between mono- and dizygotic twins with increasing latitude suggests that genetic effects may be stronger for individuals with low concentrations of vitamin D.⁶⁷ The Wellcome Trust Case Control Consortium and the International MS Genetics Consortium completed the largest MS genome-wide association study⁶⁸ and identified 2 genes involved in vitamin D metabolism that could increase susceptibility to MS. The first is CYP27B1, which encodes a rate-limiting enzyme for vitamin D synthesis. The second is CYP24A1, which encodes an enzyme that degrades 1,25-dihydroxyvitamin D. It is likely that these genes contribute to MS risk by decreasing the levels of active vitamin D. In addition, the RNA expression level of the major MS susceptibility gene HLA-DRB1*15:01 (the strongest genetic predictor of MS risk) is regulated by vitamin D. Furthermore, vitamin D receptor-binding elements have been identified in the majority of MS-associated genes, indicating that the expression of many of these genes may be regulated by vitamin D.⁶⁹

In conclusion, it is clear from observational studies that vitamin D deficiency is a modifiable risk factor

Table 1 - Summary of vitamin D interventional studies that have been carried out in multiple sclerosis patients.

Study date	Study type	Vitamin D dose, size, and duration of the study	Clinical results	Radiological results	Laboratory results
Mosayebi et al, 2011 ⁵⁵	RCT	A total of 62 MS patients enrolled; treatment group received 300,000 IU/month vitamin D3 as IM injection for 6 months	No significant difference in EDSS between the treatment and control group	No significant difference in the number of gadolinium-enhancing lesions between the treatment and control group	Levels of cell proliferation in the treatment group were significantly lower than in the control. The TGF-beta and INL-10 treatment groups were significantly higher than in the controls
Wingerchuk et al, 2005 ⁵⁶	Open-label pilot study	Fifteen MS patients received oral calcitriol (target dose: 2.5 microg/d) for 48 weeks. Dietary calcium was restricted to 800 mg/d	The on-study exacerbation rate (27%) was less than baseline. Two patients withdrew because of symptomatic hypercalcemia upon discontinuation of calcitriol at 12 months. The EDSS increased to 3.1	Brain MRI revealed enhancing lesions in 5 patients at baseline (33%), and in 4 (29%) at both 24 and 48 weeks	
Soilu-Hänninen et al, 2012 ⁵⁷	RCT	Total of 66 MS enrolled; treatment group received 20000 IU/week vitamin D3 as an add-on therapy to interferon β -1b for over one year	Tendency for reduced disability accumulation ($p=0.071$). No significant differences in adverse events or in the annual relapse rate	Significantly lower number of T1-enhancing lesions in the treatment group ($p=0.004$)	
Kimball S et al, 2011 ⁵⁸	RCT	Total of 49 patients enrolled; treatment group received increasing doses of cholecalciferol (4,000-40,000 IU/d) plus calcium (1200 mg/d), followed by 10,000 IU/d over one year			Abnormal T cell reactivities were suppressed in vivo by cholecalciferol at serum 25(OH)D concentrations higher than 100 nmol/L
Shaygannejad et al, 2012 ⁵⁹	RCT	Total of 50 patients enrolled; treatment group received escalating calcitriol doses up to 0.5 μ g/day over one year	In the placebo group, the mean EDSS increased from 1.70 at baseline to 1.94 at the end of the study ($p<0.01$). Average EDSS and RR at the end of the trial did not differ between the 2 groups. The EDSS was higher following high-dose D2 than following low-dose D2 ($p=0.05$). There were 4 relapses with high-dose D2 versus none with low-dose D2 ($p=0.04$)		
Stein et al, 2011 ⁶⁰	RCT	A total of 23 MS patients; treatment group received high-dose vitamin D2 (6,000 IU) over 6 months. All received daily low-dose (1,000 IU) D2 to prevent deficiency		No significant treatment differences were detected in the primary MRI endpoints	
Burton et al, 2010 ⁶¹	RCT	A total of 49 MS patients enrolled in the 52-week trial; treatment group received escalating vitamin D doses up to 40,000 IU/day over 28 weeks, followed by 10,000 IU/day (12 weeks), and further down titrated to 0 IU/day; calcium (1,200 mg/day) was given	No significant adverse events occurred. Non-significant reduction in RR and EDSS in the treatment group		

MS - multiple sclerosis, IM intramuscular, EDSS - Expanded Disability Status Scale, RR - relapse rate, TGF - transforming growth factor, INL - interleukin, 25(OH)D - 25-hydroxyvitamin D, ON - optic neuritis, ARR - annualized relapse rate, RCT - randomized controlled trials

Table 1 - Summary of vitamin D interventional studies that have been carried out in multiple sclerosis patients. cont'd.

