Vitamin D and multiple sclerosis

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The hypothesis that adequate vitamin D nutrition can contribute to the prevention of multiple sclerosis (MS) was originally proposed to explain the geographical distribution of MS, but only recently has the relation between various measures of vitamin D (eg, sun exposure, dietary sources, and serum concentrations of 25-hydroxyvitamin D) and risk of developing MS been rigorously investigated. Overall, the results of these studies support a protective effect of vitamin D, but there are uncertainties and many unanswered questions, including how vitamin D exerts a protective effect, how genetic variations modify the effect, and whether vitamin D can influence the course of MS progression.

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Introduction

The risk of developing multiple sclerosis (MS), a relatively common cause of disability among young adults, is determined by a combination of genetic and environmental factors. The latter include Epstein-Barr virus (EBV) infection, cigarette smoking, and inadequate serum concentrations of vitamin D.[1,2] Although EBV is nearly ubiquitous, there are no effective vaccines or treatments for EBV infection, leaving smoking cessation and vitamin D supplementation as the only available interventions that might result in a reduction in the global burden of MS. Because vitamin D deficiency is endemic worldwide,[3] the potential impact of vitamin D supplementation on MS incidence is profound.

The hypothesis that vitamin D deficiency is a risk factor for MS was first proposed over 30 years ago,[4] and gained credibility after the discovery of the immunomodulatory effects of vitamin D.[5] However, over the past few years, the epidemiological evidence of an increased MS risk among individuals with low vitamin D concentrations has achieved substantial strength, thus approaching a threshold that calls for important decision-making in terms of experimental investigations or public-health interventions. In this Review, we will provide a critical analysis of the epidemiological studies on vitamin D and MS risk or severity, and their implications for MS prevention and treatment.

Background on vitamin D

Source and metabolism

The primary form of vitamin D, colecalciferol (vitamin D3), is available from two sources: skin exposure to ultraviolet B radiation (UVB) in sunlight and diet (figure 1). UVB in the 290–315 nm range photolyses 7-dehydrocholesterol in the skin to form pre-vitamin D3, which then isomerises to colecalciferol.[6] Colecalciferol (and ergocalciferol [vitamin D2]) is also available from fortified foods (eg, milk, cereal, and some orange juice and cheeses), dark fish (eg, salmon and tuna), and vitamin supplements (colecalciferol). Relative to sun exposure, diet is a poor source of colecalciferol, providing only 40–400 IU per food serving,[12] whereas whole-body UVB exposure for 20 min for a light-skinned person during the summer months will produce at least 10 000 IU.[7,13] However, increased skin pigmentation, age, use of sunscreen, built environment, and environmental factors that reduce the strength of UVB reaching the Earth's surface (eg, winter season, high latitude, pollution, cloud cover, and ozone levels) all contribute to reduce skin colecalciferol production to the point at which diet might become the primary source.[7–14]

Both forms of vitamin D, colecalciferol and ergocalciferol, are biologically inactive and are enzymatically converted in the liver to 25-hydroxyvitamin D. This molecule then undergoes a second hydroxylation in the kidney or other tissues to give the active form, 1,25-dihydroxyvitamin D (also known as calcitriol if derived from vitamin D3; figure 1), which binds and activates the vitamin D receptor (VDR), a transcription factor that regulates the expression of as many as 500 genes.[15,16] The VDR is also present in cell membranes, where it mediates some of the rapid responses to 1,25-dihydroxyvitamin D (ie, responses that occur too fast to depend on gene transcription).[15] 25-hydroxyvitamin D is used to assess an individual's overall vitamin D nutritional status, because its formation, unlike that of 1,25-dihydroxyvitamin D, is not tightly regulated and it has a relatively long half-life (20–60 days).[17,18] Thus, 25-hydroxyvitamin D is an integrated measure of vitamin D derived from both UVB exposure and diet. Most laboratory assays do not discriminate between the forms of 25-hydroxyvitamin D derived from colecalciferol and ergocalciferol, but the latter is usually a minor component, because natural sources of ergocalciferol are scarce and ergocalciferol is more rapidly catabolised than colecalciferol.[19]

Although 25-hydroxyvitamin D concentrations above 50 nmol/L have been deemed adequate, evidence suggests that a minimum of 75 nmol/L 25-hydroxyvitamin D, and perhaps more than 90 nmol/L, is optimum for many health outcomes.[20,21] Mean concentrations in most populations are substantially lower than this (figure 2).[12,22] Judicious sun exposure or daily supplemental intake of 1000–4000 IU colecalciferol would increase 25-hydroxyvitamin D to over 75 nmol/L in most individuals.[7,20]

Non-calcaemic effects

Although the best-known function of vitamin D is to regulate calcium physiology, it also has important effects on brain development and function, cell proliferation and apoptosis, regulation of blood pressure and insulin

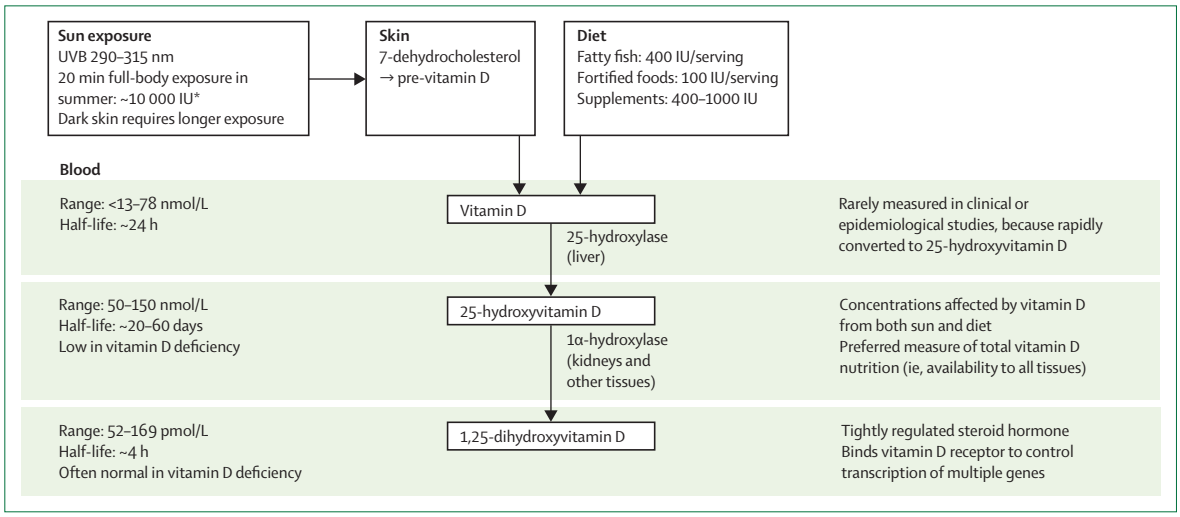

Figure 1: **Sources and metabolism of vitamin D**
Based on data from several sources.[6–11] *Dependent on geographical location. To convert nmol/L to ng/mL, multiply by 0·4. 1 IU=0·025 μg. UVB=ultraviolet B radiation.

secretion, and on the differentiation of immune cells and modulation of immune responses. These effects have been extensively reviewed,[15,27–29] and only selected observations on experimental autoimmune encephalomyelitis (EAE) or in patients with MS will be mentioned here.

