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REVIEW

Multiple sclerosis and vitamin D: an update

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MS is a chronic, immune-mediated inflammatory and neurodegenerative disease of the central nervous system (CNS), with an etiology that is not yet fully understood. The prevalence of MS is highest where environmental supplies of vitamin D are lowest. It is well recognized that the active hormonal form of vitamin D, 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D), is a natural immunoregulator with anti-inflammatory action. The mechanism by which vitamin D nutrition is thought to influence MS involves paracrine or autocrine metabolism of 25OHD by cells expressing the enzyme 1α-OHase in peripheral tissues involved in immune and neural function. Administration of the active metabolite 1,25-$(OH)_2$D in mice and rats with experimental allergic encephalomyelitis (EAE, an animal model of MS) not only prevented, but also reduced disease activity. 1,25-$(OH)_2$D alters dendritic cell and T-cell function and regulates macrophages in EAE. Interestingly, 1,25-$(OH)_2$D is thought to be operating on CNS constituent cells as well.

Vitamin D deficiency is caused by insufficient sunlight exposure or low dietary vitamin D_3 intake. Subtle defects in vitamin D metabolism, including genetic polymorphisms related to vitamin D, might possibly be involved as well. Optimal 25OHD serum concentrations, throughout the year, may be beneficial for patients with MS, both to obtain immune-mediated suppression of disease activity, and also to decrease disease-related complications, including increased bone resorption, fractures, and muscle weakness.

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Introduction

Multiple sclerosis (MS) is a slowly progressive, often disabling disease of the central nervous system (CNS), characterized by disseminated patches of demyelination in the brain and spinal cord. This disease results in multiple and varied neurologic symptoms and signs, usually with exacerbations and remissions at the onset: relapsing-remitting (RR) MS, followed in later years by a more chronic progressive course: secondary progressive (SP) MS. A primary progressive form (PP) of MS is also recognized. Women are affected more often than men. Age at onset of the clinical symptoms is typically between 20 and 40 y. It is uncertain whether MS is a single disease or whether the varying clinical patterns, for example, the relapsing and progressive forms, represent distinct entities (Noseworthy, 1999). In some MS patients (10–20%), the course of the disease can be classified as benign as they do not develop the characteristic disabilities (McAlpine, 1961; Ramsaransing *et al*, 2001). Plaques of demyelination, with perivascular inflammation and destruction of oligodendroglia, preceded by violation of the blood–brain barrier (BBB), are scattered throughout the white matter of the CNS. Apart from demyelination, axonal damage occurs in early stages of MS (Trapp *et al*, 1999; Bjartmar *et al*, 2003). Within one person, recent inflamed and more chronic lesions may coexist. Between MS patients, four basic patterns of neuropathological lesion characteristics suggest distinct, divergent disease mechanisms (Lucchinetti *et al*, 1996).

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A role for vitamin D in MS has been suggested (Goldberg, 1974a, b; Hayes *et al*, 1997; Hayes, 2000). The key questions concerning vitamin D are, one: is MS prevented by an adequate supply of vitamin D_3, two: is MS aggravated by vitamin D deficiency, three: is MS aggravated by a vitamin D metabolic disorder, including four: a genetic vitamin D-related disorder?

Etiological factors of MS

The etiology of MS is unknown. It is regarded as a complex multicausal disease. The etiological factors comprise (a) genetic factors, (b) dysfunction of the immune system (autoimmunity), and (c) environmental factors.

An increased family incidence and association with certain HLA allotypes suggests genetic susceptibility (Ebers & Sadovnick, 1994). The genetic epidemiology indicates that MS is not a single-gene disorder (Ebers, 1994; Compston, 1997; Noseworthy, 1999).

Autoimmune responses to myelin components may play an important role in the initiation of MS. The autoimmune character of MS is supported by the presence of numerous T lymphocytes in MS lesions and various deviating immune parameters for MS patients (Lucchinetti *et al*, 1996). Furthermore, the autoimmune animal model for MS, experimental allergic encephalomyelitis (EAE), has supported the role of autoimmunity in the pathogenesis of MS.

Among the postulated environmental etiological factors for MS is infection by a latent virus, possibly by a human herpes virus or retrovirus, in which viral activation and expression trigger a secondary response. However, no virus has yet been identified that causes MS (Genain & Hauser, 1997; Monteyne *et al*, 1998). Other environmental factors, possibly contributing to susceptibility for MS, are sunlight and nutrition (Agranoff & Goldberg, 1974; Alter *et al*, 1974; Goldberg, 1974a, b; Murrell *et al*, 1991; Esparza *et al*, 1995; Hutter & Laing, 1996; Hayes *et al*, 1997; Lauer, 1997; Van Noort & Amor, 1998). The vast amount of literature on nutrition and MS indicates that food intake may be an influencing factor determining the disease susceptibility. For example, the intake of grain (high in phytic acid) or meat, fat, and milk from animals correlated positively with the prevalence of MS (Swank *et al*, 1952; Goldberg, 1974a; Murrell *et al*, 1991; Esparza *et al*, 1995). Conversely, the intake of rice (low in phytic acid), fish, oil, skim milk, vegetables, and fruit correlated negatively with the prevalence of MS (Swank, 1953; Goldberg, 1974a; Lauer, 1997). Both phytic acid and fat may influence the bioavailability of vitamin D metabolites. Phytic acid may reduce the absorption of calcium in the gut (Mellanby, 1950). Obesity has been associated with vitamin D deficiency (Wortsman *et al*, 2000). Unfortunately, conclusive studies on the bioavailability of vitamin D_3 are rare as no validated methods for assessing the bioavailability are available (Van den Berg, 1997). An association has been reported in Norway between the relatively low risk of MS along its Atlantic coast and the relatively high dietary intake of fish oil, a rich source of vitamin D_3 (Swank *et al*, 1952; Goldberg, 1974a; Hayes *et al*, 1997).

Vitamin D metabolism

Vitamin D

Vitamin D_3, a lipid-soluble vitamin, is produced by sunlight in the skin, and can also be provided by the diet. It is a precursor of the metabolic active hormone 1,25-$(OH)_2$D. Sunlight has long been recognized as a major provider of vitamin D_3 for humans. Radiation in the UV-B (290–315 nm) portion of the solar spectrum photolyzes 7-dehydrocholesterol (provitamin D_3) in the skin to previtamin D_3, which, in turn, is converted by a thermal process to vitamin D_3 (Holick, 1987; Webb & Holick, 1988). The synthesis of vitamin D_3 in the skin is self-regulating (Webb *et al*, 1989). Excessive exposure to sunlight causes a photodegradation of previtamin D_3 and vitamin D_3 to prevent vitamin D_3 intoxication (Clemens *et al*, 1982; Matsuoka *et al*, 1987).

In addition to the production in the skin, vitamin D is supplied by food in two forms; vitamin D_2 (ergocalciferol, activated ergosterol), found in irradiated yeast, and vitamin D_3 (cholecalciferol), found in fish liver oils and fatty fish, including herring, mackerel, and sardines. The natural human diet can only be considered as a secondary source of the vitamin, when there is enough exposure to sunlight (Fraser, 1995; Vieth, 1999; Heaney *et al*, 2003a). However, in winter when UV-B in sunlight is limited, or when sunlight exposure is not adequate, dietary factors become of vital importance and dietary compensation should occur.