Study date	Study type	Vitamin D dose, size, and duration of the study	Clinical results	Radiological results	Laboratory results
Derakhshandi et al, 2013 ⁶²	RCT	Thirty optic neuritis patients with serum 25(OH)D levels of less than 30 ng/ml were enrolled; the treatment group (cases) received 50,000 IU of vitamin D3 weekly for 12 months	Risk reduction was 68.4% for the primary outcome (conversion of ON to MS) in the treatment group (relative risk=0.316, $p=0.007$)	After 12 months, patients in the treatment group had a significantly lower incidence rate of new T2, new gadolinium-enhancing lesions and black holes	
Kampman et al, 2012 ⁶³	RCT	A total of 68 MS patients enrolled; treatment group received 20,000 IU vitamin D(3) weekly for 96 weeks	No significant difference between groups in ARR EDSS		
Mahon et al, 2003 ⁶⁴	RCT	A total of 39 MS patients enrolled; treatment group received 1000 IU vitamin D mg, both groups received 800 mg supplemental calcium			Treatment group has increased serum TGF-beta 1
Smolders et al, 2010 ⁶⁵	Single group assignment	Fifteen MS patients were supplemented with 20,000 IU/d vitamin D3 for 12 weeks			Skewing towards an anti-inflammatory cytokine profile
Knippenberg et al, 2011 ⁶⁶	Cohort	Fifteen MS patients received 20,000 IU/day of vitamin D3 over 12 weeks			High doses of vitamin D(3) did not have substantial effects on phenotypic markers of B cell differentiation in circulating B cells

MS - multiple sclerosis, IM intramuscular, EDSS - Expanded Disability Status Scale, RR - relapse rate, TGF - transforming growth factor, INL - interleukin, 25(OH)D - 25-hydroxyvitamin D, ON - optic neuritis, ARR - annualized relapse rate, RCT - randomized controlled trials

for MS. Therefore, persons who are at risk for MS (for example, first-degree relatives of MS patients, or patients with a single episode demyelinating attack) should be screened for vitamin D deficiency. As stated previously, evidence for the effect of vitamin D on disease progression is lacking, but it is known that MS patients have an increased prevalence for vitamin D deficiency (due to for example, immobility, sun avoidance, corticosteroids, and anti-epileptic use). These patients are also susceptible to osteoporosis.⁷⁰ Therefore, vitamin D levels should be determined, and deficiency should be treated. The optimal serum vitamin D levels for exerting immunomodulatory effects have not been clinically established. Based on bone health criteria, the US and Canadian Institute of Medicine (IOM) recently stated that individuals are vitamin D sufficient at 25OHD levels ≥ 50 nmol/L; levels above 75 nmol/L have not consistently been associated with an increased benefit. Although risks have been identified for some outcomes at levels above 125 nmol/L, it has been suggested that up to 4000 IU/day of vitamin D intake is unlikely to cause toxicity, even in healthy individuals.⁷¹ Some experts favor maintaining 25(OH)D levels between 75 to 125 nmol/L in MS patients, as these levels are still within the

safe range of the IOM report, and immunomodulatory effects have been observed in hyper-physiologic ranges in experimental studies. However, the long-term effects of such high levels are unknown.

References

- Awad AM, Stüve O. Immunopathogenesis of multiple sclerosis: new insights and therapeutic implications. *Continuum (Minneapolis)* 2010; 16: 166-180.
- Browne P, Chandraratna D, Angood C, Tremlett H, Baker C, Taylor BV. Atlas of Multiple Sclerosis 2013: A growing global problem with widespread inequity. *Neurology* 2014; 83: 1022-1024.
- Kurtzke JF. A reassessment of the distribution of multiple sclerosis. *Acta Neurol Scand* 1975; 51: 137-157.
- Bohlega S, Inshasi J, Al Tahan AR, Madani AB, Qahtani H, Rieckmann P. Multiple sclerosis in the Arabian Gulf countries: a consensus statement. *J Neurol* 2013; 260: 2959-2963.
- Ramagopalan SV, Dobson R, Meier UC, Giovannoni G. Multiple sclerosis: risk factors, prodromes, and potential causal pathways. *Lancet Neurol* 2010; 9: 727-739.
- Holick MF. Vitamin D deficiency. *N Engl J Med* 2007; 357: 266-281.
- Hussain AN, Alkhenizan AH, El Shaker M, Raef H, Gabr A. Increasing trends and significance of hypovitaminosis D: a population-based study in the Kingdom of Saudi Arabia. *Arch Osteoporos* 2014; 9: 190.