Calcitriol is remarkably effective in the prevention and treatment of several experimental autoimmune conditions, including EAE.[30–36] The nuclear VDR[37] and functional interleukin 10 and the interleukin 10 receptor[33] seem to be important, because calcitriol did not prevent EAE in mice in which their functions were genetically disrupted. Further experiments, including the demonstration that a functional recombination activating gene (*RAG1*) was also required for calcitriol to be protective, and that calcitriol did not inhibit T-helper-1 or enhance T-helper-2 cell function in vivo, led to the hypothesis that calcitriol promotes regulatory T-cell function (a Rag-1-dependent cell) rather than directly affecting T-helper-1 or T-helper-2 cells,[38,39] although a role for the latter cannot be excluded. These findings suggest that calcitriol could be used as a treatment for MS, provided that hypercalcaemia (which is common in mice under the regimens effective in EAE) could be prevented.[40]

Unlike calcitriol, which prevents EAE in both male and female mice, colecalciferol was found to be effective in female mice (a 60% reduction in EAE), but completely ineffective in male mice, even at high doses.[33,41,42] In female mice, the effect was dependent on 17-β-oestradiol.[42] UVB also seems to reduce EAE incidence,[43,44] but because UVB also directly affects immune responses, relative attribution of its effects to vitamin D remains speculative.[45] By contrast with calcitriol,[32] neither UVB nor 25-hydroxyvitamin D administered after the onset of EAE affected disease course.[34] Overall, the results of these experiments provide some plausibility to the hypothesis that vitamin D could be beneficial in MS prevention and treatment. As discussed below, however, the sex-specific effect observed in EAE is not consistent with some key epidemiological observations.

The effects of vitamin D supplementation on immune responses in human beings have not been systematically investigated. Only two double-blind placebo-controlled trials have been published.[46,47] In one trial of individuals with MS, 1000 IU colecalciferol daily for 6 months was found to increase serum concentrations of transforming growth factor β1, but no significant changes were reported in other measured cytokines (tumour necrosis factor, interferon γ, and interleukin 13).[46] In the other trial, 437 overweight individuals (not known to have MS) were randomly assigned to receive 40 000 IU or 20 000 IU colecalciferol per week or placebo.[47] After 1 year, 25-hydroxyvitamin D concentrations were markedly increased in both intervention groups, and parathyroid hormone concentrations were significantly reduced, but no significant changes were found in concentrations of several circulating cytokines, including interleukin 2 and interferon γ (also combined in a T-helper-1 score); interleukins 4, 5, 10, and 13 (also combined in a T-helper-2 score); and interleukins 12 and 17, intercellular adhesion molecule 1, monocyte chemotactic protein 1, and high sensitivity C-reactive protein.[47]

Other published studies were cross-sectional or were done in vitro. Significant correlations between 25-hydroxyvitamin D or 1,25-dihydroxyvitamin D in blood and immunological markers such as the frequency or suppressive activity of regulatory T cells from patients with MS have been reported,[48,49] and are consistent with an effect of vitamin D on T-cell regulation. However, these correlations might partly indicate an effect of disease activity on vitamin D concentrations and immunological measures, and are thus insufficient to

claim an immunoregulatory effect of vitamin D in patients with MS. Data based on exposing T cells to 1,25-dihydroxyvitamin D in vitro are also inconclusive.[50] Whereas the effects observed in these experiments are consistent with a beneficial effect of calcitriol (enhancement of interleukin 10-producing cells, reduction in cells secreting the proinflammatory cytokines interleukins 6 and 17, and increase in number of regulatory T cells), the extent to which such effects occur in vivo is uncertain. Therefore, the immunological effects of vitamin D supplementation need to be determined experimentally—for example, whether it can correct the decreased regulatory T-cell activity observed in MS.[51]

Epidemiology

Factors affecting exposure

Because melanin pigment in human skin absorbs UVB,[52] black people have lower 25-hydroxyvitamin D concentrations than white people, and are often vitamin D deficient.[22] However, the risk of MS in black people is lower than in white people;[53] this contradiction to the vitamin D hypothesis might only occur because genetic differences could compensate for low vitamin D concentrations, as seems to be the case for bone turnover.[54] Analyses of the relation between vitamin D status and MS risk are therefore better done separately within each race/ethnic group. Among whites, a fair skin colour is not a good proxy for vitamin D status, because higher use of sunscreen and sun avoidance might result in lower rather than higher 25-hydroxyvitamin D concentrations.[55,56]

The importance of age of exposure and seasonality is uncertain. Studies in migrants implicate postnatal environmental exposures, but do not exclude prenatal effects.[57,58] We will discuss this issue in the context of specific investigations. Blood concentrations of 25-hydroxyvitamin D fluctuate with season, but the aetiological relevance of these fluctuations to MS risk is unknown.

Reliability of measures

Measures of blood concentrations of 25-hydroxyvitamin D also vary according to the type of assay used and between laboratories using the same assay.[59] Comparison of 25-hydroxyvitamin D concentrations across different assays or laboratories should thus be made cautiously. Prospective, repeated measures of 25-hydroxyvitamin D in a large population of healthy individuals would be the best method to determine the association between vitamin D and MS risk. Because this is rarely achievable, most investigations have relied on other markers of vitamin D status, including history of exposure to sunlight, skin actinic damage, dietary intake, or genes.

Study design, confounding, and bias

Because of the cost and length of prospective cohort studies, most investigations in MS are based on a traditional (non-nested) case-control study design, in which the distribution of exposure (vitamin D) among cases (MS) is compared with that of a group of controls (individuals who do not have MS). Unfortunately, these studies are prone to many sources of bias, including selection bias (ie, bias resulting from selection of a control group with a distribution of exposure that differs from that of the population that generated the cases), reverse causation (MS affecting vitamin D exposure or status), and recall bias (differential recall of past exposure between cases and controls). Reverse causation might also affect other study designs (table).