Vitamin D_3 is biologically inactive. It is either stored in fat or converted by 25-hydroxylase (25-OHase) enzyme in the liver to 25OHD. Interestingly, the presence of 25-OHase activity has also been demonstrated outside the liver in kidney, in keratinocytes in skin, and in parathyroid cells (Lehmann *et al*, 1999; Gascon-Barre *et al*, 2001; Correa *et al*, 2002).

25OHD

25OHD is the major circulating form of vitamin D. The serum half-life of 25OHD is approximately 10 days to 3 weeks. Serum 25OHD concentration is the indicator of the vitamin D status, and provides a good reflection of cumulative effects of exposure to sunlight and dietary intake of vitamin D (Food and Nutrition Board (FNB), Institute of Medicine, 1997). Its concentration is used as a diagnostic criterion of vitamin D deficiency. 25OHD is either stored in the liver or further converted by the enzyme 1α-hydroxylase (1α-OHase) to 1,25-$(OH)_2$D in the kidney, as well as in extra-renal tissues, including the brain (cerebellum, cerebral cortex) and lymph nodes (Hewison *et al*, 2000; Zehnder *et al*, 2001).

Renal 1,25-$(OH)_2$D and extra-renal 1,25-$(OH)_2$D

1,25-$(OH)_2$D is the hormonally active form of vitamin D. Accumulating reports have provided evidence that

1,25-$(OH)_2$D is a pleiotropic hormone influencing a plethora of biological actions, including regulation of calcium homeostasis, control of cell differentiation and maturation, and modification of immune responses (Casteels *et al*, 1995; Cantorna *et al*, 1996; Hayes *et al*, 1997; Verstuyf *et al*, 1998; Brown *et al*, 1999; Hewison *et al*, 2000; Hayes, 2000; Overbergh *et al*, 2000; Mathieu *et al*, 2001; Garcion *et al*, 2002). In addition, 1,25-$(OH)_2$D induces cell death, making the hormone of potential interest in the management of breast, prostate, and colon cancer, including brain tumors (Hewison *et al*, 2001; Garcion *et al*, 2002). The serum half-life of 1,25-$(OH)_2$D is 4–6 h (Kumar, 1986). The renal 1α-hydroxylation of 25OHD to 1,25-$(OH)_2$D is highly regulated by the serum concentrations of parathyroid hormone (PTH), calcium, and phosphate (Lips, 2001). Owing to its relatively short serum half-life and the tight regulation of the production of 1,25-$(OH)_2$D, it has not been proven to be a valuable marker for vitamin D deficiency, adequacy, or excess (FNB, Institute of Medicine, 1997).

It is now acknowledged that a wide variety of extra-renal cells can produce 1,25-$(OH)_2$D from 25OHD by the enzyme 1α-OHase *in vitro*, including activated macrophages, keratinocytes, and CNS cells (neurons and microglial cells) (Adams *et al*, 1985; Pillai *et al*, 1987; Neveu *et al*, 1994). The extra-renal production of 1,25-$(OH)_2$D is not regulated in the same way as its renal production. The relationship between expression of 1α-OHase activity by 1,25-$(OH)_2$D in a particular tissue probably involves two specific mechanisms, the first of these being substrate access, and the second being auto-regulation of 1α-OHase activity by 1,25-$(OH)_2$D itself (Hewison *et al*, 2000).

Exceptional levels of circulating 1,25-$(OH)_2$D are found in several clinical conditions. Lower levels have been found in severe vitamin D deficiency (Lips *et al*, 1982, 1988; Bouillon *et al*, 1987), as well as in inherited vitamin D metabolic disorders and chronic renal failure. Higher levels, caused by excessive extra-renal production, have been observed in sarcoidosis, tuberculosis, or malignant lymphoproliferation (Hewison *et al*, 2001). The gene encoding 1α-OHase is located on chromosome 12q13 and abnormal gene expression is the cause of hereditary pseudovitamin D-deficiency rickets (PDDR) (St-Arnaud *et al*, 1997).

Vitamin D catabolism

Ultimately, 25OHD and 1,25-$(OH)_2$D are metabolized by 24-hydroxylase (24-OHase), an enzyme induced by 1,25-$(OH)_2$D itself to control its own levels in circulation (Brown *et al*, 1999). Finally, calcitroic acid is the major excretory form (Esvelt and De Luca, 1981).

Vitamin D transport and function

Vitamin D-binding protein (DBP)

Vitamin D-binding protein (DBP) is a serum globulin, which is mainly produced in the liver. DBP transports vitamin D metabolites to a large number of target organs. Under normal physiological conditions, most of the circulating vitamin D metabolites are bound to DBP and albumin. DBP helps to regulate the bioavailability of 1,25-$(OH)_2$D, as it buffers the levels of the free metabolites and thus affords a degree of protection against short-term seasonally or dietary induced fluctuations (White & Cooke, 2000). The DBP gene locus 4q12 is among the most polymorphic known.

Vitamin D receptor (VDR)

When entering a target cell, 1,25-$(OH)_2$D dissociates from DBP, diffuses across the plasma membrane, connects to the vitamin D receptor (VDR) and shuttles between the cytoplasm and the nucleus (nuclear VDR, nVDR). Cellular action only follows after binding of 1,25-$(OH)_2$D by the nVDR in the target cell. The gene encoding the VDR is located on chromosome 12q14 and has several common allelic variants (Zmuda *et al*, 2000).

The nVDR is a member of the nuclear steroid, retinoid, and thyroid hormone receptor superfamily, acts as a ligand-activated transcription regulator, and 1,25-$(OH)_2$D is a ligand. The activated VDR dimerizes with another nuclear receptor, the retinoic acid receptor (RXR). The heterodimer RXR/VDR/1,25-$(OH)_2$D binds to a vitamin D responsive element (VDRE), a specific sequence of DNA, in the promoter region of target genes, regulated by 1,25-$(OH)_2$D. Upon binding to the VDRE, the heterodimer RXR/VDR/1,25-$(OH)_2$D activates or suppresses gene transcription, whereby synthesis of proteins is induced or repressed. 1,25-$(OH)_2$D thus exerts biological actions through VDR-mediated gene expression dependent on the target cell (Brown *et al*, 1999). VDR can also form homodimers, of which the functional significance is unknown (Issa *et al*, 1998). Efficient transcription requires co-activator or co-repressor proteins (Brown *et al*, 1999). For instance, Smad3, a downstream component of the transforming growth factor (TGF)-β signaling pathway, acts as a co-activator of VDR, by potentiating ligand-induced transactivation of the VDR (Yanagisawa *et al*, 1999). On the other hand, Smad-7 abrogates this Smad3-mediated VDR potentiation by inhibiting the Smad3–VDR complex. Thus, the interplay between the TGF-β and vitamin D pathways can modulate the VDR transactivation both positively and negatively by involving different Smad proteins (Yanagi *et al*, 1999). 1,25-$(OH)_2$D also mediates rapid responses via a putative membrane-bound receptor of the hormone (Norman *et al*, 1992).