8. Vieth R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. *Am J Clin Nutr* 1999; 69: 842-856.
9. Bikle DD. Vitamin D metabolism, mechanism of action, and clinical applications. *Chem Biol* 2014; 21: 319-329.
10. Christakos S, Ajibade DV, Dhawan P, Fechner AJ, Mady LJ. Vitamin D: metabolism. *Endocrinol Metab Clin North Am* 2010; 39: 243-253.
11. Hollis BW. Assessment of vitamin D nutritional and hormonal status: what to measure and how to do it. *Calcif Tissue Int* 1996; 58: 4-5.
12. Hanwell HE, Banwell B. Assessment of evidence for a protective role of vitamin D in multiple sclerosis. *Biochim Biophys Acta* 2011; 1812: 202-212.
13. Trochoutsou A, Kloukina V, Samitas K, Xanthou G. Vitamin-D in the Immune System: Genomic and Non-Genomic Actions. *Mini Rev Med Chem* 2015; 15: 953-963.
14. Provvedini DM, Tsoukas CD, Deftos LJ, Manolagas SC. 1,25-dihydroxyvitamin D₃ receptors in human leukocytes. *Science* 1983; 221: 1181-1183.
15. Garcion E, Wion-Barbot N, Montero-Menei CN, Berger F, Wion D. New clues about vitamin D functions in the nervous system. *Trends Endocrinol Metab* 2002; 13: 100-105.
16. Gottfried E, Rehli M, Hahn J, Holler E, Andreesen R, Kreutz M. Monocyte-derived cells express CYP27A1 and convert vitamin D₃ into its active metabolite. *Biochem Biophys Res Commun* 2006; 349: 209-213.
17. van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D₃: basic concepts. *J Steroid Biochem Mol Biol* 2005; 97: 93-101.
18. Aranow C. Vitamin D and the immune system. *J Invest Med* 2011; 59: 881-886.
19. Scolletta S, Colletti M, Di Luigi L, Crescioli C. Vitamin D receptor agonists target CXCL10: new therapeutic tools for resolution of inflammation. *Mediators Inflamm* 2013; 2013: 876319.
20. Simpson S Jr, Blizzard L, Otahal P, Van der Mei I, Taylor B. Latitude is significantly associated with the prevalence of multiple sclerosis: a meta-analysis. *J Neurol Neurosurg Psychiatry*. 2011; 82: 1132-1141.
21. Mansouri B, Asadollahi S, Heidari K, Fakhri M, Assarzaghan F, Nazari M, et al. Risk factors for increased multiple sclerosis susceptibility in the Iranian population. *J Clin Neurosci* 2014; 21: 2207-2211.
22. Bjørnevik K, Riise T, Casetta I, Drulovic J, Granieri E, Holmøy T, et al. Sun exposure and multiple sclerosis risk in Norway and Italy: The EnvIMS study. *Mult Scler* 2014; 20: 1042-1049.
23. Islam T, Gauderman WJ, Cozen W, Mack TM. Childhood sun exposure influences risk of multiple sclerosis in monozygotic twins. *Neurology* 2007; 69: 381-388.
24. Lucas RM, Ponsonby AL, Dear K, Valery PC, Pender MP, Taylor BV, et al. Sun exposure and vitamin D are independent risk factors for CNS demyelination. *Neurology* 2011; 76: 540-548.
25. van der Mei IA, 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.
26. Bäärnhielm M, Hedström AK, Kockum I, Sundqvist E, Gustafsson SA, Hillert J, et al. Sunlight is associated with decreased multiple sclerosis risk: no interaction with human leukocyte antigen-DRB1*15. *Eur J Neurol* 2012; 19: 955-962.