A confounder is a variable that is correlated with the chosen measure of vitamin D and is a risk factor for MS independently of vitamin D. For substantial confounding to occur, both correlations need to be strong. A schematic representation of the different measures of vitamin D status, their possible relation to MS risk, and some plausible confounders are shown in figure 3.[60,61] For example, sun exposure, in addition to being a determinant of vitamin D concentration, could confound the association between serum 25-hydroxyvitamin D and MS if UVB directly affects the risk of MS.

Biological interactions are likely to occur between vitamin D and other nutrients, such as calcium or vitamin A.[62] These potential interactions are difficult to investigate because of lack of power and adequate measurements, and have largely been ignored in epidemiological studies. In the literature, "interaction" or "effect modification" refers to the deviation from a specific statistical model, most often one that implies a multiplicative effect, whereby effects are additive on the log-risk scale (ie, odds ratio for case-control studies).[63]

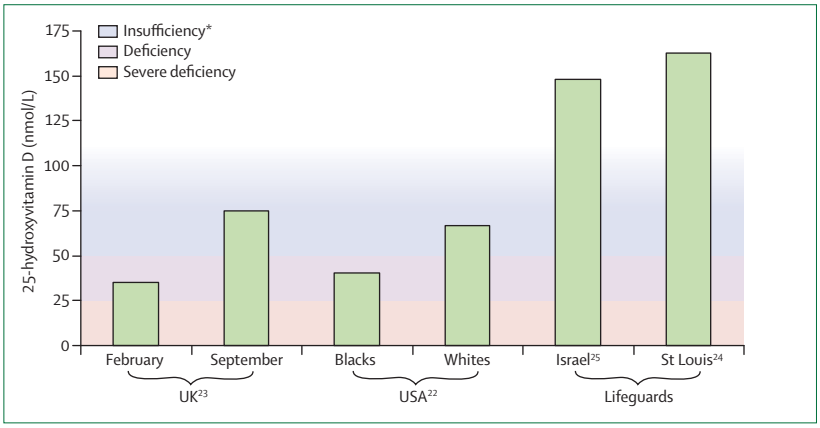

Figure 2: **Mean serum 25-hydroxyvitamin D concentrations in different populations**
Data from Looker and co-workers,[22] Hypponen and Power,[23] Haddad and Chyu,[24] and Better and co-workers.[25] *Vitamin D experts have conflicting views about the concentration below which 25-hydroxyvitamin D is considered insufficient.[20,26]

Vitamin D and MS risk

Latitude and ecological studies

The reasons why vitamin D deficiency is thought to be a risk factor for MS are as follows: (1) MS frequency

	Reverse causation	Recall bias	Selection bias	Confounding
Multiple sclerosis				
Longitudinal/prospective nested case-control studies				
Serum 25-hydroxyvitamin D	Low	Low	Low	High
Diet or sun exposure	Low	Low	Low	High
Case-control studies				
Serum 25-hydroxyvitamin D	High	Low	High	High
Actinic damage	High	Low	High	High
Diet or sun exposure	Low	High	High	High
Registry linkage studies				
Outdoor occupation	High	Low	Low	High
Skin cancer	High	Low	Low	High
Clinically isolated syndrome				
Case-control studies				
Serum 25-hydroxyvitamin D	Low	Low	High	High
Actinic damage	Low	Low	High	High
Diet or sun exposure	Low	High	High	High

Table: **Potential biases in epidemiological studies of vitamin D and multiple sclerosis, by exposure**

increases with increasing latitude, which is strongly inversely correlated with duration and intensity of UVB from sunlight and vitamin D concentrations;[53,64–69] (2) prevalence of MS is lower than expected at high latitudes in populations with high consumption of vitamin-D-rich fatty fish;[4,70,71] and (3) MS risk seems to decrease with migration from high to low latitudes.[58] Data on US veterans are particularly compelling: in analyses stratified by sex and ethnic origin, MS risk was halved among both men and women born in northern states who entered active duty in southern states.[72]

However, the latitude gradient seems to be disappearing. Although artifacts are difficult to exclude (eg, increases in MS incidence in low-risk regions, including the middle east, Mexico, and southern Europe, could be due to increased MS recognition[73,74]), the fading of the latitude gradient within the USA is most likely genuine.[75,76] Data on white women are particularly striking: a strong gradient was present among women born before 1946, but not in younger women (figure 4). Data from Olmsted County, Minnesota, suggest a constant incidence of MS (data available from 1985 to 2000);[77] thus, an increase in MS incidence in the south seems somewhat more likely than a decrease in the north, although national data are not available to confirm this assertion.

There are several possible explanations for the decline in latitude gradient within the USA. First, intestinal helminths, which were more common in the south and have been proposed as a protective factor for MS, have been decreasing.[78] Second, EBV infection is now occurring at older ages in the south, with consequent increases in mononucleosis, which is a risk factor for MS.[79,80] Gradual spread to the south of an EBV strain more likely to cause MS is also possible.[1] Third, the north–south gradient in vitamin D concentrations is disappearing owing to the fortification of milk with vitamin D and the disappearance of rickets, which was endemic among children in northern US cities until the 1940s.[81] However, because there is no evidence of a decline in MS incidence in the north, some factor causing an increase in MS incidence in both north and south has to be invoked. Finally, changes in lifestyle due to urbanisation, skin-cancer awareness, and cosmetic concerns, might have reduced sun exposure in young individuals, with greater impact in the south where sunlight contributes more to vitamin D concentrations. According to analyses of the US National Health and Nutrition Examination Survey, serum 25-hydroxyvitamin D concentrations declined significantly between 1988–1994 and 2000–2004.[82] However, laboratory differences seem to account for most of the decline, which was modest in men (about 5–9 nmol/L) and absent in women after assay calibration.[22,83] There are no data for earlier periods. In summary, latitude gradient and other ecological data provide a rationale for the vitamin D hypothesis, but remain open to many interpretations.