Serum 1,25-$(OH)_2$D concentration influences the number of VDR in the cells. VDR in cells bind 1,25-$(OH)_2$D and buffer 1,25-$(OH)_2$D concentration in serum. Action of 1,25-$(OH)_2$D through the VDR can be hindered by low 1,25-$(OH)_2$D levels, or by VDR underexpression, abnormal binding functions, and aberrant transcription (Pike, 1991). The VDR has been identified in most nucleated cells of the body, involved in countless physiological functions (Walters, 1992). VDR-containing cells, in autoimmune diseases, include β-cells in

the pancreas in insulin-dependent diabetes mellitus (IDDM), chondrocytes in the joints in rheumatoid arthritis (RA), and oligodendrocytes in the brain in MS (Casteels *et al*, 1995; Baas *et al*, 2000; DeLuca & Cantorna, 2001). In parallel to oligodendrocytes, other CNS constituent cells (microglia, neurons, and astrocytes) are VDR-expressing cells responding directly to the hormone (Garcion *et al*, 2002). The VDR has also been identified in immune-competent cells, including macrophages and activated T-lymphocytes, which implies that 1,25-$(OH)_2$D can exert effects on immune functions carried out by these cells (Bhalla *et al*, 1983; Provvedini *et al*, 1983).

Sunlight and vitamin D metabolism

Sunlight and vitamin D_3 production in skin

The production of vitamin D_3 in the skin depends on exposure to sunlight. Yet, not all sunlight is intense enough to produce vitamin D_3 in the skin. UV-B irradiance is the result of solar elevation, which in turn relies on three factors — latitude, time of year, and time of day. UV-B irradiance, necessary for vitamin D_3 production, is less when the sun is lower and its path length through the atmosphere becomes longer; additional factors influencing its intensity include cloud cover, the amount of ozone, altitude, reflectivity of the earth's surface, haze (aerosols), and other pollutions. In the tropics, sunlight is able to produce vitamin D_3 in the skin all year round. Outside the tropics at latitudes between 23:5°, the sun is never at right angles relative to the earth's surface and the seasonal influence becomes greater. At latitudes higher than around 35°, sunlight is not able to produce previtamin D_3 *in vitro* all year round (Holick, 2002). In winter, the sun is not only weaker as its elevation is lower, but people also spend less time outdoors and cover their skin with clothing (Webb & Holick, 1988). If one strives to achieve and maintain an optimal serum 25OHD concentration throughout the year, it is important to know when sunlight is able to produce vitamin D_3 in the skin and how long one needs to stay outdoors to produce a sufficient amount. Matters are being complicated, as UV-B irradiance has become a topic of increasing concern, because of its potential negative effects, including sunburn and skin cancer. Excessive UV-B irradiance needs to be avoided, without losing sight of its positive effect.

The ability to synthesize previtamin D_3 *in vitro* has been published for a number of cities in the world (Figure 1). In Los Angeles, USA, at latitude 33:56°NL with an altitude (alt.) at 38 m above sea level, sunlight can produce previtamin D_3 *in vitro* all year round. In Boston, USA (42:22° NL, alt. 6 m), little if any cutaneous vitamin D_3 production occurs in the four winter months from November to February, no matter how long one stays outdoors and in Edmonton, Canada (53:19° NL, alt. 715 m) the equivalent 'vitamin D winter' lasts 6 months, from October to March (Webb & Holick, 1988). The influence of season and latitude on the synthesis of previtamin D_3 *in vitro* in the southern hemisphere was measured in Buenos Aires (34:50 SL°, alt. 20 m), Cape Town (33:58 SL°, alt. 42 m), Johannesburg (26:08° SL, alt. 1694 m) and Ushuaoa (54:48° SL, alt. 16 m) (Holick, 2002). Only in Ushuaoa (54:48° SL, alt. 16 m) no previtamin D_3 was formed in the 6 winter months April–September. For Europe, no comparable data on cutaneous vitamin D_3 production have

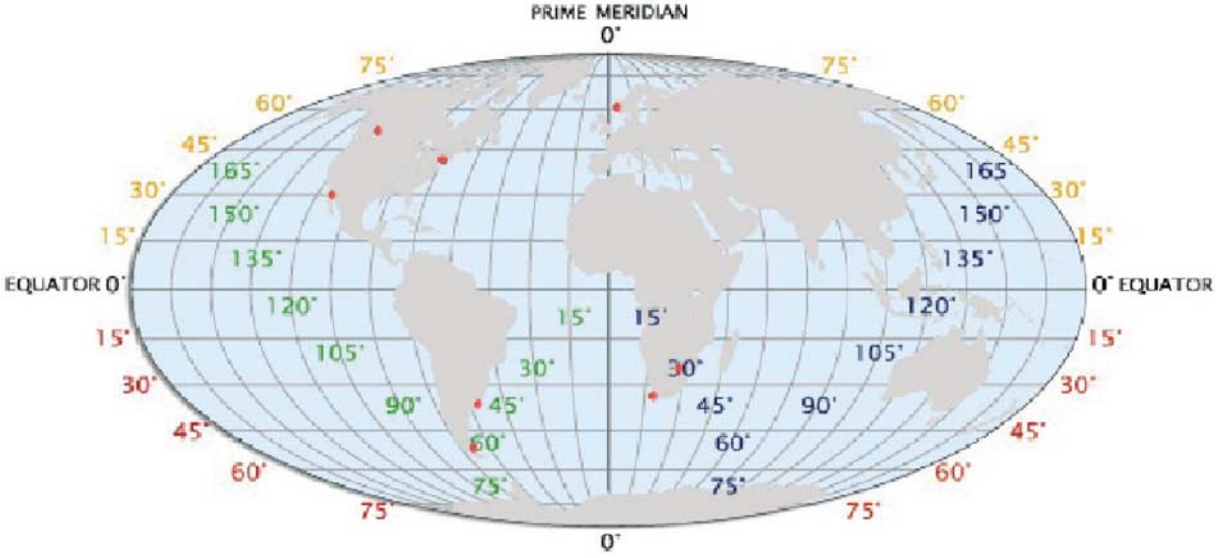

Figure 1 Cities of the world, red dots, where the length of the 'vitamin D winter' has been measured: Bergen in Norway (60:17° NL, alt. 50 m), Boston (44:22° NL, alt. 6 m), Buenos Aires (34:50 SL°, alt. 20 m), Edmonton (59:19 NL°, alt. 715 m), Cape Town (33:58 SL°, alt. 42 m), Johannesburg (26:08° SL, alt. 1694 m), Ushuaoa (54:48° SL, alt. 16 m). Note: At latitudes higher than around 35°, sunlight is unable to produce previtamin D_3 *in vitro* all year round.

been reported, except for Bergen in Norway (60:17° NL, alt. 50 m). In Bergen the 'vitamin D winter' lasted 6 months, from October to March, but in the other months of the year the previtamin D_3 formation per month is less than in Edmonton and the hours of UV-B per day are also fewer (Holick, 2002).

In the United States the vitamin D intake is much higher than in Europe, due to fortification of milk with 10 μg (400 IU) vitamin D per quart (Norman, 2000). Currently, in Europe, which is, by the way, much further away from the equator than the United States, milk is not fortified with vitamin D and recommended nutritional supplementation with vitamin D differs from country to country.

During the 'vitamin D winter', the body is dependent on its vitamin D_3 stores or on dietary intake from natural sources, food fortified with vitamin D, or supplements. As vitamin D_3 barely occurs naturally in food, and food in most countries is not fortified with vitamin D, dietary supplementation with the vitamin may be necessary for certain groups in the 'vitamin D winter' to maintain an optimal 25OHD serum level throughout the year.

Sunlight and 25OHD

The approximate normal range for serum 25OHD values is 25–130 nmol/l (Feldman *et al*, 1997). Currently, there is no consensus on what represents an optimal serum 25OHD concentration. An increasing number of reports is available on 25OHD serum levels both in healthy and unhealthy populations, from which it has become apparent that serum 25OHD levels vary from winter to summer, with lower levels in winter (Stamp, 1975; Bouillon *et al*, 1987; Lips *et al*, 1988: McKenna, 1992; Scharla *et al*, 1996; Scharla, 1998). Here we focus on two of these reports (Bouillon *et al*, 1987; Scharla, 1998).

In an age- and sex-stratified population-based sample of a normal population living in South Germany ($n=415$, 206 women and 209 men, ranging from 50 to 80 y), serum 25OHD reached its nadir of 42.5 ± 22.5 nmol/l in January, and its zenith of 67.5 ± 25 nmol/l in the months August and September (Scharla *et al*, 1996). Of the women, 40% had a subclinical vitamin D deficiency in winter, defined as $<$ 30 nmol/l 25OHD (Scharla, 1998). The serum 25OHD concentration of elderly subjects living in Belgium, who were consecutively admitted to one of the geriatric wards in Leuven ($n=240$, 137 women and 103 men, ranging from 55 to 99 y), reached its nadir of 18 nmol/l in February. The lowest levels were recorded in the 4 months from January to April (mean $<$ 25 nmol/l), and its zenith of 30 nmol/l in July (Bouillon *et al*, 1987). It was found that the frequency of very low levels of 25OHD ($<$ 12.5 nmol/l) was more pronounced in wheelchair-bound or institutionalized elderly subjects (Bouillon *et al*, 1987). From these reports, it could be concluded that the unhealthy elderly living in Belgium had lower 25OHD levels in winter than in summer. Their monthly and yearly 25OHD levels were significantly lower than those of their healthy younger control subjects. The unhealthy elderly living in Belgium also had lower monthly 25OHD levels than the healthy elderly living in South Germany. Not only in unhealthy elderly, but also in young subjects a significant difference between winter and summer 25OHD has been found (Guillemant *et al*, 1995, 1999; Docio *et al*, 1998; Zittermann *et al*, 1999). Taken together, these results emphasize a widespread seasonal variation in 25OHD levels, with low 25OHD levels in winter. This seasonal variation is reflected in the approximate normal range for serum 25OHD values 25–130 nmol/l. This wide range is used to classify individuals in vitamin D deficient and sufficient.

The Royal Dutch Meteorological Institute (KNMI) publishes the monthly and yearly mean duration of sunlight in hours of different cities in the world (Nellestijn & Dekker, 1998). The monthly mean duration of sunlight in hours in Munich in south Germany (48:21° NL, alt. 527 m) and Brussels in Belgium (50:54° NL, alt. 55 m) published by Nellestijn and Dekker (1998), as well as the monthly mean serum 25OHD concentration of healthy elderly in south Germany reported by Scharla (1998) and that of unhealthy elderly in Belgium reported by Bouillon *et al* (1987), were used to calculate the correlations. The author of the present paper compared 12 consecutive months of the year and found a significant correlation between sunlight and 25OHD, 2 months later, in healthy elderly in south Germany $r=0.86$ ($P<0.001$, $n=12$) and in unhealthy elderly in Belgium $r=0.90$ ($P<0.001$, $n=12$). This finding is in line with the time lag of 2 months between sunlight and 25OHD reported in other studies (Hine & Roberts, 1994; Need *et al*, 2000). It has been stated that the concentration as found in healthy individuals at the end of summer or as found in healthy individuals in the tropics provides a physiological indication of what might be optimal to maintain throughout the year (Vieth, 1999).

The approximate normal range for serum 1,25-$(OH)_2$D values is 36–144 pmol/l (Feldman *et al*, 1997). No seasonal variation in 1,25-$(OH)_2$D levels was observed in healthy adults (Chesney *et al*, 1981; Bouillon *et al*, 1987). No association between 25OHD and concentrations of 1,25-$(OH)_2$D was found in euthyroid patients, who previously had low 25OHD ($<$50 nmol/l) levels, but had been advised to take 25 μg (1000 IU)/day vitamin D_3 (Vieth *et al*, 2003).

Vitamin D nutrition

Dietary compensation should occur to overcome low 25OHD levels when cutaneous production of vitamin D_3 is inadequate. For their recommendations of vitamin D_3 intake, National Councils on Food and Nutrition have abandoned the older criterion of absence of disease as the definition of adequacy. Now they are confronted with two new questions, one: what concentration of serum 25OHD is adequate, and two: how much vitamin D_3 is needed each day to meet or sustain that concentration. The border between a vitamin

D-deficient and -sufficient state is represented by reported cutoff levels for the 25OHD concentration which rang widely from 12.5 to 140 nmol/l, but seem to increase over the years (Bouillon *et al*, 1987; Chapuy *et al*, 1997; Dawson-Hughes *et al*, 1997; Barger-Lux *et al*, 1998; Malabanan *et al*, 1998; Scharla, 1998; Thomas *et al*, 1998; Vieth, 1999; Need *et al*, 2000; Lips, 2001; Heaney *et al*, 2003a). Serum levels of 25OHD between 30 and 100 nmol/l have been mentioned as necessary to ensure vitamin D sufficiency (Barger-Lux *et al*, 1998; Lips, 2001; Heaney, 2003b; Vieth *et al*, 2003). In elderly nursing home residents, vitamin D_3 10 μg (400 IU)/day increased serum 25OHD from 22 to 62 nmol/l in 12 weeks (Chel *et al*, 1998). Others have observed higher doses to ensure adequate serum 25OHD levels (Barger-Lux *et al*, 1998; Heaney *et al*, 2003a; Vieth *et al*, 2003). Recently, a daily supplement of 25 μg (1000 IU) vitamin D_3 has been advocated for all adults to ensure a serum 25OHD level of at least 40 nmol/l (Vieth *et al*, 2001, 2003). There is no consensus on this, but this dose is well below the Tolerable Upper Intake Level (UL) of vitamin D for adults of 50 μg (2000 IU)/day set by the FNB of the Institute of Medicine for the USA, as well as by the Scientific Committee on Food of the European Commission (SCF) for the European Union (FNB, Institute of Medicine, 1997; SCF, 2002).

MS and vitamin D

MS prevalence and sunlight

MS is more common in temperate climates than in the tropics, with a prevalence of 100/100 000 and 10/100 000, respectively (Martyn, 1991; Gale & Martyn, 1995). Of all the climatic variables analyzed, insolation, in terms of annual and winter hours of sunlight, exhibited the strongest negative correlation with the prevalence of MS (Acheson *et al*, 1960; Norman *et al*, 1983). The incidence of MS is low in areas with at least 3000 h sunlight annually or with sufficient vitamin D_3 intake (Goldberg, 1974a). Unlike mortality from skin cancer, mortality from MS was negatively associated with residential exposure to sunlight (Freedman *et al*, 2000). A negative correlation between ultraviolet radiation (UVR) and MS prevalence was found in Australia (Van der Mei *et al*, 2001). The suggestion that the risk of developing MS is largely determined before the age of 15 y has been questioned by Australian epidemiological data. The prevalence in the migrant population from the UK and Ireland in the different regions in Australia showed a significant correlation with latitude and was considerably less than in their countries of origin (Hammond *et al*, 2000). The epidemiological evidence suggests that UVR may play a protective role in three autoimmune diseases: MS, insulin-dependent diabetes mellitus, and rheumatoid arthritis has been reviewed (Ponsonby *et al*, 2002). New evidence has been reported that increased sun exposure during ages 6–15 y is associated with a decreased risk of multiple sclerosis (Van der Mei *et al*, 2003). Interestingly, the prevalence of MS among Sardinians presents evidence against the latitude gradient theory (Pugliatti *et al*, 2001) and could be explained by a high susceptibility of the population to MS (Montomoli *et al*, 2002).

MS and 25OHD serum concentration

Only a few reports have investigated the association between MS and 25OHD serum concentration. Vitamin D deficiency was detected in a group of female MS patients who were subjects of a study on osteoporosis. Of these patients ($n = 52$), 70% had a subclinical vitamin D deficiency, defined as serum 25OHD < 50 nmol/l (Nieves *et al*, 1994). This study consecutively recruited female MS patients who were admitted to a tertiary care hospital because of deterioration in their clinical status, and there was no appropriate control group. Results suggested that low circulating 25OHD levels contributed to low bone mineral density (BMD) (Nieves *et al*, 1994).

Another group of MS patients ($n = 54$), of whom 64% had a subclinical vitamin D deficiency, defined as serum 25OHD $<$ 50 nmol/l, had more rapid bone loss and more frequent fractures than healthy age- and gender-matched controls (Cosman *et al*, 1998). Levels of 25OHD were on average 20–37.5 nmol/l lower in MS patients than they were in the three control groups: men, pre- and postmenopausal women. In the analyses, lack of sunlight exposure, a vitamin D-deficient diet, immobility, and corticosteroid treatment contributed to the low 25OHD serum concentrations (Cosman *et al*, 1998). However, in this study, it was not clear whether the vitamin D deficiency was merely a result of immobility, as it has been reported that immobility may be the strongest risk factor for vitamin D deficiency (Gloth *et al*, 1995).

Mahon *et al* (2003) reported that 48% of MS patients ($n = 39$) had a subclinical vitamin D deficiency at baseline, defined as serum 25OHD < 50 nmol/l.

No reports have been found on the 1,25-$(OH)_2$D serum concentration in MS patients. Case–control studies with sufficient power on patients with MS with respect to serum 25OHD and 1,25-$(OH)_2$D concentration are lacking. Moreover, seasonal variation of 25OHD serum concentration has not yet been established in MS patients.

MRI and season

Various studies have investigated the association between number of active magnetic resonance imaging (MRI) lesions and season (Auer *et al*, 2000; Embry *et al*, 2000; Rovaris *et al*, 2001; Killestein *et al*, 2002). Active MRI lesions are the gadolinium-enhancing lesions on MRI scans, which reflect subclinical disease activity. A statistical significant seasonal fluctuation, measured as active MRI lesions, has been demonstrated in MS patients ($n = 53$) living in south Germany. The number of active MRI lesions was the highest in April and lowest in October (Auer *et al*, 2000). The seasonal variation of active MRI lesions in MS patients has since been re-addressed in other studies. The monthly mean number of MRI lesions was pooled in four seasons: spring (March, April, May),

summer (June, July, August), autumn (September, October, November), and winter (December, January, February). No statistical significant difference could be detected between the number of active MRI lesions in these four seasons (Rovaris *et al*, 2001; Killestein *et al*, 2002). However, these calendar months do not necessarily correspond with the 'vitamin D winter' and more importantly do not necessarily represent the circulating 25OHD levels of the individuals under investigation. In addition, Rovaris *et al* (2001) used data from MS patients living in different parts of the world.

Embry *et al* (2000) have graphically combined two separate studies and showed close correspondence between the curve representing monthly mean serum 25OHD concentrations in the group of non-MS individuals provided by Scharla and the curve representing the monthly number of MRI lesions, 2 months later, in MS patients provided by Auer (Scharla, 1998; Auer *et al*, 2000). Of course, there exists an inherent weakness in combining data from different studies to reach a new conclusion. In this case, the 25OHD serum levels were measured in a group of non-MS individuals. These levels may not be representative of a cohort of MS patients, whose vitamin D metabolite levels may be influenced by their MS.

The monthly mean duration of sunlight in hours in Munich published by Nellestijn and Dekker (1998) and the monthly mean number of active MRI lesions reported by Auer *et al* (2000) were used to calculate the correlation. The author of the present paper compared the 12 consecutive months of the year and found a statistical significant inverse correlation between sunlight and active MRI lesions, 4 months later, $r=-0.90$ ($P<0.001$, $n=12$).

Further research is required; currently no reports are available on mean serum 25OHD concentrations, and active MRI lesions by months of the year in patients with MS living in the same area.

MS and vitamin D-related genetic polymorphism

Genetic factors may contribute to the disease course in MS patients (Goldberg, 1974a, b; Hayes *et al*, 1997). Vitamin D receptor gene (VDRG) polymorphism could be a factor in determining susceptibility to MS or disease modulation of MS. An association with MS for VDRG polymorphism or linkage disequilibrium of VDRG to other pathologic gene loci has been found among Japanese women (Fukazawa *et al*, 1999). In another Japanese study, DBP polymorphism showed no differences in distribution between MS patients and non-related healthy individuals (Niino *et al*, 2002). In a Canadian population, no evidence was found for association or linkage of genes involved in the metabolism and function of vitamin D with MS, including the 1α-OHase gene, the DBP gene, and the VDR gene (Steckley *et al*, 2000).

Neuro-immunology and vitamin D metabolites

The CNS as a target tissue for vitamin D metabolites is supported by discovery of VDR in the rat forebrain, hippocampus, cerebellum, brainstem, spinal cord, and perivascular tissue and the discovery of 1α-OHase in cerebellum and cerebral cortex (Neveu *et al*, 1994, Issa *et al*, 1998, Hewison *et al*, 2000; Zehnder *et al*, 2001). In addition, the possibility of a local synthesis of 1,25-$(OH)_2$D in brain has been postulated (Garcion *et al*, 2002). Profound alterations in the brain at birth have been demonstrated in rats born to vitamin D_3-deficient mothers (Eyles *et al*, 2003).

The influences of 1,25-$(OH)_2$D on cells of the nervous system were recently reviewed by Garcion (Garcion *et al*, 2002). It appeared that 1,25-$(OH)_2$D had effects on neurons, oligodendrocytes, as well as astrocytes, but the exact pathways of these effects remain to be established. In general, the influences of 1,25-$(OH)_2$D on these cells seem to be neuroprotective and anti-inflammatory (Garcion *et al*, 2002).

Immune system and 1,25-$(OH)_2$ D

The active form of vitamin D, 1,25-$(OH)_2$D, is a potent regulator of the immune system (Bouillon *et al*, 1995; Casteels *et al*, 1995; Cantorna *et al*, 1996, 1998, 1999; Hayes *et al*, 1997; Nashold *et al*, 2000, 2001; Gregori *et al*, 2001; Griffin *et al*, 2001). Here we focus on the autoimmune animal model for MS EAE. The preventive and curative effects of vitamin D-related treatment on the clinical course of EAE are described (Table 1). The effects on the cellular level, which are relevant for EAE, and may have implications for MS and other autoimmune diseases, are summarized. The possible actions of vitamin D metabolites on immune cells relevant for EAE are portrayed in Figure 2. Finally, the influences of vitamin D metabolites on the production of cytokines and nitric oxide (NO), and on the BBB are described.

Experimental autoimmune encephalomyelitis (EAE)

EAE is a useful (although not perfect) animal model of human MS (Van Etten *et al*, 2003). EAE is induced by immunization of rodents or primates with myelin or myelin components. This results in the generation of autoreactive, myelin-specific T lymphocytes. In the CNS of EAE animals perivascular inflammatory lesions are present and, depending on the immunization protocol, a variable degree of demyelination is observed. The lesions in the brain and spinal cord are accompanied by transient clinical signs such as paralysis of the tail and hind limbs. EAE can also be induced by transferring T lymphocytes from rats immunized with myelin components into naïve rats, indicating the crucial role of T lymphocytes in this model (Paterson & Hanson, 1969). In particular, the interferon-gamma (IFN-γ)-producing T helper 1 (Th1) lymphocytes are required for induction of EAE. Macrophages are crucial for the effector phase of EAE, the phase in which actual tissue damage is caused by an immune response. In EAE, macrophage depletion leads to complete suppression of clinical signs (Huitinga *et al*, 1990; Tran *et al*, 1998).

Table 1 Preventive and curative effects of vitamin D-related treatment of EAE Administration of (treatment before/during or after induction of EAE)

		Effect of treatment given		
	Vitamin D-related treatment	*Before/during induction of EAE*	*After induction of EAE*	*Species*
EAE and UV				
Hauser *et al* (1984)	UV full spectrum	↓	=	Mouse
EAE and 1,25-$(OH)_2$D				
Lemire & Archer (1991)	1,25$(OH)_2$D	↓	↓↓	Mouse
Cantorna *et al* 1996	1,25$(OH)_2$D	↓	↓↓	Mouse
Nataf *et al* (1996)	1,25$(OH)_2$D		↓↓	Rat
EAE and vitamin D deficiency				
Cantorna *et al* (1996)	Vitamin D-deficient diet	↑		Mouse
Garcion *et al* (2003)	Vitamin D-deficient diet		↑↑	Rat
Cantorna *et al* (1996)	Withdrawal of 1,25$(OH)_2$D		↑↑↑	Mouse
EAE and 1,25-$(OH)_2$D combined treatment				
Branisteanu *et al* (1995)	1,25$(OH)_2$D and cyclosporine	↓	↓↓	Mouse
Branisteanu *et al* (1997)	1,25$(OH)_2$D and sirolimus	↓	↓↓	Mouse
Cantorna *et al* (1999)	1,25$(OH)_2$D and Ca^{2+}	↓	=	Mouse
EAE and synthetic 1,25-$(OH)_2$D analogs				
Lemire *et al* (1994)	1,25-$(OH)_2$-16eneD3	↓	↓↓	Mouse
Mattner *et al* (2000)	Ro 63-2023	↓	↓↓	Mouse
Garcion *et al* (2003)	MC1288		↓↓	Rat
EAE and synthetic 1,25-$(OH)_2$D analog combined treatment				
Van Etten *et al* (2000)	Analogs and other substances	↓	↓↓	Mouse
Van Etten *et al* (2003)	TX527 and bisphosphonate pamidronate	↓	↓↓	Mouse

Effect of treatment: ↓, prevention of EAE effective; ↑, susceptibility to EAE and clinical signs of EAE increased; =, modification of EAE ineffective; ↓↓, clinical signs of EAE decreased; ↑↑, clinical signs of EAE increased; ↑↑↑, resumption of clinical signs of EAE; sirolimus, Rapamycin (RAP).

EAE and vitamin D-related treatment

Table 1 summarizes the observed effects of vitamin D-related treatment on EAE. Exposure of mice to whole body (full spectrum) UV was effective in preventing EAE when administered before immunization, but was ineffective in modifying ongoing EAE or in preventing relapses of EAE induced by re-immunization (Hauser *et al*, 1984). However, this report does not mention vitamin D metabolites at all. The first study on 1,25-$(OH)_2$D treatment of EAE was by Lemire and Archer (1991) and showed that administration of 1,25-$(OH)_2$D during the immunization phase in mice significantly prevented the onset and development of EAE. The preventive effect of 1,25-$(OH)_2$D on EAE in mice given before EAE induction was complete (Cantorna *et al*, 1996). When treatment with 1,25-$(OH)_2$D on EAE was started after the appearance of clinical signs, progression and severity was decreased in mice (Cantorna *et al*, 1996) and rats (Nataf *et al*, 1996). A vitamin D-deficient diet resulted in an increased susceptibility to EAE, an accelerated onset of paralytic symptoms and aggravated clinical symptoms (Cantorna *et al*, 1996). In rats deprived of vitamin D, the clinical signs of EAE increased (Garcion *et al*, 2003). Withdrawal of 1,25-$(OH)_2$D after EAE induction resulted in a resumption of clinical signs (Cantorna *et al*, 1996). The effect of 1,25-$(OH)_2$D can be potentiated by cyclosporine, sirolimus (Rapamycin, RAP), and calcium (Branisteanu *et al*, 1995, 1997; Cantorna *et al*, 1999). In EAE in mice, calcium was required in addition to 1,25-$(OH)_2$D to prevent the appearance of this disease, and the higher the calcium intake the lower the 1,25-$(OH)_2$D dose needed (Cantorna *et al*, 1999). These results suggest that 1,25-$(OH)_2$D and dietary calcium are both involved in the prevention of symptomatic EAE (Cantorna *et al*, 1999; DeLuca & Cantorna, 2001). Interestingly, changes in dietary calcium and phosphate levels resulted in changes in target tissue VDR expression (Issa *et al*, 1998). VDR itself is essential for the immunosuppressive ability of 1,25-$(OH)_2$D during EAE (Meehan & DeLuca, 2002). From studies on vitamin D deficiency in the elderly, it is understood that a low calcium intake causes secondary hyperparathyroidism, which increases vitamin D turnover and aggravates vitamin D deficiency and its consequences, while high calcium intake may reduce vitamin D requirement (Lips, 2001).

Treatment with synthetic 1,25-$(OH)_2$D analogs has also been reported (Lemire *et al*, 1994; Mattner *et al*, 2000; Van Etten *et al*, 2000, 2003; Garcion *et al*, 2003). Curative treatment of vitamin D-deprived rats with the nontoxic-1,25-$(OH)_2$D analog MC1288 strongly inhibited EAE symptoms, thus suggesting that these compounds may be a suitable treatment for MS (Garcion *et al*, 2003). The 1,25-

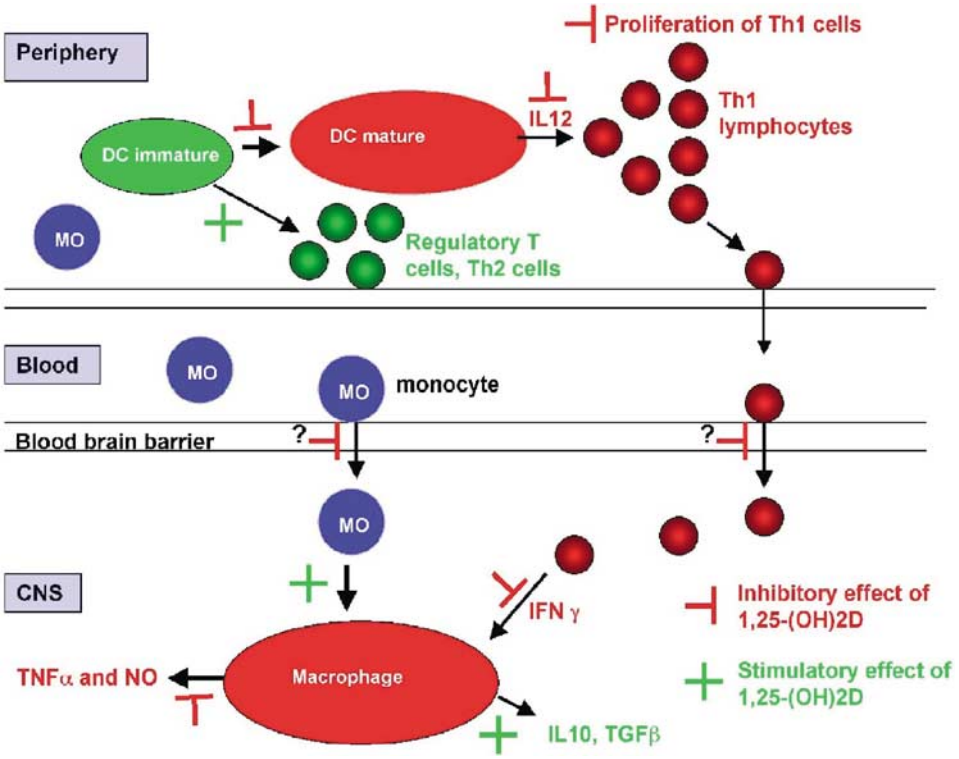

Figure 2 Downregulation by 1,25-$(OH)_2$D of pro-inflammatory dendritic cell and T-cell function and macrophage activity and migration, in experimental allergic encephalomyelitis (EAE, an animal model of MS). Effects on the CNS-constituting cells are not incorporated in this figure, but are reviewed in Garcion *et al* (2002). The effect of 1,25-$(OH)_2$D on the cells of the BBB is unknown, but EAE data suggest that cellular infiltration is inhibited (Nashold *et al*, 2000). Further references on which this figure is based are mentioned in the text under Neuro-immunology and vitamin D metabolites. Dendritic cell, DC; monocyte, MO; T helper 1 lymphocyte, Th1; T helper 2 lymphocyte, Th2; interferon gamma, IFNγ; interleukin, IL; nitric oxide, NO; transforming growth factor β, TGFβ; tumor necrosis factor alpha, TNFα.

$(OH)_2$D analog TX527 decreased disease severity and postponed onset in mice with EAE, and adding the bisphosphonate pamidronate prevented the side effects of this analog (Van Etten *et al*, 2003).

Cellular effects

T lymphocytes and dendritic cells. The effect of 1,25-$(OH)_2$D on the acquired, antigen-specific immune response is initiated by exposure of antigen in the groove of MHC class II molecules to T lymphocytes. This so-called antigen presentation results in proliferation and cytokine production by antigen-specific T lymphocytes. Based on the cytokine production profile, two populations of T lymphocytes can be distinguished. Th1 cells produce IFN-γ, a pro-inflammatory cytokine that promotes macrophage activation and MHC class II expression. T helper 2 (Th2) cells produce interleukin (IL)-4 and IL-5, promoting antibody production in particular IgE. Antigen-presenting cells can influence the cytokine production profile of T lymphocytes upon antigen recognition. By production of the cytokine IL-12, antigen-presenting cells can induce a shift towards the Th1 cytokine profile. Classical antigen-presenting cells are the so-called dendritic cells, which are derived from monocytes. They occur in almost all tissues of the body and in large numbers in lymphoid organs where they present antigen to T lymphocytes. 1,25-$(OH)_2$D inhibits antigen-induced T-lymphocyte proliferation (Bhalla *et al*, 1984; Lemire & Adams, 1992) and prevents Th1 development in EAE (Mattner *et al*, 2000). Various reports have shown that 1,25-$(OH)_2$D exerts major effects on dendritic cells (DC), by inhibition of DC maturation (Penna & Adorini, 2000; Griffin *et al*, 2001). Accordingly, DC of VDR-deficient mice fail to respond to maturational stimuli (Griffin *et al*, 2001). Mature DC are required for the induction of an efficient Th1 response, in particular by their production of the pro-inflammatory cytokine IL-12. The production of IL-12 by DC is downregulated by 1,25-$(OH)_2$D, whereas the production of the anti-inflammatory cytokines IL-10 is enhanced (Penna & Adorini, 2000) and the production of TGF-β is unaffected (Griffin *et al*, 2001). Thus, by reduction of IL-12 production by DC, 1,25-$(OH)_2$D may inhibit the development of pro-inflammatory Th1 cells.

Furthermore, 1,25-$(OH)_2$D treatment results, alone or when combined with the selective inhibitor of lymphocyte proliferation mycophenolate mofetil (MMF), in

the generation of a population of CD4 + CD25-regulatory T cells (Gregori *et al*, 2001). The potency of this effect of 1,25-$(OH)_2$D is illustrated by the fact that tolerance is induced by 1,25-$(OH)_2$D/MMF treatment to fully mismatched pancreatic islet allografts in mice (Gregori *et al*, 2001). Altogether, these data show that 1,25-$(OH)_2$D inhibits DC maturation and inhibits the induction of pro-inflammatory Th1 cells. In addition, the formation of tolerogenic T cells, an active mechanism for natural immune suppression, and the production of anti-inflammatory cytokine IL-10 are promoted by 1,25-$(OH)_2$D.

Macrophages. In addition to its effect on T lymphocytes, the effect of 1,25-$(OH)_2$D on macrophages contributes to its immunomodulatory potential. Almost two decades ago, it has been reported that 1,25-$(OH)_2$D promoted the induction of (pro)monocytic differentiation to macrophages (Koeffler *et al*, 1984). 1,25-$(OH)_2$D increases the antigen-presenting activity of macrophages and enhances the phagocytic activity of macrophages (Goldman, 1984; Amento & Cotter, 1988).

Cytokines and nitric oxide (NO)

The cellular effects of 1,25-$(OH)_2$D include effects on production of immunoregulatory molecules such as cytokines and NO. 1,25-$(OH)_2$D decreases the production of pro-inflammatory cytokines IL-2, IFN-γ and TNF-α *in vitro* and *in vivo* (Manolagas *et al*, 1985; Reichel *et al*, 1989; Lemire & Adams, 1992), and IL-12 *in vivo* (Lemire *et al*, 1994; D'Ambrosio *et al*, 1998; Mattner *et al*, 2000). On the other hand, it promotes the *in vivo* production of anti-inflammatory cytokines such as IL-4 and TGF-β (Cantorna *et al*, 1998). An increase of TGF-β1 expression in lymph nodes at the periphery may explain the beneficial effect of 1,25-$(OH)_2$D in EAE and has been re-emphasized in MS (Cantorna *et al*, 1998; Mahon *et al*, 2003). In contrast, TGF-β1 increase was not found in the rat CNS (Garcion *et al*, 2003), suggesting that the effects of 1,25-$(OH)_2$D in EAE are due to effects on the peripheral immune system rather than on local immune suppression. 1,25-$(OH)_2$D triggers the production of inducible nitric oxide synthase (iNOS) by a human macrophage cell line *in vitro* (Figure 2) (Rockett *et al*, 1998), but decreases iNOS expression during rat EAE (Garcion *et al*, 1997, 1998, 2003). The macrophage enzyme iNOS is required for the inducible production of NO by macrophages. The role of NO in EAE and MS is not yet fully clarified, but several studies indicate a worsening effect due to NO production in the brain (Cross *et al*, 1994, 2000; Zhao *et al*, 1996). Others indicate that NO has an immune-downregulating effect in EAE (Ruuls *et al*, 1996; Willenborg *et al*, 1999).

Blood–brain barrier (BBB)

Inflammatory cells can only cause damage in the CNS after they have migrated from the peripheral blood into the CNS parenchyma. This involves passage of these cells across the BBB. A direct effect of 1,25-$(OH)_2$D on the BBB has, to our knowledge, not been described thus far. In 1,25-$(OH)_2$D-treated EAE rats, a reduced number of infiltrated macrophages in the CNS was observed (Nataf *et al*, 1996, Nashold *et al*, 2000), suggesting that 1,25-$(OH)_2$D suppresses the transendothelial migration of monocytes (Nashold *et al*, 2000).

MS and vitamin D supplementation

Only few reports are available on the effect of vitamin D supplementation in MS patients. A group of MS patients ($n=16$) was treated with dietary supplements containing vitamin D 125 μg (5000 IU), calcium (16 mg/kg/day), and magnesium (10 mg/kg/day) (Goldberg *et al*, 1986). The results after 1 y showed that exacerbations were not eliminated, but their number was reduced by 59% compared with the number of the previous year(s). Vitamin D was given in the form of cod liver oil (20 g/day). Apart from vitamin D, cod liver oil may contain vitamin A and the amount of vitamin A in cod liver oil 20 g/day is six times its toxic dose. The limited number of patients in this study and the methodological bias (six out of 16 patients dropped out) do not allow conclusions.

Mahon *et al* (2003) studied the cytokine profile in patients with MS following 6 months supplementation with calcium 800 mg/day and vitamin D 25 μg (1000 IU)/day ($n=17$) or calcium 800 mg/day and placebo ($n=22$). The serum 25OHD levels in the vitamin D treatment group significantly increased from 42.5 ± 15 to 70 ± 20 nmol/l. Vitamin D supplementation also significantly increased serum TGF-β1 levels.

Double-blind randomized placebo-controlled studies on vitamin D supplementation in patients with MS with sufficient power are lacking.

Future prospects: MS and 1,25-$(OH)_2$D treatment

Given its immune-modulatory and anti-inflammatory effects, treatment with 1,25-$(OH)_2$D, or its analogs, may be valuable in the management of MS (Cantorna *et al*, 1996; Hayes *et al*, 1997; Verstuyf *et al*, 1998; Mathieu *et al*, 2001; Mathieu & Adorini, 2002). The calcemic side effects of 1,25-$(OH)_2$D make its use in high doses, needed for immunomodulation, unattractive. 1,25-$(OH)_2$D analogs, which might block MS without affecting the blood calcium level, have been identified and synthesized (DeLuca *et al*, 2000). Until now, only one of these 1,25-$(OH)_2$D analogs, 19-nor-1,25-dihydroxyvitamin D_2 (19-nor), was given in an oral dose for more than 9 months to 11 newly diagnosed MS patients with RRMS. This analog, however, did not reduce the number of active MRI lesions (Flemming *et al*, 2000). More research and clinical trials are needed to assess the usefulness of vitamin D compounds for the treatment of MS.

Conclusion

This review provides some epidemiological and ecological evidence for the preventive role that vitamin D nutrition may play in decreasing susceptibility to MS. The putative preventive effect of adequate supply of vitamin D_3 is supported by results obtained in EAE. In EAE 1,25-$(OH)_2$D prevents the onset when administered before EAE induction and ameliorates the severity and duration of EAE when given after EAE induction (Table 1).

Widespread seasonal variation in serum 25OHD levels has been reported especially in temperate climates, with low 25OHD levels in winter. A vitamin D-deficient diet in mice and rats resulted in an increased susceptibility to EAE, and 1,25-$(OH)_2$D deprivation aggravated the clinical signs of EAE (Cantorna *et al*, 1996; Garcion *et al*, 2003). Likewise, once MS is apparent, low 25OHD levels may aggravate its severity. Living in a temperate climate may cause annually recurring seasonal low serum 25OHD concentrations in MS patients. Low serum 25OHD concentrations may be responsible for upsetting the balance in the neuro-immune system of MS patients, causing reversible and irreversible neuro-immunological damage aggravating RRMS. The cumulative negative effects over the years may contribute to the secondary progressive course of MS. Further studies are required to establish the seasonal fluctuations in serum concentrations of vitamin D metabolites in MS patients. The effects of sunlight on the clinical manifestations of MS may be influenced by the fact that this may not be a direct effect, but indirect. There might be a time lag of 2 months between sunlight and 25OHD and a time lag of 4 months between sunlight and MRI lesions. A 25OHD reference interval may need to be determined to distinguish inadequate from adequate levels. The quantitative relation between vitamin D_3 input and the resulting serum 25OHD concentration needs to be investigated, as it has been speculated that patients with MS may have a higher vitamin D requirement (Goldberg, 1974a; Cantorna *et al*, 1996; Hayes *et al*, 1997; Hayes, 2000; Vieth, 1999; DeLuca & Cantorna, 2001; Holick, 2002; Mahon *et al*, 2003). More research is also needed to address the question if MS might be aggravated by a vitamin D-related metabolic or genetic disorder. It is hypothesized that vitamin D deficiency might only lead to MS in susceptible individuals, and a poor vitamin D status might expose an unknown, possibly gene-related, etiology.

Finally, we need to answer the question: 'Do we need 1,25-$(OH)_2$D analogs for the treatment of MS, as pharmacological doses of 1,25-$(OH)_2$D are accompanied by adverse side effects, or is it simply a matter of enough vitamin D_3 all year round and enough time for it to take effect?'

Until more evidence is provided, it is suggested that MS patients living in temperate climates should have their serum 25OHD concentration checked in winter, January–March in the northern and July–September in the southern hemisphere, respectively, or use a vitamin D_3 supplement and follow the recommendations for vitamin D_3 and calcium published by their National Council on Food and Nutrition. The dietary reference intakes on vitamin D and calcium for the USA and Europe have been published by the FNB, Institute of Medicine in 1997 and by the SCF of the European Commission in 2002, respectively, and have since been updated (FNB, Institute of Medicine, 1997; SCF, 2002; Heaney *et al*, 2003a). Alternatively, the reader is referred to the most recent recommendations for the required daily intake of vitamin D_3 and calcium given for bone loss, osteoporosis, and fractures (Chapuy *et al*, 1992; Lips, 2001). For the moment, it would be wise to aim at a serum 25OHD level >50 nmol/l either by augmenting sunlight exposure or by a vitamin D_3 supplement of 10 μg (400 IU) per day. Such a dose is safe, and side effects are virtually nonexistent (Lips, 2001). Further studies should be done to evaluate if higher levels of 25OHD are necessary in the management of MS to prevent exacerbations. In contrast, the use of the active metabolite 1,25-$(OH)_2$D carries the danger of hypercalcemia, hypercalciuria, and renal failure, and should be restricted to clinical investigational use under close supervision.

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