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Abstract

Inheriting genetic susceptibility is not sufficient to cause MS; exposure to environmental factors is also required. MS may be preventable if these factors can be identified and avoided. MS prevalence increases with decreasing solar radiation, suggesting that inadequate sunlight may be an MS risk factor. Sunlight catalyzes vitamin D synthesis, and MS prevalence is highest where vitamin D supplies are lowest, suggesting that vitamin D may inhibit MS. Evidence consistent with this hypothesis comes from geographic, climatologic, genetic, and biological studies. The vitamin D receptor gene *b* allele was over-represented in Japanese MS patients, suggesting an association with susceptibility. Fish oil, an excellent vitamin D source, may lower MS prevalence and severity. MS patients show low bone mass, high fracture rates, indicating vitamin D deficiency, and MS disease activity varied seasonally and inversely with vitamin D levels. Experiments with murine experimental autoimmune encephalomyelitis also support the hypothesis that vitamin D may inhibit MS. Administering the vitamin D hormone completely inhibited EAE induction and progression. The hormone stimulated the synthesis of interleukin-4 and transforming growth factor beta-1, and influenced inflammatory cell trafficking and/or apoptosis. If vitamin D is a natural inhibitor of MS, providing supplemental vitamin D would be advisable.

Environmental risk factors and MS

The biological mechanisms leading to MS are uncertain, but both genetic and environmental factors contribute to establishment and progression of the disease.¹ Compared to unrelated individuals, biological first-degree relatives of MS patients show a 20- to 40-fold increased risk of disease, and this increased risk is attributable to genetic factors, rather than a transmissible agent.² However, 70% of monozygotic twin pairs are discordant for MS indicating that inheriting MS susceptibility genes is not sufficient for disease development.³ Thus, MS development requires exposure to one or more environmental risk factors. This observation suggests that MS may be preventable if these risk factors can be identified and avoided.

The geographic distribution of MS prevalence is striking and provides a clue to environmental risk factors. The disease prevalence increases with increasing latitude in both hemispheres from a low of 1-2 cases per 10⁵ population near the equator to a high of >200 cases per 10⁵ population at latitudes >50°.⁴ Of all the latitude-linked variables examined, average annual hours of sunlight and average December daily solar radiation showed the most significant inverse correlation with MS prevalence, suggesting that sunlight might be protective in MS.⁴ A very recent epidemiological study has supported this possibility.⁵ The very large and thorough study found that individuals exposed to the highest levels of residential and occupational sunlight had a significantly lower risk of mortality from MS (odds ratio 0.24), and a significantly higher risk of mortality from melanoma (odds ratio 1.38), which has been causally linked to sunlight.

Separating genetic and environmental influences is difficult in epidemiological studies. However, studies involving genetically similar populations have reinforced the possibility that sunlight may be protective in MS. The study by Freedman and colleagues examined subsets of MS patients and controls, particularly as regards a northern European descent, and showed that

the lower mortality risk from MS among individuals exposed to residential and occupational sunlight was independent of country of origin, age, sex, race, or socioeconomic status.⁵ Earlier studies are consistent with this conclusion. In Switzerland, where the population is relatively genetically homogeneous, low altitude districts (≤ 1000 m) with decreased solar radiation intensity had high MS rates, whereas high altitude districts (≥ 2000 m) with higher solar radiation intensity had low MS rates, despite the relative genetic similarity of the two populations.⁶ Migration studies also support a protective effect of sunlight in MS, independently of genetic risk factors. A very recent study of migrants from the United Kingdom and Ireland to Australia found that the migrants showed a reduced MS prevalence similar to native-born Australians, compared to the higher MS prevalence rate for non-migrants in the United Kingdom and Ireland.⁷ Strikingly, the migrants showed the same strong correlation between MS prevalence and latitude as the native-born Australians, regardless of their age at migration. The conclusions from this recent migration study are consistent with a large number of previous migration studies.⁸ Together, these epidemiological studies suggest that low exposure to sunlight may be a significant environmental risk factor for MS, and further, that sunlight may be beneficial in those with a genetic risk of MS over a period of many years, not just in childhood and early adulthood.