Serum 25-hydroxyvitamin D

If vitamin D had any effect on MS risk, we would expect MS incidence to decrease with increasing 25-hydroxyvitamin D concentrations. Because serum 25-hydroxyvitamin D concentrations decline after MS onset,[55,84] studies that measure 25-hydroxyvitamin D in patients with MS are uninformative as to whether vitamin D decreases MS risk.[50,85–87] Longitudinal studies based on 25-hydroxyvitamin D concentrations before the onset of MS are thus needed. The only study satisfying this condition used a nested case-control design to sample an underlying prospective cohort comprising over 7 million individuals who served in the US military and had at least two serum samples stored in the US Department of Defense Serum Repository (figure 5).[84] Individuals with at least 99·2 nmol/L 25-hydroxyvitamin D (top quintile) had a 62% lower odds of MS than those in the bottom quintile (<63·3 nmol/L). The study concluded that serum concentration of 25-hydroxyvitamin D in healthy young white adults is an important predictor of their risk of developing MS, independently from their place of birth and latitude of residence during childhood.[84]

This association could be due to either a protective effect of vitamin D or to confounding by some factor affecting both 25-hydroxyvitamin D concentration and MS risk. A possible confounder is UVB exposure, which is strongly correlated with 25-hydroxyvitamin D concentration and could have direct immunosuppressive effects.[45] However, genes seem unlikely confounders, because according to a recent genome-wide association study, neither the *HLA-DRB1*1501* risk alleles nor other predictors of MS risk are associated with 25-hydroxyvitamin D concentrations.[88] Similarly, confounding by anti-EBV antibodies or cigarette smoking, both MS risk factors, is unlikely because neither is consistently associated with vitamin D concentrations.[60,84,89–92]

Dietary vitamin D intake

In a prospective investigation comprising approximately 200 000 women in the USA, vitamin D intake was measured every 4 years by a comprehensive semi-quantitative food frequency questionnaire.[93] The validity of the estimated vitamin D intake was assessed in a subgroup of over 300 participants. Women in the top quintile of vitamin D intake had higher plasma concentrations of 25-hydroxyvitamin D (mean 75 nmol/L *vs* 55 nmol/L) and a 37% lower risk of hip fracture compared with those in the bottom quintile.[93] The incidence of MS during the 30-year follow-up decreased with increasing vitamin D intake (p=0·03 for trend) and was 33% lower among women in the highest quintile of vitamin D intake versus those in the lowest quintile.[94] Furthermore, MS incidence was 41% lower among women taking 400 IU per day or more from supplements compared with non-users (figure 6).[94] These results were not explained by confounding by other known risk factors for MS, including smoking and latitude,[94] or by UVB exposure, which is only weakly correlated with vitamin D intake. Although confounding by other vitamins or micronutrients is more difficult to exclude (most vitamin D was taken in the form of multivitamin supplements), there is no convincing evidence that other micronutrients are independent predictors of MS risk.[94]

Attempts to relate vitamin D intake to MS risk have also been made in case-control studies. In Norway, a study comprising 119 MS cases and 251 controls living above the Arctic Circle, where fatty fish is a major contributor to vitamin D intake, reported a lower risk of MS for individuals who ate fish three or more times per week at 16–20 years of age compared with those with a lower consumption (odds ratio 0·57 [95% CI 0·33–0·93]; p=0·024).[95] Supplementation with cod-liver oil was also associated with lower risk of MS, but only among individuals with low summer outdoor activity. However, these findings were not supported by

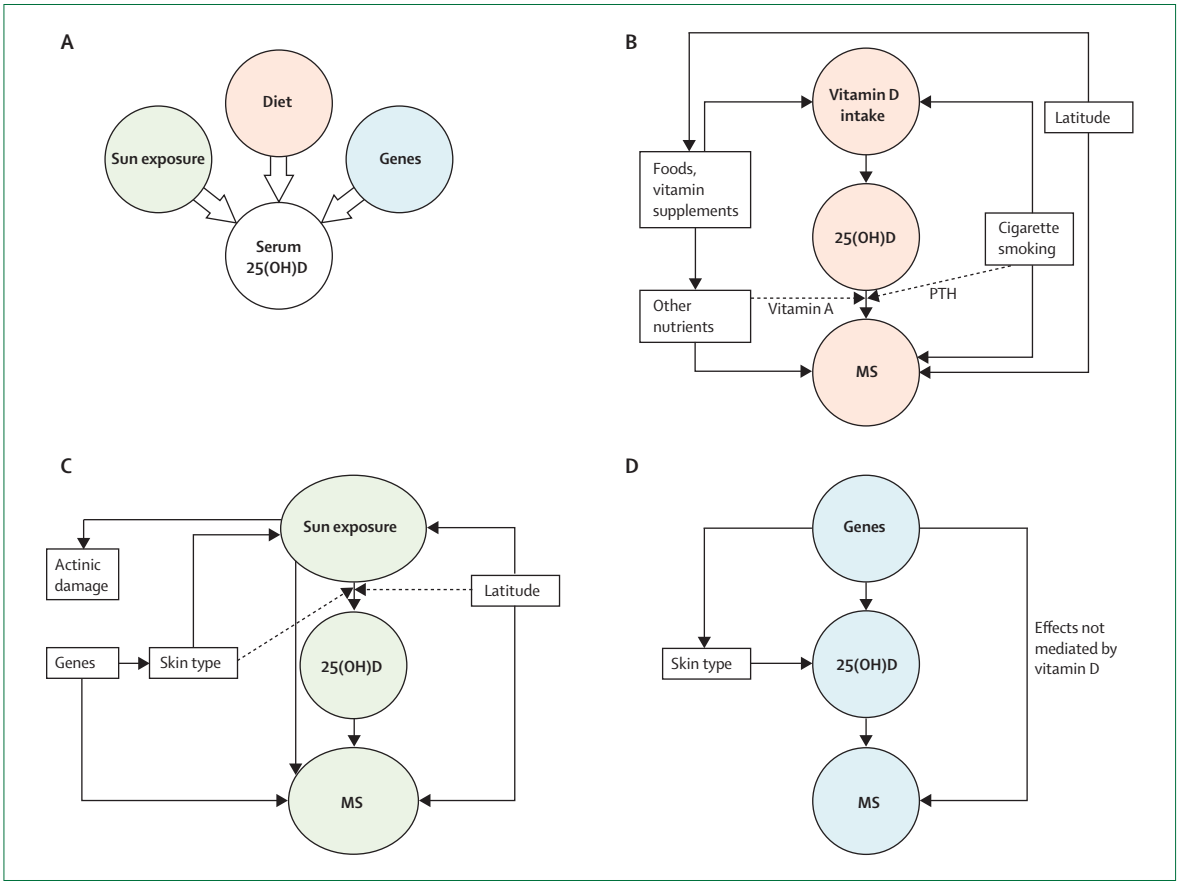

Figure 3: **Potential confounding factors and interactions in studies of vitamin D and MS risk**
Only selected links are shown. (A) Major contributors to 25(OH)D concentrations. (B) The association between vitamin D intake and MS risk could be confounded by latitude, smoking, or intake of other nutrients. Smoking and some nutrients could also modify the effects of vitamin D intake on MS risk (dashed lines) because of effects of smoking on parathyroid hormone concentrations,[60] or, for example, binding of vitamin A to retinoic-X receptors that form heterodimers with the VDR.[61] (C) Direct effects of sunlight exposure could confound the association between 25(OH)D and MS risk. (D) Confounding from genetic factors could occur if genes that determine skin type (and thus both sun exposure and its effects on 25(OH)D concentrations) were independently correlated with MS risk. Because serum 25(OH)D indicates vitamin D from sunlight and diet as well as genetic effects, the association between 25(OH)D and MS could be confounded by multiple factors. However, each factor contributes only modestly to the between-person variation in 25(OH)D concentrations, and potential for bias is therefore limited. Although confounding is difficult to eliminate, residual bias from uncontrolled confounding is generally modest, unless the confounder is strongly associated with both the exposure and the outcome of interest and no information is available to control for it. 25(OH)D=25-hydroxyvitamin D. PTH=parathyroid hormone. MS=multiple sclerosis. VDR=vitamin D receptor.

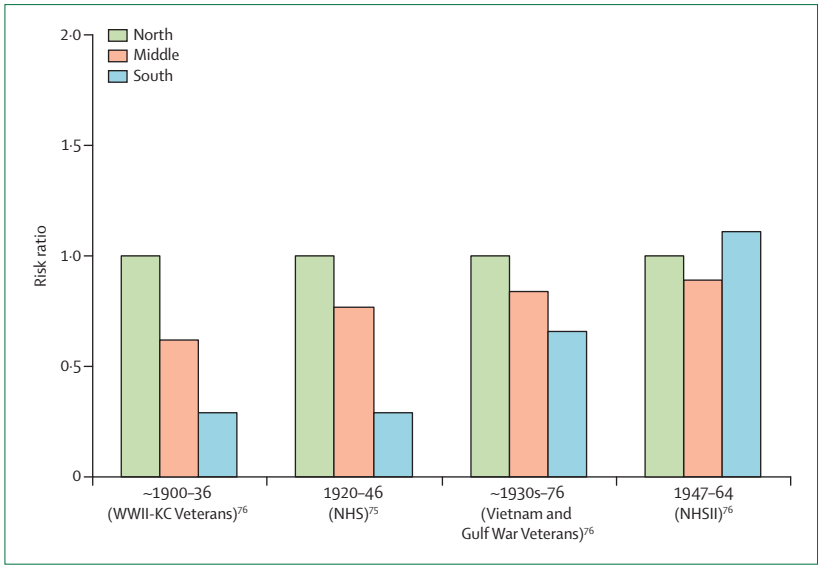

Figure 4: **Risk ratios for MS by latitude at birth in different birth cohorts of white US women**
Birth cohort dates for veterans are approximate. North is reference category. Data from Hernán and co-workers[75] and Wallin and co-workers.[76] MS=multiple sclerosis. WWII-KC=World War II-Korean Conflict. NHS=Nurses' Health Study.

studies from a Tasmanian study of vitamin D supplement use at ages 10–15 years[96] or a Canadian study of fish intake in adults,[97] although even modest differences in recall between cases and controls could cause substantial bias.[98]

Sun exposure and related measures

Actinic damage

Actinic damage is an objective measure of cumulative sun exposure, and is thus unaffected by recall bias. However, a lower degree of actinic damage could indicate lower sun exposure after MS onset (reverse causation). The inverse association between actinic damage and MS risk in a Tasmanian study (odds ratio for grades 4–6 *vs* grade 3, 0·32 [95% CI 0·11–0·88]) should thus be interpreted cautiously,[96] although its robustness to adjustment for self-reported sun exposure after MS onset and restriction of the analyses to MS cases of more recent onset provides some evidence against reverse causation.

History of sun exposure

The effect of sun exposure on vitamin D synthesis depends on many factors, including some that are difficult to assess retrospectively (time of day, clothing, and use of sunscreen). Therefore, the correlation between recalled duration of sun exposure and 25-hydroxyvitamin D concentration is typically modest.[99,100] In prospective studies, this error would tend to bias any existing association between vitamin D and MS risk towards the null hypothesis. However, in case-control studies, the lack of an objective measure also increases the vulnerability to recall bias. Not surprisingly, results of these studies have been rather inconsistent.

In a case-control study in Israel that included 241 individuals with MS and 964 controls, a significantly higher proportion of cases (89%) than controls (82%) reported spending at least 2 h per day outdoors in the summer during childhood (p=0·02), contrary to the prediction of the vitamin D hypothesis.[101] However, an excess of controls with chronic illnesses, who were more likely to be at home at survey time than healthy individuals, suggested evidence of selection bias. In a study involving 300 MS cases and 300 individuals with sciatica (controls), no association was found between time spent outdoors in the summer at age 15 years and MS risk.[102] The relevance of this study is uncertain because analyses were not adjusted for age and sex, and the proportion of men among controls was higher than among cases (data not reported).

By contrast with these early investigations, results generally consistent with a protective effect of vitamin D were found in more recent studies.[95,96,103] One of these studies, which comprised 136 individuals with MS and 272 age-matched and sex-matched controls in Tasmania,[96] collected information on sun exposure at different ages, and measured skin colour (a potential confounder) and actinic damage. The participation rate among controls was 76%. The odds ratios of MS among individuals who reported spending at least 2 h per day in the sun during holidays and at weekends at ages 6–10 years were 0·47 (95% CI 0·26–0·84) for winter and 0·50 (0·24–1·02) for summer exposure; inverse but weaker associations were observed with sun exposure at older ages. The study from Norway was based on mailed questionnaires, which were returned by 83% of the patients with MS and 65% of population-based controls, matched to the cases by age, sex, and place of birth.[95] Increased outdoor activities in the summer were associated with a decreased risk of MS, most pronounced at age 16–20 years (odds ratio 0·55 [95% CI 0·39–0·78]; p=0·001). Finally, a study in the USA based on 81 monozygotic twin pairs who were discordant for MS showed that twins with MS reported significantly lower levels of sun exposure than their healthy sibling.[103] The twin design removes selection bias, but recall bias remains a potential concern.

Outdoor occupation

If sun exposure were to decrease risk, MS should be less common than expected among individuals with outdoor occupations. The results of two investigations on occupation and MS mortality (USA and Sweden) seem to support this conclusion.[104,105] In the US study, a lower risk of MS mortality among outdoor workers was only observed in areas of high sunlight intensity.[104] However, the results of these studies could be explained by reverse causation: individuals with MS tend to avoid outdoor work due to heat-related fatigue, particularly in areas of intense sunlight.[106]

Skin cancer

Non-melanoma skin cancer, and, less strongly, melanoma,[107] are more common in individuals with high

levels of sun exposure. Thus, if vitamin D were protective, these cancers would be expected to be rare among individuals with MS. A lower than expected occurrence of skin cancer was reported among individuals with MS in a study in the UK,[108] but not in independent investigations in Norway, Denmark, France, and Sweden.[109–112] Even if consistent results had been obtained, separating the effect of sun exposure on MS risk from the effects of MS on sun exposure would remain difficult.

Month of birth

Month of birth has also been suggested as a factor that affects MS risk. In a pooled analysis of data from Canada, UK, Denmark, and Sweden including more than 40 000 individuals with MS, significantly fewer (8·5%) people with MS were born in November and significantly more (9·1%) were born in May.[113] This finding suggests that prenatal exposures or exposures in the first months of life could be important in MS aetiology, but the link to vitamin D is unclear.

In addition to the multiple sources of potential bias discussed, the association between all measures of sun exposure and MS risk could potentially be explained by a direct protective effect of UVB on MS risk.[45]

Obesity and gestational diabetes

Obesity has been associated with lower serum 25-hydroxyvitamin D concentrations.[114,115] The difference in serum 25-hydroxyvitamin D between people with a body-mass index (BMI) above 30 kg/m² and those of normal weight is less than 37·5 nmol/L in most studies,[114,115] but this might be important if it occurs during an aetiologically relevant age. The relation between pre-diagnostic BMI and MS risk has been examined in one longitudinal study, in which obesity at age 18 years (BMI >30 kg/m²), but not obesity later in life, conferred a two times higher risk of developing MS.[116]

Low vitamin D concentrations during pregnancy were associated with an increased risk of gestational diabetes in a prospective study.[117] Two studies have reported a 3–10 times increased risk of MS among individuals whose mothers had gestational diabetes during pregnancy with the affected individual.[118,119]

Genetic factors

Genes can affect vitamin D metabolism, skin colour, and behaviour, all of which can influence circulating 25-hydroxyvitamin D concentrations. Furthermore, genetic variations in *VDR* and other genes might influence the effects of vitamin D on the immune system. Therefore, genetic variations in vitamin-D-related genes might also affect MS risk, either directly or by modifying the effects of vitamin D. The increasing ratio in the concordance of MS risk between monozygotic and dizygotic twins with increasing latitude suggests that genetic effects may be stronger at low concentrations of vitamin D.[103] However, little is known about the role of vitamin-D-related genes or specific genetic interactions with vitamin D in determining MS risk. No information on vitamin D is available for the cases and controls included in the large genetic studies done to date, and existing epidemiological studies on vitamin D have insufficient power to address these questions.

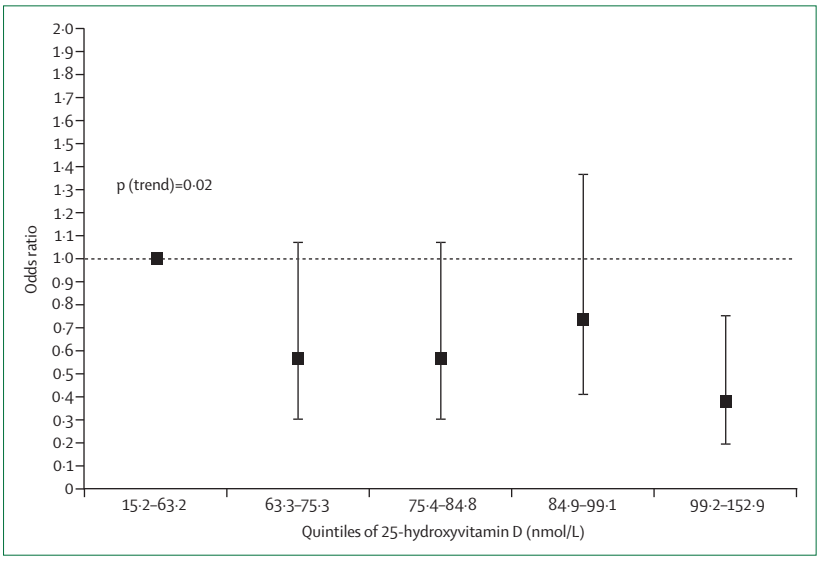

Figure 5: **25-hydroxyvitamin D concentration and MS risk among young, white adults in the US military**
Mean serum 25-hydroxyvitamin D concentration in two or three samples, adjusted for season of blood collection, age, and sex, was used to characterise the average vitamin D status in 257 US Army, Navy, or Marines active duty personnel who developed MS during follow-up (mean 5 years after the first blood collection) and 514 controls matched for age, sex, and ethnic origin. Among whites, MS risk, adjusted for latitude of the place of entry into active duty, declined with increasing 25-hydroxyvitamin D concentration: for individuals in the top 20% (25-hydroxyvitamin D ≥99·2 nmol/L), odds were 62% lower than in those in the bottom 20% (<63·3 nmol/L). Preliminary dose-response analyses suggested that high 25-hydroxyvitamin D concentrations are needed to provide a measurable benefit, thus possibly explaining the lack of association in the same investigation between 25-hydroxyvitamin D and MS risk among black people,[84] who, as expected,[22] had low 25-hydroxyvitamin D concentrations (mean 45·5 nmol/L). Reproduced from Munger et al,[84] with permission from the American Medical Association. MS=multiple sclerosis.

Predictors of serum 25-hydroxyvitamin D

According to the vitamin D hypothesis, and assuming that all other factors are equal, individuals carrying genotypes associated with lower 25-hydroxyvitamin D would be expected to have a higher MS risk (mendelian randomisation).[120] However, the assumption that other factors remain equal might not apply because adaptive changes could evolutionarily compensate for genetically low vitamin D concentrations.[121] Furthermore, although any difference in 25-hydroxyvitamin D concentrations across genotypes could be important, the effect of small differences could be difficult to detect above the background of wider environmentally determined variations in 25-hydroxyvitamin D concentration.