27. Dobson R, Giovannoni G, Ramagopalan S. The month of birth effect in multiple sclerosis: systematic review, meta-analysis and effect of latitude. *J Neurol Neurosurg Psychiatry* 2013; 84: 427-432.
28. Norval M, Halliday GM. The consequences of UV-induced immunosuppression for human health. *Photochem Photobiol* 2011; 87: 965-977.
29. Wang Y, Marling SJ, Beaver EF, Severson KS, Deluca HF. UV light selectively inhibits spinal cord inflammation and demyelination in experimental autoimmune encephalomyelitis. *Arch Biochem Biophys* 2015; 567: 75-82.
30. Becklund BR, Severson KS, Vang SV, DeLuca HF. UV radiation suppresses experimental autoimmune encephalomyelitis independent of vitamin D production. *Proc Natl Acad Sci U S A* 2010; 107: 6418-6423.
31. Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. *JAMA* 2006; 296: 2832-2838.
32. Salzer J, Hallmans G, Nyström M, Stenlund H, Wadell G, Sundström P. Vitamin D as a protective factor in multiple sclerosis. *Neurology* 2012; 79: 2140-2145.
33. Munger 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: 60-65.
34. Kampman MT, Wilsgaard T, Mellgren SI. Outdoor activities and diet in childhood and adolescence relate to MS risk above the Arctic Circle. *J Neurol* 2007; 254: 471-477.
35. Bäärnhielm M, Olsson T, Alfredsson L. Fatty fish intake is associated with decreased occurrence of multiple sclerosis. *Mult Scler* 2014; 20: 726-732.
36. Mirzaei F, Michels KB, Munger K, O'Reilly E, Chitnis T, Forman MR, et al. Gestational vitamin D and the risk of multiple sclerosis in offspring. *Ann Neurol* 2011; 70: 30-40.
37. Munger KL, Chitnis T, Frazier AL, Giovannucci E, Spiegelman D, Ascherio A. Dietary intake of vitamin D during adolescence and risk of multiple sclerosis. *J Neurol* 2011; 258: 479-485.
38. Ueda P, Rafatnia F, Bäärnhielm M, Fröbom R, Korzunowicz G, Lönnerbro R, et al. Neonatal vitamin D status and risk of multiple sclerosis. *Ann Neurol* 2014; 76: 338-346.
39. Pandit L, Ramagopalan SV, Malli C, D'Cunha A, Kunder R, Shetty R. Association of vitamin D and multiple sclerosis in India. *Mult Scler* 2013; 19: 1592-1296.
40. Neau JP, Artaud-Urior MS, Lhomme V, Bounaud JY, Lebras F, Boissonnot L, et al. [Vitamin D and multiple sclerosis. A prospective survey of patients of Poitou-Charentes area]. *Rev Neurol (Paris)* 2011; 167: 317-323. French
41. Shahbeigi S, Pakdaman H, Fereshtehnejad SM, Nikravan E, Mirabi N, Jalilzadeh G. Vitamin d₃ concentration correlates with the severity of multiple sclerosis. *Int J Prev Med* 2013; 4: 585-591.
42. Mazdeh M, Seifirad S, Kazemi N, Seifrabie MA, Dehghan A, Abbasi H. Comparison of vitamin D₃ serum levels in new diagnosed patients with multiple sclerosis versus their healthy relatives. *Acta Med Iran* 2013; 51: 289-292.
43. Kubicka K, Pierzchała K. Concentration of 25(OH)D₃ and calcium and phosphorus metabolism in patients suffering from relapsing-remitting multiple sclerosis. A pilot study. *Neurol Neurochir Pol* 2013; 47: 126-130.
44. Yildiz M, Tettenborn B, Putzki N. Vitamin D levels in Swiss multiple sclerosis patients. *Swiss Med Wkly* 2011; 141: w13192.

