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REVIEW ARTICLE

Is hypovitaminosis D one of the environmental risk factors for multiple sclerosis?

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The role of hypovitaminosis D as a possible risk factor for multiple sclerosis is reviewed. First, it is emphasized that hypovitaminosis D could be only one of the risk factors for multiple sclerosis and that numerous other environmental and genetic risk factors appear to interact and combine to trigger the disease. Secondly, the classical physiological notions about vitamin D have recently been challenged and the main new findings are summarized. This vitamin could have an important immunological role involving a number of organs and pathologies, including autoimmune diseases and multiple sclerosis. Furthermore, human requirements for this vitamin are much higher than previously thought, and in medium- or high-latitude countries, they might not be met in the majority of the general population due to a lack of sunshine and an increasingly urbanized lifestyle. Thereafter, the different types of studies that have helped to implicate hypovitaminosis D as a risk factor for multiple sclerosis are reviewed. In experimental autoimmune encephalomyelitis, vitamin D has been shown to play a significant immunological role. Diverse epidemiological studies suggest that a direct chain of causality exists in the general population between latitude, exposure to the sun, vitamin D status and the risk of multiple sclerosis. New epidemiological analyses from France support the existence of this chain of links. Recently reported immunological findings in patients with multiple sclerosis have consistently shown that vitamin D significantly influences regulatory T lymphocyte cells, whose role is well known in the pathogenesis of the disease. Lastly, in a number of studies on serum levels of vitamin D in multiple sclerosis, an insufficiency was observed in the great majority of patients, including at the earliest stages of the disease. The questionable specificity and significance of such results is detailed here. Based on a final global analysis of the cumulative significance of these different types of findings, it would appear likely that hypovitaminosis D is one of the risk factors for multiple sclerosis.

Keywords: hypovitaminosis D; multiple sclerosis; vitamin D

Abbreviations: EAE = experimental autoimmune encephalomyelitis; HLA = human leucocyte antigen; UVB = ultraviolet B radiation; 1,25(OH)2D = 1,25-dihydroxyvitamin D2 and D3; 25(OH)D = 25-hydroxyvitamin D

Introduction

Vitamin D and its effects on bone have been known for a long time. However, nowadays we are progressively discovering that major actions of this vitamin involve a number of other organs and pathologies, most likely including multiple sclerosis. The recent considerable increase in publications on vitamin D, involving almost all medical specialities, is without precedent in the history

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of vitamin research. New findings may even prompt changes in medical practice in the very near future, not only in the field of general medical primary prevention but perhaps also in the treatment of some specific pathologies. In multiple sclerosis, although reliable results on a curative effect of vitamin D are still lacking, the notion that hypovitaminosis D may be one of the risk factors has greatly progressed in the last few years (Hayes, 2000; van Amerongen *et al*., 2004; Ascherio and Munger, 2007*b*). After a brief review of the main currently suspected risk factors for multiple sclerosis, we will discuss the different physiological, experimental, epidemiological, immunological and biological arguments that suggest that hypovitaminosis D is one of the risk factors.

Risk factors for multiple sclerosis

Multiple sclerosis is considered to be an autoimmune disease, although its precise pathogenesis remains obscure. It is generally accepted that upstream to the disease different types of risk factors exist, even if we do not know exactly how these lead to the disease itself (Fig. 1). Among the risks, numerous genetic factors have been identified; in particular some susceptibility appears to exist in the histocompatibility complex of the human leucocyte antigen (HLA) (Sawcer and Compston, 2006; Compston and Coles, 2008; Chao *et al*., 2009; International Multiple Sclerosis Genetics Consortium, 2009; Ramagopalan *et al*., 2009*a*). Genetic factors will not be reviewed here, but we shall often refer to their actions, which appear to be of primary importance in influencing the risk of developing multiple sclerosis.

Environmental risk factors are also strongly related to multiple sclerosis (Giovannani and Ebers, 2007; Ebers, 2008; Handel *et al*., 2010) (Fig. 1). The effects of latitude, climate and, most recently, hypovitaminosis D have successively been considered, even if the latter had previously been envisaged a long time ago (Goldberg, 1974). There are also infectious environmental risk factors, principally involving past infections with the Epstein-Barr—or related—virus (Bagert, 2009). Thus, after such infections and a variable but usually quite long latency, an immunological cascade could eventually trigger the disease, with a risk of multiple sclerosis multiplied by 20 or 30 if infectious mononucleosis is clinically expressed during adolescence (Thacker *et al.,* 2006; Ascherio and Munger, 2007*a*, 2008; Zaadstra *et al.,* 2008; Ramagolapan *et al.,* 2009*d*). Furthermore, smoking could be both a premorbid risk factor and a deleterious factor influencing the course of the disease (Hernan *et al.,* 2005; Ascherio and Munger, 2007*a*; Mikaeloff *et al*., 2007; Pittas *et al*., 2009). There may well be other, as yet undiscovered, environmental risk factors.

Moreover, it seems likely that a combination of several different risk factors is needed in order to trigger the disease (Compston and Coles, 2008; Goodin, 2009; Handel *et al*., 2010) (Fig. 1). For example, hypovitaminosis D (Hayes and Donald Acheson, 2008; Holmoy, 2008) or smoking (Simon *et al.,* 2010) may potentiate the immunological stigmata of a past infection with Epstein-Barr virus and an increased susceptibility to the disease may result from the coexistence of some HLA groups with hypovitaminosis D