The proportion of variation in 25-hydroxyvitamin D that is caused by genetic factors is estimated to be 28·8–80·3%,[122–126] but few genetic determinants have been identified. The most convincing are polymorphisms in the group-specific component (vitamin D binding protein; *GC*).[127–134] In general, the effects of these

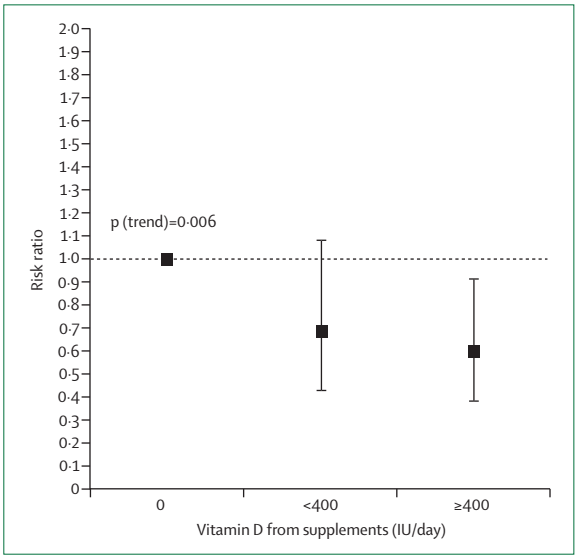

Figure 6: **Multiple sclerosis risk and supplemental vitamin D intake among women in the Nurses Health Studies**
Reproduced from Ascherio and Munger,[2] with permission from John Wiley & Sons.

polymorphisms on 25-hydroxyvitamin D concentrations are small. For example, two studies reported a 7 nmol/L mean difference between the homozygous wild-type and recessive alleles of the functional polymorphism rs7041.[131,132] This difference is probably too small to produce a measurable difference in MS risk across genotypes, and it is thus not surprising that three small investigations of *GC* polymorphisms found no association with MS risk.[135–137]

Other genes investigated for possible association with 25-hydroxyvitamin D concentration include *CYP27B1, CYP27A1, CYP2R1, CYP24A1, IL10, IL12B, IL12RB1, IL4R, SPP1, ADRB2, RXRA, NOD2,* and *VDR*.[125,127,128,130–132,138–142] Further investigation into the role of these genes is warranted because many of the polymorphisms have been investigated only in one or two studies or in specific populations, or the findings are inconsistent. In any case, the genetic contribution of any of these polymorphisms to MS risk, including *GC*, is small relative to that of *HLA-DRB1*1501*.

HLA-DRB1

The *HLA-DRB1*1501* risk haplotype is the strongest genetic predictor of MS risk in individuals of northern European ancestry, conferring an approximately three times increased risk of MS.[143] However, the association between *HLA-DR* haplotypes and MS risk is complicated by observed heterogeneity between populations and more recent work showing that some individual haplotype risks are modified by epistatic interactions with other *HLA-DR* haplotypes.[144] A highly conserved vitamin-D-responsive element (VDRE) has recently been identified in the promoter region of the *HLA-DRB1*1501* haplotype. In ex-vivo experiments, the conserved VDRE was found to be functional and bound VDR at a higher affinity than other VDREs. Stimulation with 1,25-dihydroxyvitamin D of cells transiently transfected with gene constructs including the consensus *HLA-DR15* sequence increased *HLA-DRB1*1501* expression by 1·6 times, whereas no change was seen for constructs bearing sequences specific to other *DR* haplotypes.[145] This suggests that the effects of 25-hydroxyvitamin D on MS risk might be dependent on the presence of the *HLA-DRB1*1501* haplotype—a hypothesis that has yet to be tested in epidemiological studies.

Other genetic factors

If vitamin D had a role in MS aetiology, functional variations in genes in the vitamin D pathway would be expected to influence MS risk. The most studied gene is *VDR*, which has been extensively investigated in relation to bone mineral density, osteoporosis, and fractures, but with inconclusive results.[146–150] The relation between *VDR* polymorphisms and MS is similarly inconsistent,[125,135,137,140,141,151–157] although this might be related to differences in environmental exposures that could possibly modify a genetic association with MS.

A polymorphism in the 5' region of *CYP27B1* (encoding mitochondrial 25-hydroxyvitamin D 1α hydroxylase), which is in linkage disequilibrium with other single-nucleotide polymorphisms in *CYP27B1*, has been associated with MS in one genome-wide association study,[158] but not in others.[159,160] Of interest, the co-occurrence of vitamin-D-dependent rickets type 1 (due to a mutation in *CYP27B1*) has been reported in three patients with MS.[161] Other intriguing findings include possible interactions between dietary vitamin D intake and the *VDR Fok*I polymorphism,[137] and between sun exposure and two vitamin-D-related polymorphisms: *CDX2* 3'-untranslated region in *VDR*, and alleles of *MC1R* (which encodes melanocortin 1 receptor), associated with red hair.[156,162] These observations support the potential importance of environmental factors when considering the risk of MS associated with genetic polymorphisms.

Association versus causation

A causal effect of vitamin D concentrations on MS risk is supported by the temporality, moderate strength, biological gradient, and plausibility of the observed association. The overall evidence is also reasonably consistent, given that methodological flaws might explain most discordant results. A protective effect of vitamin D is also in agreement with most data on the geographical distribution of MS, including the latitude gradient, the low prevalence among residents of northern regions with high fish consumption, and the effects of migration. The recent decline in the latitude gradient could in part be attributed to interventions against vitamin D deficiency and rickets in childhood. Although the inverse association between serum 25-hydroxyvitamin D concentrations and

MS risk could be explained by a direct immunosuppressive effect of UVB, the latter could not explain the lower risk of MS among women who take vitamin D supplements. This lack of overlap in potential unmeasured confounders makes the results on dietary intake strongly complementary to those on serum 25-hydroxyvitamin D concentrations—a protective effect of vitamin D is the most direct common explanation for both findings. However, the overall evidence for causality is only moderate, primarily because of the scarcity of large longitudinal studies. Further prospective investigations are crucial to obtain stronger evidence on causality and to identify the optimum range of 25-hydroxyvitamin D and relevant ages of exposure. Other potential modifiers, such as genetic susceptibility and ethnic origin, should also be explored.

Implications for future research and MS prevention

On the basis of the results of the only longitudinal study of serum 25-hydroxyvitamin D and MS onset,[84] and assuming that these results are unbiased and vitamin D is truly protective against MS, over 70% of MS cases in the USA and Europe could be prevented by increasing the serum 25-hydroxyvitamin D concentration of adolescents and young adults to above 100 nmol/L.[28,163] These concentrations are commonly found only in individuals with outdoor lifestyles in sunny regions (figure 1), but could be reached in most people by taking 1000–4000 IU colecalciferol daily.[8,26,164,165] Although these doses are largely considered safe and potentially beneficial for other outcomes,[8,20] confirmation of safety and efficacy in a large randomised trial is needed before making general recommendations. One option would be a national or multinational study based on randomisation of school districts or other suitable units in regions of low sunlight intensity, perhaps including multiple outcomes that might be affected by vitamin D, such as diabetes, obesity, respiratory infections, and asthma.[166–173] Alternatively, high-risk first-degree relatives of individuals with MS could be targeted in a smaller trial,[174] although randomisation and compliance could be more challenging. In either design, contamination of the control group would be a potential concern.

Vitamin D and MS activity and progression

Many patients with MS have deficient or insufficient vitamin D concentrations. In addition, serum 25-hydroxyvitamin D concentrations in patients with MS are lower during MS relapses than during remissions[50,87,175] and correlate inversely with disease severity.[55,85,176,177] All but one[175] of these results were from cross-sectional studies, and might indicate lower sun exposure in patients with severe MS, rather than a beneficial effect of vitamin D on immune responses. Although serum 25-hydroxyvitamin D concentrations indicate UVB exposure or vitamin D intake over several weeks, even a modest systematic effect of an MS relapse on 25-hydroxyvitamin D concentrations could be sufficient to induce a spurious inverse correlation between 25-hydroxyvitamin D and disease activity.[98] CSF 25-hydroxyvitamin D concentrations, which could provide a better indication of 25-hydroxyvitamin D availability to brain tissue, were found to correlate with serum concentrations, but did not differ between patients with MS and controls, and were not associated with presence of relapses or gadolinium-enhanced lesions.[178]

Stable individual characteristics that predict vitamin D status could be used to prevent reverse causation bias, but at a cost. For example, the more severe course of MS in black patients has been cited as evidence of an adverse effect of vitamin D deficiency, but it could also indicate genetic differences.[179–181] Among whites, a lower level of disability among patients with sun-sensitive skin has been deemed compatible with a protective effect of vitamin D,[182] but this conclusion seems unwarranted because sun-sensitive skin is not predictive of higher serum 25-hydroxyvitamin D concentrations.[55,56]

The results of some,[183–187] but not all,[188,189] studies support an increase in MS relapses during the months when vitamin D concentrations are at their lowest, but they could be confounded by respiratory infections.[190,191] Similarly, increase in serum calcitriol during pregnancy and its fall after delivery could explain the parallel variation in frequency of relapses,[192,193] but other hormones follow a similar pattern and provide plausible alternative explanations.[194] Large longitudinal studies of patients with MS or individuals presenting with a first demyelinating episode are thus needed to strengthen and refine the hypothesis in terms of the amount of vitamin D required for optimum protection and the identification of patients who are most likely to benefit from supplementation.

Only small safety trials of vitamin D supplementation have been done. In a year-long pilot tolerability study of oral calcitriol (2·5 μg daily), exacerbation rates were 27% lower than baseline rates.[195] Another safety and tolerability trial examined the effect of high doses of oral colecalciferol (28 000–280 000 IU weekly) and 1·2 g calcium daily in 12 individuals with MS.[196] Over the 28-week study, no cases of hypercalcaemia or hypercalciuria were observed, and although disease severity and activity were not affected, the mean number of gadolinium-enhancing lesions per patient fell from 1·75 at baseline to 0·83. Although these results are only slightly informative in terms of vitamin D efficacy, they are reassuring in terms of the safety of giving high doses of vitamin D to patients with MS, and strengthen the rationale for a large phase 3 trial. Individuals with a first demyelinating episode and those with MS could both theoretically benefit from colecalciferol, but separate trials would be desirable because disease stage could modify the response to treatment. In either case, such a trial should have sufficient power to detect a moderate beneficial effect (such as a 20–25% reduction in risk of conversion to MS, relapse rates, or disease progression), and oral colecalciferol should be given at a

Search strategy and selection criteria

References for the Review were identified through searches of PubMed from 1949 to February, 2010, and Embase from 1974 to February, 2010, by use of the following search string: ("vitamin D" OR "sun exposure" OR "diet" OR "latitude") AND "multiple sclerosis". Bibliographies of papers and book chapters were also reviewed. Only papers published in English were considered. We did not include results presented only as abstracts. Studies were selected on the basis of relevance as judged by the authors.

dose equivalent to 4000–10 000 IU daily, which should be sufficient to maintain 25-hydroxyvitamin D concentrations of most participants at 100–150 nmol/L, while retaining a low risk of toxic effects.

Conclusions

Vitamin D supplementation in healthy individuals is emerging as a promising approach for MS prevention. In utero and early-life exposure could also be important, but there is strong evidence that vitamin D concentrations during late adolescence and young adulthood have a major effect in determining MS risk. Whereas future observational epidemiological studies, and genetic and molecular investigations, will be useful to strengthen and refine the hypothesis, evidence is approaching equipoise, at which the soundest decision might be to do a large randomised trial to establish the safety and efficacy needed to promote large-scale vitamin D supplementation. Although substantial evidence supports the safety of even large doses of vitamin D, such evidence is based on studies of limited size and duration, which were mostly done in older adults. A test of the hypothesis that vitamin D could reduce MS risk will require the administration of relatively high doses of vitamin D to hundreds of thousands of young adults for several years, and careful monitoring for unforeseen adverse effects is mandatory. Given the financial, logistical, and scientific complexity, and the limited societal experience with large-scale population experiments, we suggest that an international multi-disciplinary working group should be set up to oversee the design of future prevention or supplementation studies.

Evidence supporting a therapeutic effect of vitamin D in modifying the course of MS is less compelling than evidence of a preventive effect. However, given the safety of high doses of vitamin D, there is sufficient evidence to support the need for large randomised trials to determine whether vitamin D supplementation could delay the time to progress from a first demyelinating episode to MS or to MS treatment. Furthermore, screening of serum 25-hydroxyvitamin D concentrations is likely to identify a large proportion of patients who are vitamin D deficient or insufficient, who might benefit from vitamin D supplementation for prevention of osteoporosis and other complications.

Contributors

AA conceived the overall scope of this Review. AA, KLM, and KCS contributed to the initial draft of the Review, and to the critical review and editing of the final manuscript.

Conflicts of interest

We have no conflicts of interest.

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