45. Martinelli V, Dalla Costa G, Colombo B, Dalla Libera D, Rubinacci A, Filippi M, et al. Vitamin D levels and risk of multiple sclerosis in patients with clinically isolated syndromes. *Mult Scler* 2014; 20: 147-155.
46. Simpson S Jr, Taylor B, Blizzard L, Ponsonby AL, Pittas F, Tremlett H, et al. Higher 25-hydroxyvitamin D is associated with lower relapse risk in multiple sclerosis. *Ann Neurol* 2010; 68: 193-203.
47. Mowry EM, Krupp LB, Milazzo M, Chabas D, Strober JB, Belman AL, et al. Vitamin D status is associated with relapse rate in pediatric-onset multiple sclerosis. *Ann Neurol* 2010; 67: 618-624.
48. Runia TF, Hop WC, de Rijke YB, Buljevac D, Hintzen RQ. Lower serum vitamin D levels are associated with a higher relapse risk in multiple sclerosis. *Neurology* 2012; 79: 261-266.
49. Smolders J, Menheere P, Kessels A, Damoiseaux J, Hupperts R. Association of vitamin D metabolite levels with relapse rate and disability in multiple sclerosis. *Mult Scler* 2008; 14: 1220-1224.
50. van der Mei IA, 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: 581-590.
51. Weinstock-Guttman B, Zivadinov R, Qu J, Cookfair D, Duan X, Bang E, et al. Vitamin D metabolites are associated with clinical and MRI outcomes in multiple sclerosis patients. *J Neurol Neurosurg Psychiatry* 2011; 82: 189-195.
52. Harandi AA, Shahbeigi S, Pakdaman H, Fereshtehnejad SM, Nikravesh E, Jalilzadeh R. Association of serum 25(OH) vitamin D3 concentration with severity of multiple sclerosis. *Iran J Neurol* 2012; 11: 54-58.
53. Thouvenot E, Orsini M, Daures JP, Camu W. Vitamin D is associated with degree of disability in patients with fully ambulatory relapsing-remitting multiple sclerosis. *Eur J Neurol* 2015; 22: 564-569.
54. Ascherio A, Munger KL, White R, Köchert K, Simon KC, Polman CH, et al. Vitamin D as an early predictor of multiple sclerosis activity and progression. *JAMA Neurol* 2014; 71: 306-314.
55. Mosayebi G, Ghazavi A, Ghasami K, Jand Y, Kokhaei P. Therapeutic effect of vitamin D3 in multiple sclerosis patients. *Immunol Invest* 2011; 40: 627-639.
56. Wingerchuk DM, Lesaux J, Rice GP, Kremenutzky M, Ebers GC. A pilot study of oral calcitriol (1,25-dihydroxyvitamin D3) for relapsing-remitting multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2005; 76: 1294-1296.
57. Soilu-Hänninen M, Aivo J, Lindström BM, Elovaara I, Sumelahti ML, Färkkilä M, et al. A randomised, double blind, placebo controlled trial with vitamin D3 as an add on treatment to interferon β -1b in patients with multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2012; 83: 565-571.
58. Kimball S, Vieth R, Dosch HM, Bar-Or A, Cheung R, Gagne D, et al. Cholecalciferol plus calcium suppresses abnormal PBMC reactivity in patients with multiple sclerosis. *J Clin Endocrinol Metab* 2011; 96: 2826-2834.
59. Shaygannejad V, Janghorbani M, Ashtari F, Dehghan H. Effects of adjunct low-dose vitamin d on relapsing-remitting multiple sclerosis progression: preliminary findings of a randomized placebo-controlled trial. *Mult Scler Int* 2012; 2012: 452541.
60. Stein MS, Liu Y, Gray OM, Baker JE, Kolbe SC, Ditchfield MR, et al. A randomized trial of high-dose vitamin D2 in relapsing-remitting multiple sclerosis. *Neurology* 2011; 77: 1611-1618.
61. Burton JM, Kimball S, Vieth R, Bar-Or A, Dosch HM, Cheung R, et al. A phase I/II dose-escalation trial of vitamin D3 and calcium in multiple sclerosis. *Neurology* 2010; 74: 1852-1859.
62. Derakhshandi H, Etemadifar M, Feizi A, Abtahi SH, Minagar A, Abtahi MA, et al. Preventive effect of vitamin D3 supplementation on conversion of optic neuritis to clinically definite multiple sclerosis: a double blind, randomized, placebo-controlled pilot clinical trial. *Acta Neurol Belg* 2013; 113: 257-263.
63. Kampman MT, Steffensen LH, Mellgren SI, Jørgensen L. 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. *Mult Scler* 2012; 18: 1144-1151.
64. Mahon BD, Gordon SA, Cruz J, Cosman F, Cantorna MT. Cytokine profile in patients with multiple sclerosis following vitamin D supplementation. *J Neuroimmunol* 2003; 134: 128-132.
65. Smolders J, Peelen E, Thewissen M, Cohen Tervaert JW, Menheere P, Hupperts R, et al. Safety and T cell modulating effects of high dose vitamin D3 supplementation in multiple sclerosis. *PLoS One* 2010; 5: e15235.
66. Knippenberg S, Smolders J, Thewissen M, Peelen E, Tervaert JW, Hupperts R, et al. Effect of vitamin D(3) supplementation on peripheral B cell differentiation and isotype switching in patients with multiple sclerosis. *Mult Scler* 2011; 17: 1418-1423.
67. Ascherio A, Munger KL, Simon KC. Vitamin D and multiple sclerosis. *Lancet Neurol* 2010; 9: 599-612.
68. International Multiple Sclerosis Genetics Consortium; Wellcome Trust Case Control Consortium 2. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* 2011; 476: 214-219.
69. Cree BA. Multiple sclerosis genetics. *Handb Clin Neurol* 2014; 122: 193-209.
70. Hearn AP, Silber E. Osteoporosis in multiple sclerosis. *Mult Scler* 2010; 16: 1031-1043.
71. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab* 2011; 96: 53-58.