Sunlight, vitamin D, and MS

The vitamin D endocrine system is exquisitely responsive to sunlight, and vitamin D is sometimes called the sunshine vitamin. All vertebrates, including humans, obtain their vitamin D requirement mainly from exposure of their skin to sunlight rather than from their diet.⁹ Sunlight catalyzes previtamin D₃ synthesis in skin.¹⁰ The UV-B photons penetrate the epidermis where their energy is absorbed by the abundant cholesterol metabolite, 7-dehydrocholesterol, rupturing the 9-10 carbon-carbon bond and yielding an unstable intermediate, previtamin D₃. This

compound spontaneously isomerizes to vitamin D₃. Vitamin D₃ is transported from the skin to the liver bound to the serum vitamin D binding protein. In hepatocytes, C-25 hydroxylation produces the major circulating form of vitamin D, 25-hydroxyvitamin D₃ (25-(OH)-D₃). In the kidney, a highly regulated C-1 hydroxylation produces a highly biologically active hormone, 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂-D₃).^{11,12}

Latitude and season affect the intensity of solar radiation reaching earth's surface, and therefore the rate of vitamin D synthesis.¹³ In winter and at high latitudes, sunlight strikes the earth at an oblique angle, and filters through a great stratospheric distance that decreases the UV-B radiation. In Boston, Massachusetts (42°N), there was insufficient sunlight to support vitamin D synthesis from November through February. Further north in Edmonton, Canada (52°N), previtamin D synthesis stopped from October through March. In Los Angeles, California (34°N), previtamin D synthesis occurred year-round. Consequently, people living at northerly or southerly latitudes, who do not eat vitamin D-rich foods or supplements, are at a significant risk of becoming vitamin D deficient during the winter.

MS prevalence is high in geographic areas with low supplies of vitamin D due to low intensity solar radiation and inadequate dietary vitamin D. Conversely, MS prevalence is low where vitamin D is abundant, as in sunny climates, high altitudes, and areas with diets rich in fish oil. This correlation led Goldberg and us to hypothesize that the sunshine vitamin, vitamin D, may be a natural inhibitor of MS.¹⁴⁻¹⁶

Genetic and biological evidence correlating vitamin D and MS inhibition

Evidence consistent with the hypothesis that vitamin D may be a natural inhibitor of MS derives from genetic studies, human biological investigations, and animal experiments. A well documented mechanism of vitamin D hormone action is the binding of 1,25-(OH)₂-D₃ to the

vitamin D hormone receptor (VDR), and the subsequent transcriptional control of target genes through vitamin D response elements.¹⁷ Thus, mutations in the VDR gene, or in the genes coding for enzymes involved in vitamin D metabolism, might be associated with MS susceptibility. We looked for associations between MS and genetic markers at the VDR locus on chromosome 12q14, the nearby gene encoding the 25-hydroxyvitamin D₃ 1- α -hydroxylase (12q13), and the vitamin D binding protein locus (4q12) in Canadian families, but found none.¹⁸ However, in Japanese families, Fukazawa and colleagues found that the *b* allele of the VDR gene was over-represented in MS patients, and may be associated with MS susceptibility.¹⁹

Fish oil is a rich source of vitamin D, and there is limited evidence that diets rich in fish may lower the incidence and/or severity of MS. Lower MS prevalence rates were found along the Atlantic coast of Norway than inland.²⁰ The coastal Norwegians consumed about 1300 IU of vitamin D₃ daily, about 3-fold higher than individuals living inland.¹⁴ Furthermore, in a small clinical trial, MS patients ingesting cod liver oil (20 g/day; 5000 IU/day of vitamin D), along with calcium and magnesium supplements, reportedly lowered their rate of exacerbations.²¹ This trial involved very few subjects and had other methodological shortcomings, but the results are intriguing in the context of all the evidence that is consistent with the vitamin D - MS hypothesis.

There is very good data indicating that MS patients exhibit long-term vitamin D deficiency, as characterized by low bone mass and high fracture rates. The best indicator of near-term vitamin D nutrition is the serum 25-(OH)-D₃ level, which was less than adequate (<50 nmol/L) in 69% of MS patients.²² Compared to their age- and gender-matched healthy peers, the MS patients had significantly reduced bone mass, which is indicative of long-term vitamin D malnutrition.²² Finally, MS patients lost bone mass at a 3- to 7-fold higher rate and experienced fractures at a 10-fold higher rate than their peers.²³ These findings indicate that significant

vitamin D deficiency of some duration exists in most MS patients, consistent with the theory that this deficiency may be an MS risk factor.