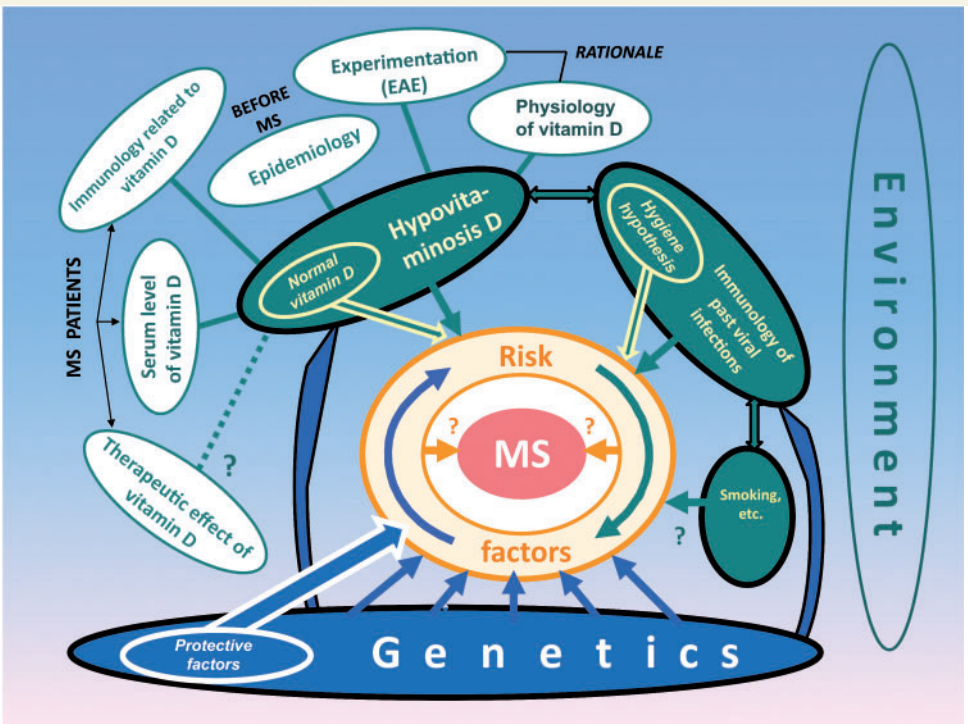

Figure 1 Main risk and protective factors (arrows) for multiple sclerosis and arguments (bars) supporting a role of hypovitaminosis D among the environmental risk factors. Note that the mixing of all risk (and protective) factors is schematized in the orange ring surrounding multiple sclerosis.

(Niino *et al*., 2000; Ramagopalan *et al.,* 2009*c*) or with the effects of a past infection with Epstein-Barr virus (De Jager *et al.,* 2008, 2009; Sundström *et al.,* 2008, 2009). Furthermore, protective genetic and environmental factors (Fig. 1) may counterbalance some of the deleterious effects of risk factors e.g. a climate offering a normal vitamin D status and, in the infectious field, the so-called 'hygiene' hypothesis, in which multiple infections occurring in early childhood (versus later in the life) could have a subsequent protective effect against autoimmune diseases (Ascherio and Munger, 2007*a*, 2008). Lastly, several crucial epochs for risk acquisition from the environment appear to exist (Ebers, 2009; Handel *et al*., 2010; McDowell *et al*., 2010). Environmental risk factors (i) could have affected previous generations, leaving a susceptibility for future generations via the HLA system (Chao *et al*., 2009); (ii) may also be present during pregnancy (see below influence of month of birth); (iii) may be important during childhood and adolescence (see below exposure to sun during this time of life); or (iv) could affect young adults (Munger *et al.*, 2006), including after migrations (Ascherio and Munger, 2007*b*) (see below). Accordingly, generally speaking, it may be suggested that depending on the ethnic group, individual genetics, the familial environmental history, the month of birth, the latitude and climate of the country where a person has lived, the individual lifestyle in that country, infections that occurred in early childhood or during adolescence and other possible environmental factors, including smoking, multiple sclerosis will either start one day or never occur (Fig. 1). Finally, within the realm of all these risk factors, the potential pathogenic role of hypovitaminosis D appears to be relatively limited, possibly accounting for a significant effect at the scale of a population but not for the whole range of individual situations, in which genetics and several other environmental risk factors could interact in a very variable way without always requiring hypovitaminosis D to trigger the disease.

Physiology of vitamin D

Metabolism and general effects of vitamin D

Great advances have recently been made in our knowledge of the physiology of vitamin D (Borradale and Kimlin, 2009; Adams and Hewison, 2010). There are two forms of vitamin D: vitamin D3 (cholecalciferol) i.e. the animal or human vitamin D, and vitamin D2 (ergocalciferol), which is of plant or mushroom origin. Vitamins D2 and D3 are both available in dietary form but only vitamin D3 is synthesized in the skin by ultraviolet B (UVB) radiation from sunlight. Vitamin D and its metabolites are transported in the plasma, bound to the vitamin D binding protein. Vitamin D is transformed in the liver into 25-hydroxyvitamin D [25(OH)D], which is regulated by the supply of synthesized and ingested vitamin D. Under stimulation by parathyroid hormone, this metabolite is transformed in the renal proximal tubule to form 1,25-dihydroxyvitamin D [1,25(OH)2D], which is the active metabolite. 1,25(OH)2D is released into the bloodstream with a half life of several hours, binds to vitamin D receptors in its target tissues and is considered a 'hormone' (Adams and Hewison, 2010). Vitamin D receptors are present not only in the intestine, bone and kidney i.e. the classical target tissues of vitamin D, but also in gonads, breast, pancreas, cardiovascular system, brain (microglia) and circulating immunity cells i.e. macrophages, monocytes and activated lymphocyte T and B cells (Bhalla *et al.,* 1983; Vedman *et al*., 2000; Mathieu and Adorini, 2002; Holick, 2004; Lips, 2006; Chen *et al*., 2007; Holick, 2008*a*, *b*). All these 'non-classical' target tissues are able to transform 25(OH)D into 1,25(OH)2D, which exerts autocrine/paracine effects within these cells and, possibly, neighbouring cells. The physical link between vitamin D and the basic cells of immunity is of particular interest given the potential immunological role of this vitamin in autoimmunity in general and in multiple sclerosis in particular. Furthermore, single nucleotide polymorphisms of the CYP27B1 and the vitamin D receptor genes influence the metabolism and effects of vitamin D (Uitterlinden *et al*., 2004) and the risk of multiple sclerosis (Niino *et al*., 2000; Tajouri *et al.,* 2005; Orton *et al.,* 2008; Smolders *et al*., 2008*a*; Torkildsen *et al.,* 2008; Dickinson *et al.,* 2009; Smolders *et al.,* 2009*a*). Vitamin D binding protein is also genetically influenced, which affects 25(OH)D concentrations (Bouillon *et al*., 1981; Sinotte *et al*., 2009; Ahn *et al*., 2010) and may potentially affect the risk of multiple sclerosis. Finally, a considerable body of literature published during the last 10 years, comprising multiple intervention studies on the effects of vitamin D in bone pathology and numerous association studies on non-classical effects of this vitamin in other organs and pathologies, has revolutionized our knowledge of vitamin D (Holick, 2004, 2007; Vieth, 2007; Cannell *et al.,* 2008; Kimlin, 2008; Borradale and Kimlin, 2009; Bischoff-Ferrari, 2010). The main non-classical effects of vitamin D [via vitamin D receptors and 1,25(OH)2D] appear to be anti-inflammatory, anti-infectious, immunomodulatory, antiproliferative and as a neurotransmitter involving not only many autoimmune diseases—including, among others, multiple sclerosis (see below), type 1 diabetes (Mathieu *et al*., 2005; Forouhi *et al*., 2008; Zipitis and Akobeng, 2008; Danescu *et al*., 2009), rheumatoid arthritis (Merlino *et al*., 2004; Patel *et al*., 2007) and systemic lupus erythematosus (Amital *et al*., 2010)—but also some cancers, in particular colon and breast cancer (Lappe *et al*., 2007; Abbas *et al*., 2008; Chen *et al*., 2009; Yin *et al*., 2009; Jenab *et al*., 2010; Kawase *et al*., 2010), diseases of the cardiovascular system (Dobnig *et al*., 2008; Forman *et al*., 2008; Wang *et al*., 2008), infection (Nnoaham and Clarke, 2008; Ginde *et al*., 2009*b*; Urashima *et al*., 2010; Youssef *et al*., 2010) and other general symptoms such as muscle weakness and falls (Bischoff-Ferrari *et al*., 2004*a*, 2009*a*; Zhu *et al*., 2006; Broe *et al*., 2007; Pfeifer *et al*., 2009).

Optimal serum levels of vitamin D

25(OH)D, with a half life of several weeks, is representative of the overall vitamin D store in the body (D2 + D3) and is, therefore, the serum component that must be measured to evaluate vitamin D status (Heaney, 2000; Zitterman, 2003; Souberbielle *et al.*, 2008; Zerwekh, 2008). There is not yet a standardized 25(OH)D assay, but according to the UK-based Danish External Quality Assessment Scheme (DEQAS), the main methods give roughly

similar mean results, differing by not much more than 7% (Carter *et al*., 2010). Assays measuring both 25(OH)D2 and D3 (Cavalier *et al*., 2008) are recommended. According to review and position papers published by many experts during the last decade, the minimum 25(OH)D serum level required to achieve an optimal vitamin D status would be somewhere between 50 and 100 nmol/l (i.e. 20 and 40 ng/mg), though the minimum level most frequently recommended is ~75–80 nmol/l (Lips, 2001; Zitterman, 2003; Holick, 2004; Dawson-Hughes *et al*., 2005; Hollis, 2005; Vieth, 2005; Biscoff-Ferrari *et al*., 2006, 2009*b*; Roux *et al*., 2008; Souberbielle *et al*., 2008; Adams and Hewison, 2010; Dawson-Hughes *et al*., 2010). This limit is not 'population-based', since this has no real sense in countries with limited sunshine, but has been determined using 'health-based reference values' i.e. from both metabolic and pathological bases regarding various outcomes that can grossly be separated into 'bone/calcium-related' and 'not bone/calcium-related' outcomes. When considering 'bone/calcium-related' outcomes, the threshold of 75 nmol/l corresponds to the serum level below which (i) parathyroid hormone secretion is generally stimulated by the lack of vitamin D (Chapuy *et al*., 1996; Holick, 2007; Durazo-Arvizu *et al*., 2010); (ii) initial signs of mineralization defect are observed (Premiel *et al*., 2010); and (iii) calcium absorption by the gut is not yet optimal (Heaney *et al*., 2003*b*). Other recent original findings suggest that peak bone density in young adults becomes optimal when 25(OH)D is above the level of 90 nmol/l (Bischoff-Ferrari *et al*., 2004*b*) and a recent meta-analysis of 12 placebo-controlled randomized controlled trials concluded that non-vertebral fracture prevention in patients aged 65 and older was optimal in trials with mean 25(OH)D serum levels of 75–110 nmol/l (Bischoff-Ferrari, 2009*c*). Furthermore, due to a progressive decrease in sensitivity to 1,25(OH)2D and also in the capacity of the kidney to hydroxylate 25(OH)D into 1,25(OH)2D, the minimum optimal 25(OH)D level for bone health probably varies with age and should be higher in the elderly than in the young, namely at least above 75 nmol/l in the former (Baraké *et al*., 2010; Dawson-Hughes *et al*., 2010; Whiting and Calvo, 2010). For the 'non-calcium/bone' endpoints, the minimum 25(OH)D target levels are not yet well determined since large randomized controlled trials are still lacking. However, a multitude of epidemiological (association) studies, for example in the cancer and the cardiovascular fields that cannot be reviewed in detail here (see above), suggest a protective effect of vitamin D in people with relatively high 25(OH)D serum levels (usually above 75 or 100 nmol/l) compared to people with low serum levels (usually between 20 and 40 nmol/l) (see Bischoff-Ferrari *et al*., 2009*b*). Accordingly, an absolute consensus does not yet exist on the recommended minimum level of 25(OH)D, since some authors recommend a minimum level of 50 nmol/l (Lips *et al*., 2009), whereas others argue in favour of at least 80 or 100 nmol/l (Zitterman, 2003; Holick, 2004; Hollis, 2005; Bischoff-Ferrari *et al*., 2006; Vieth *et al*., 2007; Niino *et al*., 2008; Bischoff-Ferrari *et al*., 2009*b*, *c*). However, the question of what 25(OH)D serum level should be defined as the minimum needed to achieve an optimal vitamin D status i.e. between 50 and 100 nmol/l depending on the authors, does not radically change the general problem of vitamin D insufficiency, since currently between a third and a half of the 'normal' population in temperate countries do not even reach the threshold of 50 nmol/l (Mithal *et al*., 2009; Adams and Hewison, 2010) (see below, 'Vitamin D status in the general population'). Concerning the upper limit for the reference values of 25(OH)D serum level, it must be mentioned that physiologically, in outdoor workers, the serum level is generally between 75 and 175 nmol/l (rarely exceeding 200 nmol/l) (Haddad and Chyu, 1971; Haddock *et al*., 1982; Barger-Lux and Heaney, 2002), and there is no true risk of vitamin D intoxication up to 375 nmol/l (Hathcock *et al*., 2007; Burton *et al*., 2010); this represents a considerable safety margin in cases of simple vitamin D supplementation, assuming a target serum 25(OH)D level between 75 and 125 nmol/l i.e. around 100 nmol/l on average (Bischoff-Ferrari *et al*., 2009*b*).

Requirements

On the basis of these recent metabolic and pathological findings, the daily requirement of vitamin D has been reassessed and is now thought to be far higher than the 300–400 IU/day that, until a few years ago, was estimated to be sufficient. The daily requirement does of course depend on what the optimal target 25(OH)D serum level is considered to be: for a 25(OH)D serum level of 50 nmol/l, 800 IU/day of vitamin D appears sufficient, but to bring most people above the 75 nmol/l level, a dosage of between 1000 and 4000 IU/day (depending upon the individual, but on average 2000 IU/day) is required (Heaney *et al*., 2003*a*; Grant and Holick, 2005; Hollis, 2005; Bischoff-Ferrari *et al*., 2006, 2009*b*, *c*; Vieth, 2006; Heaney *et al*., 2009; Hall *et al*., 2010; Schwalfenberg *et al*., 2010; Whiting and Calvo, 2010). Vitamin D intake via (unfortified) food is very marginal in normal Western diets, even in those considered well balanced, and generally provides < 100 IU/day. Even diets that include oily fish, as in traditional Scandinavian food (Mark and Carson, 2006; Kampman and Brustad, 2008), or fortified food (Calvo *et al*., 2004; Moore *et al*., 2005; Välimäki *et al*., 2007; O'Donnell *et al*., 2008; Vatanparast *et al*., 2010), rarely exceed a few hundred IU/day and this usually remains markedly below the daily requirement. Sunshine therefore remains the principal natural source of vitamin D, providing 80–90% of the requirement in the absence of fortified food. Although exposing a part of the body (for example the face, trunk and arms) to the sun in summer can provide 10 000 IU of vitamin D in less than half an hour, this supply disappears within a few weeks and cannot readily be replenished throughout the year except in tropical countries (Vieth, 1999; Hollis, 2005; Vieth, 2005; Diffey, 2010). Moreover, elderly and dark-skinned subjects are less able to synthesize vitamin D than young, light-skinned subjects who, if they protect themselves too much from the sun (by clothing or sun-block), may also rapidly find themselves in a state of vitamin D insufficiency (Vieth, 1999; Armas *et al*., 2007; Binkley *et al*., 2007).

Geography and sunshine

Accordingly, a major problem of vitamin D supply exists for many populations, namely those who live beyond the 40th parallels North or South (Holick, 2004, 2008*a*, *b*; van Amerongen *et al*., 2004) (Fig. 2). These geographical parallels mark the line

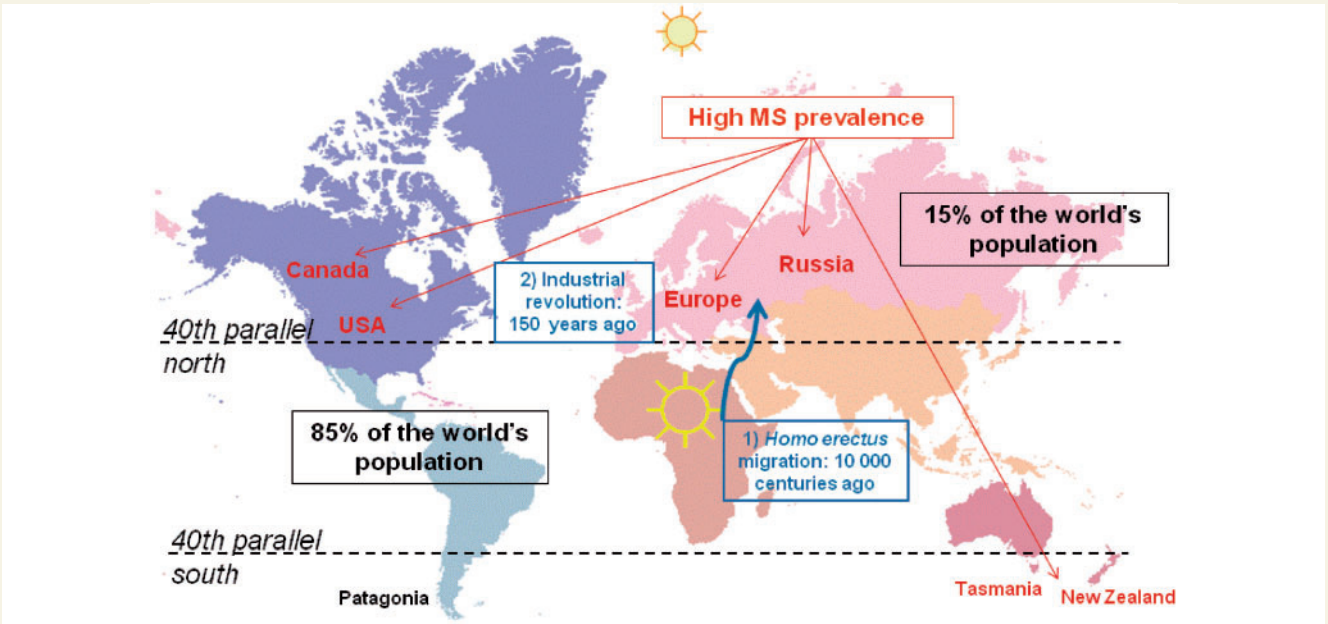

Figure 2 Geographical and historical considerations on vitamin D and multiple sclerosis. MS = multiple sclerosis.

at which the sun at its zenith becomes seasonally so low that for ~4 months of the year UVB levels are insufficient to synthesize vitamin D (Webb *et al*., 1988). At even higher latitudes, periods without a solar source of vitamin D may reach 6–8 months per year. By contrast, at low latitudes, in particular between the tropics, there is no problem with sunshine. However, it should also be taken into account that UVB is only available for a few hours a day, either side of mid-day i.e. the period during which we are currently advised by dermatologists to limit exposure to the sun, of course for excellent dermatological reasons (Diffey, 2010). Be that as it may, relatively limited amounts of sunshine mainly concern Canada, the Northern half of the USA, almost all of Europe (the 40th parallel passing through the middle of Spain), Russia and a few areas in the Southern hemisphere, such as New Zealand, Tasmania and Patagonia i.e. involving only ~15% of the world's population, the remaining 85% live in regions well endowed with sunshine (Fig. 2). It is well known that, except for Patagonia (Melcon *et al*., 2008), which is sparsely populated, the regions with limited amounts of sunshine are also those with the highest prevalence of multiple sclerosis (Goodin, 2009) (Fig. 2), even if other environmental risk factors may also be involved in these countries.

History

A brief look at the history of humanity suggests that two main events may have been important for vitamin D, the first extremely old and the second quite recent. The first event happened ~1 million years ago when *Homo erectus* began to migrate from their birthplace in East Africa to Northern regions of the globe, with a much less sunny climate (Fig. 2). In 10 000 centuries, the *Homo erectus* family and their descendants have had sufficient time to evolve and adapt, in a Darwinian sense, to limited sunshine (Jablonski and Chaplin, 2000, 2010). Evolving into *Homo sapiens*, humanity has undergone many changes, but one of the most visible alterations in Northern people has been the lightening of their skin (Diamond, 2005; Vieth, 2006; Yuen and Jablonski, 2010). Light skins are remarkably effective at synthesizing vitamin D with only small amounts of sunshine, being about five times more efficient in this respect than dark skins. Even so, light skins still have to be exposed to sunshine in order to synthesize vitamin D. This consideration leads to the second historical event of importance relating to vitamin D, i.e. the so-called 'industrial revolution', which happened only a few generations ago. During the second half of the 19th century, many people in what are now developed countries left an essentially rural way of life—in which they were almost constantly exposed to nature, the climate and sunshine—to colonize towns and live and work indoors. The result has probably been a drastic fall in vitamin D levels, without the possibility of any physiological adaptation in such a short-time scale (Vieth, 2006). Devastating epidemics of rickets were observed in the main Northern industrial cities (e.g. London, Paris, New York), in which it is estimated that ~80% of children were to some extent affected at the end of the 19th century (Hess and Hunger, 1921; Holick, 2007). It was not understood until the beginning of the 20th century that rickets was caused by a lack of sunshine and vitamin D itself was not formally identified until the early 1930s.

Nowadays, in countries with limited sunshine, paediatricians usually prescribe a vitamin D supplement for infants to prevent rickets and geriatricians prescribe it for the elderly to reduce the risk of falls, fractures and osteomalacia. However, nothing is usually done for people between these two extremities of life, although such age groups are just as lacking in vitamin D as infants and the elderly, as shown by recent epidemiological studies (see below). Although there are no apparent bone stigmata

suggesting a lack of vitamin D in all these intermediate age groups, a chronic vitamin D insufficiency could have pernicious delayed effects on the development of osteoporosis and a wide range of serious diseases. Therefore, during the past few years, a growing part of the medical community has advocated a systematic supplementation, at least during winter, for the general population living in temperate or Nordic countries (Holick, 2004; Hollis, 2005; Vieth, 2006; Binkley, 2009; Cavalier *et al.*, 2009; Edlich *et al*., 2009; Grant *et al*., 2009; Stechschulte *et al*., 2009; Gillie, 2010; Zittermann *et al*., 2010). To sum up these historical aspects, it took almost a century to understand that rickets observed in infants in Northern industrial countries was due to vitamin D deficiency, and it has now taken almost another century to realize that all age groups in these countries suffer from a lack of vitamin D.

Vitamin D status in the general population

As a probable result of these diverse physiological, geographical and historical considerations, recent epidemiological studies in temperate countries (mainly beyond the 40th parallels) on the adult population (>15–18 years, involving both genders and mostly Caucasian people) have shown that serum levels of 25(OH)D are low, whatever the assays used. For example, in the USA, the mean serum level of 25(OH)D was 74 nmol/l in a large cohort of 15 000 adults (over 18 years) distributed throughout the country and studied between 1988 and 1994, with samples collected all year round (Zadshir *et al.,* 2005). However, in a more recent analogous American cohort of 20 000 adults studied between 2000 and 2004, the mean serum level was 60 nmol/l, which suggests, after accounting for assay differences, a global decrease of ~10 nmol/l in 10 years (Looker *et al.,* 2008; Ginde *et al.,* 2009*a*). This marked and rapid decrease has mainly been attributed to an increase in the degree of urbanization and in body fat. In the UK, the mean serum level of vitamin D was 51 nmol/l (with 41–60 nmol/l from winter to summer) in a cohort of 7437 British adults, who were 45 years old in 2003, with a North–South gradient existing within the results (Hyppönen and Power, 2007). The authors concluded that there was an urgent need for preventive action. Similarly, low mean serum levels of 25(OH)D were recently reported in normal adults in Australia (51–75 nmol/l depending upon the region, skin colour and lifestyle; van der Mei *et al.,* 2007*b*), Canada (67 nmol/l; Langlois *et al*., 2010), New Zealand (mean = 50 nmol/l, with 32 nmol/l in winter and 74 nmol/l in summer; Rockell *et al.,* 2006) and Germany (42 nmol/l in winter and 67 nmol/l in summer; Scharla *et al*., 1996), with, therefore, serum levels usually 20–40 nmol/l lower in winter than in summer in these countries. In France, a study involving 1579 adults in nine different regions during the winter of 1994–95, found a mean serum level of 61 nmol/l and a North–South gradient (Chapuy *et al.,* 1996); with serum levels of 40–50 nmol/l in the North and 80–90 nmol/l in the South (Fig. 4A). Significant correlations existed in this study between the regional serum levels of vitamin D and both latitude ($r = -0.79$; $P < 0.01$) and the annual local amount of sunshine ($r = 0.72$; $P = 0.003$) (Fig. 3, links B–C and A–C).

On a world-wide scale, in a meta-analysis based on 394 studies, a significant correlation existed between 25(OH)D serum levels and latitude in Caucasian subjects (Hagenau *et al.*, 2009). In another meta-analysis, involving Europe and Asia, the factors affecting the 25(OH)D serum levels in adults were (i) age, the synthesis of vitamin D being less efficient in older people; (ii) gender, women generally having lower levels than men; (iii) skin colour, dark skins synthesizing vitamin D less efficiently than light skins; (iv) type of clothing and the extent to which it covers the body; (v) food, whether or not supplemented with vitamin D; and, most importantly, (vi) the degree of urbanization, with nowadays less and less time spent outdoors with exposure to sun (Lips, 2007). In Nordic countries, the serum levels of vitamin D are often lower than those of temperate countries (Välimäki *et al*., 2004; Andersen *et al*., 2005), whereas in tropical regions the serum levels are generally higher (Linhares *et al*., 1984; Chailurkit *et al*., 1996; Ho-Pham *et al*., 2010). However, frequent exceptions may be observed to these main trends due to differences in lifestyle or diet with, for example, the possibility of low serum levels of vitamin D in people of sunny countries, if they avoid the sun or, conversely, relatively high serum levels in people of Northern regions, who may take more advantage of the sun in summer and partly compensate the lack of sunshine by a diet rich in vitamin D in winter (van der Wielen *et al*., 1995; Lips *et al*., 2001; Lips, 2010). Accordingly, in temperate and Nordic countries, 50–90% of the general population (depending on the cut-off <50 or 75 nmol/l) are more or less permanently in a state of vitamin D insufficiency, a situation that cannot be ignored, whatever the cut-off considered.

Experimental results

Role of 1,25(OH)2D

Experimental autoimmune encephalomyelitis (EAE) is the best experimental model of multiple sclerosis. Although experimental findings in EAE may seem to be of debatable relevance when principally discussing a risk factor for a human disease, they do at least contribute to the rationale involving vitamin D in the immunology of a central inflammatory neurological pathology. Based on more than 20 original studies published between the early 1990s and 2010, it emerges that 1,25(OH)2D has both a preventive and a curative effect in EAE (Lemire and Archer, 1991; Cantorna *et al*., 1996; Mehan and DeLuca, 2002), this effect requiring the presence of calcium (Cantorna *et al*., 1999) and possibly existing only in females (if using vitamin D3, probably via a potentiation by oestrogens) (Spach and Hayes, 2005; Nashold *et al*., 2009), with the involvement of various (not mutually exclusive) immunological mechanisms such as an anti-inflammatory effect (Spach *et al*., 2004), actions on macrophages (Nashold *et al*., 2000), on different types of cytokines (Cantorna *et al*., 1998; Spach *et al*., 2006; Pedersen *et al*., 2007) and on regulatory T lymphocyte cells, lymphocytes Th1 and Th2 (Mattner *et al*., 2000; Muthian *et al*., 2006). The latter

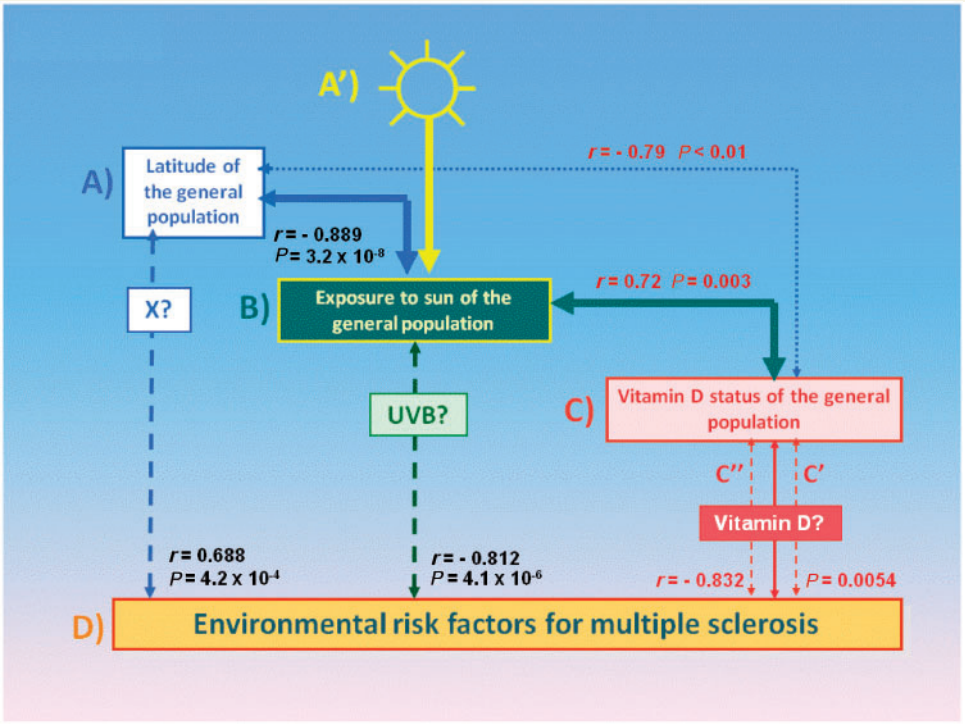

Figure 3 Environmental climatic risk factors for multiple sclerosis and links between them. The *r*- and *P*-values illustrate the example of France and correspond to the Pearson correlation tests reviewed in this article or performed by the authors, based on data for French regions concerning (**A**) mean latitude, (**B**) mean global annual sunshine (Suri *et al*., 2007), (**C**) mean serum level of vitamin D in normal adults (Chapuy *et al*., 1996) and (**D**) multiple sclerosis prevalence in French farmers (Vukusic *et al*., 2007); *r* and *P* in black = data from 22 regions; *r* and *P* in red = data from nine regions. Modified from Pierrot-Deseilligny (2009).

mechanism is favoured by some authors, who suggest that vitamin D positively influences the activity of regulatory T lymphocyte cells, restoring a better ratio between the lymphocytes Th2 (protective) and Th1 (aggressive); the overall effect being a decrease in inflammation (Cantorna, 2006, 2008; Smolders *et al.*, 2008*a*). It should be noted that this mechanism is analogous to the mechanism of interferon-β, used as an immunomodulator in multiple sclerosis therapy, and that a potentiation exists between the beneficial effects of interferon-β and 1,25(OH)2D analogue used together in EAE (Van Etten *et al.*, 2007). However, it may be that the effects of 1,25(OH)2D in EAE result from other mechanisms.

Possible specific effect of UVB, independent of vitamin D

It has recently been reported that UVB itself may also have a beneficial effect in EAE that could be independent of the 25(OH)D serum level and vitamin D mechanism, the authors suggesting that this immunological UVB effect could account for the assumed immunological effect of vitamin D previously reported in EAE as well as in multiple sclerosis (Becklund *et al*., 2010). However, this as yet unique study will need additional confirmation since a transitory significant increase in the 25(OH)D serum level was nevertheless observed in the mice treated with UVB. Furthermore, UVB could have produced 1,25(OH)2D directly in the mouse skin (Lehman *et al*., 2001; Reichrath, 2007), this finally resulting, via the draining lymph nodes and the general immune system, in a general positive immunosuppressive effect (Gorman *et al*., 2007; Loser and Beissert, 2009) whatever the 25(OH)D serum level. Lastly, a possible specific action of UVB does not exclude a parallel immunological effect of 1,25(OH)2D in EAE, an effect that has previously been shown in many different studies in which UVB did not play any role (see above and Niino *et al*., 2008).

Epidemiological findings

Effect of latitude on the risk of multiple sclerosis

The effect of latitude on the risk of multiple sclerosis has long been known and is universally acknowledged, the prevalence of the disease being minimal at the equator and increasing with either North or South latitude (Handel *et al*., 2010) (Fig. 3). This effect is observed on a world scale (Gale and Martyn, 1995; Alonso and Hernan, 2008; Sloka *et al.*, 2009), at a continental level (Kurtzke, 1995; Puggliatti *et al.*, 2006), in large countries, such as the USA (Kurtzke *et al.*, 1985, Kurtzke, 2008), the former Soviet Union (Boiko *et al*., 1995) and Australia (Van der Mei *et al.*, 2001; Taylor *et al*., 2010) and even in comparatively

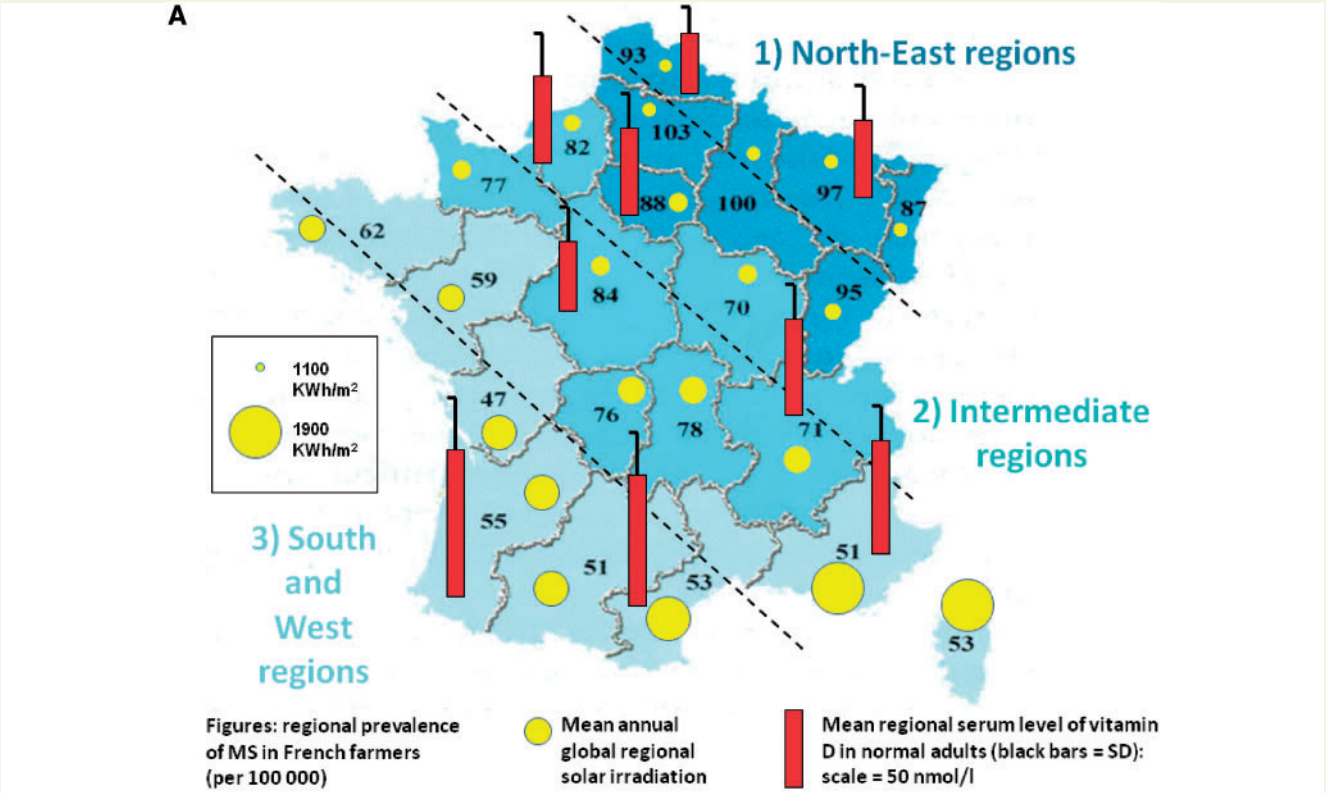

Figure 4 Epidemiological studies on multiple sclerosis prevalence, exposure to sun and serum levels of vitamin D in normal adults in the administrative regions of France. (**A**) Map of France showing the 22 administrative regions, figures for regional multiple sclerosis prevalence (per 100 000 inhabitants) in the farmer population (Vukusic *et al.*, 2007), the average annual amount of global solar irradiation (yellow spots) per region determined from European environmental data (Suri *et al*., 2007) and the average serum vitamin D levels in normal adults (red bars) per region from Chapuy *et al.* (1996). Vukusic *et al.* (2007) divided France into three main zones of regions (various shades of blue) and showed that a significant gradient existed between the North–East, intermediate and South–West zones in terms of regional multiple sclerosis prevalence. (**B**) Correlation performed by the authors of the present article between the regional multiple sclerosis prevalence in French farmers (Vukusic *et al*., 2007) and the average global annual (between 1981 and 1990) solar irradiation in the French regions (Suri *et al*., 2007), expressed in KWh/m^2, using the Pearson test. This correlation is highly significant; note also that the three main zones of regions identified by Vukusic *et al*. (2007) are still relatively distinct in this comparison (ellipses). (**C**) Correlation performed by the authors of the present article between the regional multiple sclerosis prevalence in French farmers (Vukusic *et al.*, 2007) and the average serum levels of vitamin D in normal adults living in nine roughly analogous French regions (Chapuy *et al.*, 1996), using the Pearson test (modified from Pierrot-Deseilligny, 2009); this correlation was also significant and the three main zones of regions (as in **A** and **B**) were still relatively distinct in this comparison (ellipses). MS = multiple sclerosis.

small countries such as New Zealand (Taylor *et al*., 2008) and France (Vukusic *et al.*, 2007). The study by Vukusic *et al.* (2007) involved French farmers, who represent 7% of the French population, and the mean multiple sclerosis prevalence was 65 per 100 000. For the 22 administrative regions of France (Fig. 4A), a geographical gradient of prevalence existed between North–East regions, intermediate and South and West regions ($P < 0.001$). However, the marked obliquity of the main geographical axes of these three zones of regions (Fig. 4A) suggests that this significance was not simply due to the latitude of these regions but also resulted from a more complicated factor, such as the climate (Ebers, 2008, 2009). French farmers represent a 'nearly ideal' (Ebers, 2008) population for the discussion of a possible climatic impact on multiple sclerosis since (i) being mostly Caucasian, they have a degree of ethnic homogeneity; (ii) they usually remain in the same region throughout their lives; (iii) they are evenly distributed throughout the country; and (iv) they spend a large part of their time outdoors, with consequently a marked exposure to the effect of climate. Thus, in this relatively homogeneous population, the only notable variable that might have influenced multiple sclerosis prevalence would appear to be the climate, since there is a very marked sunshine contrast between the North–East and South–West of France (see below). In another recent study also involving multiple sclerosis patients in France, more extensive data were obtained from national health insurance records, representing 82% of the French population and 46 926 patients with multiple sclerosis, with a mean prevalence of 95 per 100 000 (Fromont *et al.*, 2009). In this study, the results were analysed in the 95 administrative divisions of France (called '*départements*'), which are much smaller than the 22 administrative regions referred to above. A difference in prevalence also existed between the North–East and the South–West but, even

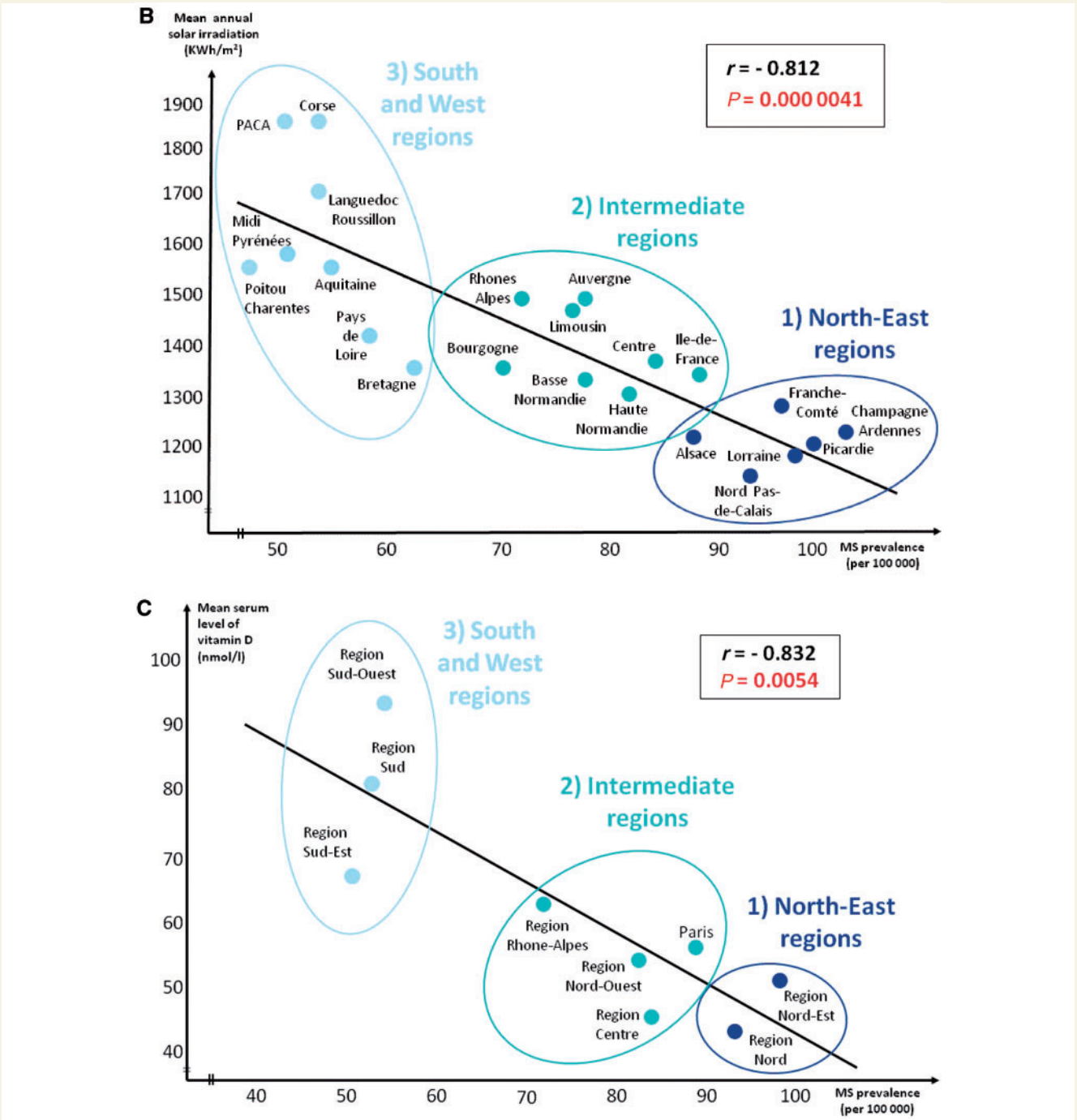

Figure 4 Continued.

though the data were more complete, no geographical gradient was found, in contrast to the farmers' study: this is possibly because the general population is (i) less ethnically homogeneous, (ii) less geographically stable, (iii) more unevenly distributed throughout the country and (iv) much more diversified in terms of lifestyle, with therefore a less marked climatic impact than in farmers. This last factor might also explain the overall lower prevalence of multiple sclerosis in farmers. