

Vitamin D: a natural inhibitor of multiple sclerosis

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Inheriting genetic risk factors for multiple sclerosis (MS) is not sufficient to cause this demyelinating disease of the central nervous system; exposure to environmental risk factors is also required. MS may be preventable if these unidentified environmental factors can be avoided. MS prevalence increases with decreasing solar radiation, suggesting that sunlight may be protective in MS. Since the vitamin D endocrine system is exquisitely responsive to sunlight, and MS prevalence is highest where environmental supplies of vitamin D are lowest, we have proposed that the hormone, 1,25-dihydroxycholecalciferol (1,25-(OH)₂D₃), may protect genetically-susceptible individuals from developing MS. Evidence consistent with this hypothesis comes not only from geographic studies, but also genetic and biological studies. Over-representation of the vitamin D receptor gene *b* allele was found in Japanese MS patients, suggesting it may confer MS susceptibility. Fish oil is an excellent vitamin D source, and diets rich in fish may lower MS prevalence or severity. Vitamin D deficiency afflicts most MS patients, as demonstrated by their low bone mass and high fracture rates. However, the clearest evidence that vitamin D may be a natural inhibitor of MS comes from experiments with experimental autoimmune encephalomyelitis (EAE), a model of MS. Treatment of mice with 1,25-(OH)₂D₃ completely inhibited EAE induction and progression. The hormone stimulated the synthesis of two anti-encephalitogenic cytokines, interleukin 4 and transforming growth factor β-1, and influenced inflammatory cell trafficking or apoptosis. If vitamin D is a natural inhibitor of MS, providing supplemental vitamin D to individuals who are at risk for MS would be advisable.

Vitamin D: 1,25-Dihydroxycholecalciferol: Multiple sclerosis: Experimental autoimmune encephalomyelitis: Autoimmunity

Multiple sclerosis (MS) is a central nervous system demyelinating disease of uncertain aetiology (Ebers, 1998). Genetic epidemiological studies demonstrate that genetic risk factors determine susceptibility and account for familial clustering of MS (for review, see Ebers & Dyment, 1998). Biological first-degree relatives of MS patients show a 20- to 40-fold increased risk of disease compared with unrelated individuals. However, no single locus controlling MS development has been identified, suggesting that genetic MS susceptibility may be polygenic, as originally postulated by Pratt *et al.* (1951).

Importantly, inheriting MS susceptibility factors is not sufficient for disease development, as evidenced by the finding that 70 % of monozygotic twin pairs are discordant for MS (Ebers *et al.* 1986). Thus, exposure to one or more environmental risk factors is necessary for MS development. Since infections often precede the onset of MS

symptoms and/or exacerbations (Sibley *et al.* 1985; Anderson *et al.* 1993; Panitch, 1994), an infectious MS aetiology has been proposed (for review, see Noseworthy, 1999). Despite intense investigation, compelling evidence for this hypothesis has not been forthcoming, and other disease-determining environmental risk factors must be sought.

MS prevalence shows a striking geographic distribution. It increases with increasing latitude in both hemispheres (for example, see Davenport, 1922; Ulett, 1948; Limburg, 1950; Kurland, 1952), from a low of one to two cases per 10⁵ population near the equator to a high of >200 cases per 10⁵ population at latitudes >50°. This peculiar distribution suggests that one disease-determining environmental risk factor is somehow linked to latitude. Acheson *et al.* (1960) examined many latitude-linked variables, and showed that average annual hours of sunlight and average

Abbreviations: EAE, experimental autoimmune encephalomyelitis; 1,25-(OH)₂D₃, 1,25-dihydroxycholecalciferol; MS, multiple sclerosis; VDR, vitamin D receptor.

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December daily solar radiation showed a highly significant inverse correlation ($r=0.80$) with MS prevalence. They concluded that sunshine 'could conceivably act directly – a certain skin dose of sunshine per unit time protecting the individual in some way'.

Epidemiological studies are confounded by the problem of separating genetic and environmental influences. However, some evidence from studies involving genetically similar populations reinforces the possibility that sunlight may be protective in MS. In Switzerland, districts at low altitudes (≤ 1000 m) have high MS rates, whereas districts at high altitudes (≥ 2000 m) have low MS rates, despite the relative genetic similarity of the two populations (Kurtzke, 1967). This anomaly may reflect the increased short-wavelength u.v. radiation received at high altitudes compared with low altitudes (Geiger, 1965). Further, within genetically similar migrating populations, those migrating to regions with increased solar radiation had reduced MS rates, whereas those migrating to regions with decreased solar radiation had increased MS rates (for review, see Ebers & Sadovnick, 1994). The benefits of sunlight accrued to migrating individuals of all ages (Hammond *et al.* 2000). Together these epidemiological studies indicate that MS may be a preventable disease in individuals who are genetically at risk, if the pertinent environmental risk factors can be correctly identified and avoided. Sunlight appears to be the protective environmental factor linked to latitude.

Sunlight, the vitamin D endocrine system and multiple sclerosis

The vitamin D endocrine system is exquisitely responsive to sunlight. Sunlight is required for precholecalciferol synthesis in skin (Velluz & Amiard, 1949), and precholecalciferol is the precursor of the biologically active hormone, 1,25-dihydroxycholecalciferol ($1,25\text{-(OH)}_2\text{D}_3$; Holick *et al.* 1971; Norman *et al.* 1971). Goldberg (1974a, b) first proposed that sunlight might protect individuals from developing MS through the actions of $1,25\text{-(OH)}_2\text{D}_3$, since geographic areas with low supplies of vitamin D due to low-intensity solar radiation and inadequate dietary vitamin D correlated with regions of high MS prevalence. Conversely, MS prevalence is low where vitamin D is abundant, as in sunny climates, high altitudes and areas with diets rich in fish oil. We reinforced this hypothesis (Hayes *et al.* 1997), and provided strong experimental evidence from the experimental autoimmune encephalomyelitis (EAE) model in mice, summarized later (p. 533), showing that $1,25\text{-(OH)}_2\text{D}_3$ is a natural inhibitor of the autoimmune-mediated processes that underlie MS (Cantorna *et al.* 1996, 1998; Nashold *et al.* 2000).

All vertebrates, including human subjects, obtain their vitamin D requirement mainly from exposure of their skin to sunlight rather than from their diet (Holick, 1995). The energy of u.v. B photons that penetrate the epidermis is absorbed by the abundant cholesterol metabolite 7-dehydrocholesterol, rupturing the C-9–C-10 bond and yielding an unstable intermediate, precholecalciferol. This compound spontaneously undergoes an internal sigmatropic shift of a proton and isomerization to the thermodynamically-stable

cholecalciferol. Cholecalciferol 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-hydroxycholecalciferol, which is biologically inactive at physiological concentrations. In the kidney, a further C-1 hydroxylation forms the biologically active hormone, $1,25\text{-(OH)}_2\text{D}_3$ (Holick *et al.* 1971; Norman *et al.* 1971). The production of the hormone is highly regulated by the need for Ca and P.

Latitude and season affect the intensity of solar radiation reaching the earth's surface and, therefore, the rate of cholecalciferol synthesis (Webb *et al.* 1988). In Boston, MA (42°N), human skin exposed to sunlight produced no precholecalciferol from November to February due to insufficient intensity of solar radiation. Further north in Edmonton, Canada (52°N), this period of no precholecalciferol synthesis extended from October to March. In Los Angeles, CA (34°N), cholecalciferol synthesis occurred throughout the year. Individuals living at northerly or southerly latitudes who do not eat vitamin D-rich foods or supplements become vitamin D deficient during the winter.

Genetic evidence for the vitamin D – multiple sclerosis hypothesis

Evidence consistent with the hypothesis that vitamin D may be a natural inhibitor of MS comes from geographic (discussed earlier), genetic and biological studies. The hormone exerts most of its biological effects only after it has bound to the vitamin D hormone receptor (VDR; Haussler *et al.* 1997). New genetic evidence indicates that one allele of the VDR gene may be associated with MS susceptibility. Fukazawa *et al.* (1999) found over representation of the VDR gene *b* allele (chromosome 12q14) in Japanese MS patients. This allele is distinguished by a *BsmI* endonuclease site, and it was also implicated in susceptibility to insulin-dependent diabetes mellitus in Indian Asians (McDermott *et al.* 1997). In Canadian families Steckley *et al.* (2000) found no association between MS and genetic markers at the VDR locus, the nearby gene encoding the 25-hydroxycholecalciferol 1- α -hydroxylase (12q13), or the vitamin D-binding protein locus (4q12). However, the report by Fukazawa *et al.* (1999) indicates that a VDR polymorphism may contribute to MS susceptibility, consistent with the vitamin D – MS hypothesis.

Biological evidence for the vitamin D – multiple sclerosis hypothesis

Fish oil is a rich source of cholecalciferol, and there is limited evidence that diets rich in fish may lower the incidence and/or severity of MS. Lower MS prevalence rates are found along the Atlantic coast of Norway than inland (Swank *et al.* 1952; Presthus, 1960; Westlund, 1970). The coastal Norwegians consumed the equivalent of approximately $32.5\ \mu\text{g}$ cholecalciferol daily, about 3-fold higher than individuals living inland (Goldberg, 1974a). Furthermore, in a small clinical trial MS patients ingested cod liver oil (20 g/d; $125\ \mu\text{g}$ vitamin D/d), along with Ca and Mg supplements, which reportedly lowered their rate of exacerbations (Goldberg *et al.* 1986). A second small trial of

fish oil also lessened MS symptoms (Bates *et al.* 1989). These trials involved very few subjects and had other methodological shortcomings. Nevertheless, the available evidence on fish oil consumption and MS is consistent with the vitamin D – MS hypothesis.

There is clear evidence that the majority of MS patients exhibit long-term vitamin D deficiency, as characterized by low bone mass and high fracture rates. The serum 25-hydroxycholecalciferol level, the best indicator of near-term vitamin D nutrition, was less than adequate (<50 nmol/l) in 69 % of MS patients tested (Nieves *et al.* 1994). In addition, MS patients had significantly reduced bone mass compared with their age- and gender-matched healthy peers (Nieves *et al.* 1994), indicative of long-term vitamin D malnutrition. A follow-up study found that 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 (Cosman *et al.* 1998). These findings indicate that significant vitamin D deficiency of some duration exists in most MS patients, consistent with the hypothesis that this deficiency may pose a risk factor for MS.

Experimental evidence for the vitamin D – multiple sclerosis hypothesis

The clearest evidence that vitamin D may be a natural inhibitor of MS comes from experiments done using the EAE system as a model of MS. Immunizing mice with spinal cord homogenate containing myelin basic protein induces a progressively paralytic autoimmune disease, EAE, with strong similarities to MS (Olitsky & Yager, 1949). Lemire & Archer (1991) and Branisteanu *et al.* (1995) used the EAE model to show that feeding a low-Ca diet and injecting 1,25-(OH)₂D₃ prolonged the survival of SJL/J mice with severe EAE, but these treatments did not completely inhibit the morbidity or mortality of the disease. We studied relapsing–remitting EAE in B10.PL mice and demonstrated that 1,25-(OH)₂D₃ pretreatment completely eliminated EAE, while hormone treatment at the first sign of symptoms inhibited EAE progression (Cantorna *et al.* 1996). The lower the dietary Ca level, the higher was the 1,25-(OH)₂D₃ dose needed to completely prevent EAE symptoms, suggesting that adequate dietary Ca is important for EAE inhibition (Cantorna *et al.* 1999).

In further experiments we tested the hypothesis that 1,25-(OH)₂D₃ might stimulate the development of anti-encephalitogenic cells and the cytokines they produce (Cantorna *et al.* 1998). Consistent with this hypothesis, we detected interleukin 4 and transforming growth factor β-1 transcripts in the lymph nodes and central nervous system of hormone-treated mice, where EAE was prevented, but not in those of mock-treated mice with EAE. The interleukin 4 and transforming growth factor β-1 cytokines have strong anti-inflammatory activity in EAE (Johns *et al.* 1991; Kuruvilla *et al.* 1991; Racke *et al.* 1991; Inobe *et al.* 1996). In other experiments we induced severe EAE, administered hormone treatment or mock treatment, and examined the central nervous system for infiltrating inflammatory cells (Nashold *et al.* 2000). Whereas the mock-treated mice remained severely paralysed, most hormone-treated animals regained the partial use of both hind limbs within 72 h of hormone

treatment. Histopathological examination and flow cytometric analyses indicated that about 5 × 10⁶ inflammatory macrophages were lost from the inflamed central nervous system within 24 h of the 1,25-(OH)₂D₃ treatment, suggesting a possible influence of the hormone on inflammatory cell trafficking or apoptosis.

Vitamin D nutrition and multiple sclerosis

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. It is noteworthy that vitamin D supplementation during childhood significantly decreased the risk of type I diabetes mellitus, an autoimmune disease (EURODIAB Substudy 2 Study Group, 1999). A reassessment of vitamin D nutrition is underway, and there is good evidence that the currently recommended adequate intakes are too low (Vieth, 1999). The adequate intake for adults is currently 5 µg/d, but this does not prevent osteoporosis and secondary hyperparathyroidism (Holick, 1998; Malabanan *et al.* 1998). To prevent secondary hyperparathyroidism a serum 25-hydroxycholecalciferol concentration >50 nmol/l is required (Malabanan *et al.* 1998). Adults living or working in sunny environments, where MS prevalence is lowest, have circulating 25-hydroxycholecalciferol levels between 105 and 163 nmol/l (Vieth, 1999). Thus, a serum 25-hydroxycholecalciferol concentration ≥ 100 nmol/l may be optimal to prevent MS. To maintain serum 25-hydroxycholecalciferol at approximately 100 nmol/l an adult who is not exposed to sunlight would need to ingest 100 µg/d (Vieth, 1999). This estimate is between the 95 µg/d that Goldberg (1974b) calculated might prevent MS, and the 125 µg/d that was given in the small clinical trial of fish oil (Goldberg *et al.* 1986).

Very high doses of vitamin D can cause hypercalcaemia, which is potentially fatal. Accordingly, a tolerable safe upper limit for vitamin D supplementation has been set at 50 µg/d for age > 1 years, and 25 µg/d for age < 1 years (Holick, 1998). 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 >250 µg vitamin D/d through sun exposure without adverse effects, so the safe upper limit for total vitamin D nutrition is at least 250 µg/d (Vieth, 1999). Moreover, all documented cases of vitamin D toxicity with hypercalcaemia involved intakes ≥ 1000 µg/d (Vieth, 1999). Thus, the 100 µg/d needed by an adult without sun exposure to maintain serum 25-hydroxycholecalciferol at approximately 100 nmol/l would be safe, since it is well below the 250 µg/d generated by adults living or working in sunny environments.

The 100 µg vitamin D/d supplementation that might be needed to prevent MS is significantly higher than the intake required to prevent rickets, a metabolic bone disease attributable to vitamin D deficiency. The intake recommended to prevent rickets in children (0–18 years) living in far northern and southern latitudes is 5 µg/d (Holick, 1998). It is interesting to note that the geographies of rickets (Hess, 1929) and MS are very similar; the geography of rickets led Sniadecki (cited by Holick, 1995) to suggest in 1822 that sunlight might cure rickets. Regrettably, rickets continued to

cripple children for a full century before investigators proved the benefits of sunlight or cod liver oil (Hess & Unger, 1921; Chick *et al.* 1922), and cod liver oil became a winter staple for children living in northerly latitudes. The evidence that vitamin D might be a natural inhibitor of MS is compelling. Examining the benefit of vitamin D supplementation for MS prevention will require a major effort on the part of the scientific community, but it is clearly justified.

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