Critically important evidence that vitamin D deficiency may exacerbate MS, whereas vitamin D sufficiency may inhibit MS comes from a study on the seasonal fluctuations of gadolinium-enhancing, magnetic resonance imaging (MRI) lesions in MS patients.²⁴ In the south German study population, disease activity peaked in spring and was lowest in autumn, closely resembling the frequency of disease onset and exacerbations in Swiss and Arizona MS patients.^{25,26} Significantly, in a south German control population, serum 25-(OH)-D₃ levels reached a nadir shortly before MS disease activity peaked, and rose before MS disease activity subsided.²⁷ Thus, MS disease onset, physical exacerbations, and MRI activity all correlated strongly and inversely with presumed serum 25-(OH)-D₃ levels. It will be important to measure serum 25-(OH)-D₃ levels in the MS patients themselves to substantiate the evidence correlating MS disease activity with seasonal variations in vitamin D synthesis.

Additional support for a possible protective role of vitamin D in MS comes from animal studies. We and others showed that the vitamin D hormone, 1,25-(OH)₂-D₃, is a strong inhibitor of experimental autoimmune encephalomyelitis (EAE), a model of MS. Immunizing mice with spinal cord homogenate containing myelin basic protein induces a progressively paralytic autoimmune disease similar to MS.²⁸ Lemire and Archer and Branisteanu et al. fed SJL/J mice a low calcium diet and injected 1,25-(OH)₂-D₃, thereby prolonging survival of the mice with severe EAE, but the morbidity and mortality of the disease were not completely inhibited.^{29,30} We studied relapsing-remitting EAE in B10.PL mice and demonstrated that 1,25-(OH)₂-D₃ pre-treatment completely blocked EAE induction, while hormone treatment at the first signs of EAE inhibited disease progression.³¹ The lower the dietary calcium level, the higher was the 1,25-

(OH)₂-D₃ dose needed to completely prevent EAE symptoms, suggesting that adequate dietary calcium is important for EAE inhibition.³²

The mechanism whereby 1,25-(OH)₂-D₃ inhibits EAE is not known, but may involve enhancing the synthesis of the anti-inflammatory cytokines interleukin-4 (IL-4) and transforming growth factor beta-1 (TGF-β1).³³ Additional experiments examined hormone treatment in mice with severe EAE.³⁴ EAE was induced, 1,25-(OH)₂-D₃ or mock treatment was administered, and clinical disease, histopathological disease, and encephalitogenic cells in the CNS were analyzed within 24-72 hr of the treatment. The mock-treated mice remained paralyzed (stage 3 EAE) while most hormone-treated animals regained the partial use of both hind limbs (stage 2 EAE) within 72 hr of treatment. A histopathological examination showed the hormone-treated mice had a 50% decrease in white matter and meningeal inflammation at 72 hr post treatment. A flow cytometric analysis of cell surface markers on spinal cord cells recovered 24 hr post treatment showed the mock-treated mice with EAE had millions of inflammatory Mac-1⁺ cells/cord, whereas the hormone-treated mice were not significantly different from the unmanipulated control mice in this regard. These results suggest an influence of the hormone on inflammatory cell trafficking or apoptosis.³⁴ Further experiments suggest a role for the hormone in promoting peripheral T cell tolerance (Nashold, FE, KA Hoag, J Gorman, and CE Hayes, unpublished).

Vitamin D inhibition of Type I diabetes mellitus and other autoimmune diseases

It is important to note that vitamin D and the hormone 1,25-(OH)₂-D₃ have been implicated in the inhibition of other human autoimmune diseases and many mouse autoimmune disease models. Type I diabetes, like MS, shows a strong latitude gradient, its prevalence increasing with increasing latitude, and an inverse correlation between incidence and mean monthly sunshine hours.³⁵⁻³⁹ Importantly, a recent study correlated vitamin D supplementation in early

childhood with significantly reduced risk for Type I (insulin-dependent) diabetes mellitus.⁴⁰ Also, like MS in Japanese families, the VDR gene *b* allele was implicated in susceptibility to insulin-dependent diabetes in Indian Asians.⁴¹ Moreover, 1,25-(OH)₂-D₃ prevented insulinitis and diabetes in the non-obese diabetic mouse.⁴² Finally, the vitamin D hormone 1,25-(OH)₂-D₃ inhibited experimental autoimmune thyroiditis, experimental murine lupus, and both collagen-induced arthritis and Lyme arthritis.⁴³⁻⁴⁵ Underlying these diverse observations may be a fundamental biological requirement for vitamin D to sustain immunological health, in particular to maintain self tolerance.

Vitamin D nutrition and immunological health

To summarize, the evidence that vitamin D may inhibit MS is diverse and circumstantial, but compelling. It derives from geographic, climatologic, genetic, nutritional, and biological, investigations. To our knowledge, there is no evidence that is inconsistent with this hypothesis. The level of vitamin D nutrition that may inhibit MS development is not known. The serum 25-(OH)-D₃ level is the most reliable indicator of near-term vitamin D nutrition. Individuals living and working in sunny environments, where MS prevalence is lowest, have circulating 25-(OH)-D₃ levels between 105 and 163 nmol/L.⁴⁶ The parathyroid hormone level increases with decreasing vitamin D nutrition, and parathyroid hormone levels do not plateau until serum 25-(OH)-D₃ levels rise to about 70 nmol/L.^{47,48} In contrast, the currently accepted threshold for adequate vitamin D nutrition with respect to bone health is 50 nmol/L.⁴⁸ It is likely that the serum 25-(OH)-D₃ level required to maintain bone health will prove to be lower than the serum 25-(OH)-D₃ level required for immunological health. Studies explicitly measuring serum 25-(OH)-D₃ levels and correlating these with MS prevalence and MS disease activity are needed to provide guidance on optimal 25-(OH)-D₃ levels from the perspective of MS.

If vitamin D is a natural inhibitor of MS, it would be reasonable to provide supplemental vitamin D to individuals who are at risk for MS. National Academy of Sciences of the United States, and The Food and Nutrition Board of the Institute of Medicine in the United States determined that an adequate vitamin D intake for individuals age 0 to 50 yrs is 200 IU/day (5 µg/day) to maintain bone health.⁴⁹ This intake is sufficient to prevent rickets, a metabolic bone disease, but it does not prevent secondary hyperparathyroidism and osteoporosis in adults.^{48,49} Furthermore, an oral intake of 600 IU/day was not enough to prevent vitamin D deficiency in veiled ethnic Danish Moslems who were sunlight deprived; an intake of 1000 IU/day would probably be required to achieve normal 25-(OH)-D₃ levels in this population.⁵⁰ To maintain a serum 25-(OH)-D₃ level ~100 nmol/L, similar to the level in individuals who live and work in the sun and exhibit the lowest risk of MS, an adult who is not exposed to sunlight would need to ingest 4000 IU/day.⁴⁶ This estimate is between the 3800 IU/day that Goldberg calculated might prevent MS, and the 5000 IU/day that was given in the small clinical trial of fish oil, and 200-fold higher than the suggested adequate intake of 200 IU/day.^{14,21} Further studies are needed at or shortly after the nadir of solar radiation wherein vitamin D intakes are varied and serum 25-(OH)-D₃ levels are measured in populations who are seasonally deprived of sunlight because they live at high latitudes. Such studies will provide guidance on optimal vitamin D intakes to maintain a serum 25-(OH)-D₃ level of in the range of 70-100 nmol/L, which may be protective in MS.

Very high doses of vitamin D can cause hypercalcemia, which is potentially fatal. Accordingly, a tolerable safe upper limit for vitamin D supplementation has been set at 2000 IU/day for age ≥1.⁴⁹ However, the panel that established this limit overlooked information indicating that the safe upper limit is actually much higher. Adults living or working in sunny

environments easily generate >10,000 IU/day of vitamin D through sun exposure without adverse effects, so the safe upper limit for total vitamin D nutrition is at least 10,000 IU/day.⁴⁶ Moreover, all documented cases of vitamin D toxicity with hypercalcemia involved intakes $\geq 40,000$ IU/day.⁴⁶ Thus, a reappraisal of vitamin D supplementation in light of immunological health, seasonal deprivation of sunlight, and safety is clearly needed.

Before new information is forthcoming, it would be important to monitor 25-(OH)-D₃ levels and bone health in MS patients, since many of these patients show deficient serum 25-(OH)-D₃ levels, reduced bone mass, high bone mass loss rates, and high fracture rates compared to controls.^{22,23} The analysis should be done a month or two after the nadir of solar radiation, and should include measures of serum 25-(OH)-D₃, bone mass, bone turnover, and MS disease activity. Supplementation with calcium and vitamin D to correct poor bone health would be advisable.

In closing, it is interesting to note that MS and rickets show very similar geographies.⁵¹ The geography of rickets led Sniadecki to suggest in 1822 that sunlight might cure rickets.⁹ Regrettably, rickets continued to cripple children for a full century before the benefits of sunlight and/or cod liver oil were proven, and cod liver oil became a winter staple for children living in northerly latitudes.^{52,53} The evidence linking sunlight, vitamin D, and MS is compelling. As a scientific community, let us move rapidly to test the hypothesis that vitamin D may be a natural inhibitor of MS, and possibly other autoimmune diseases.

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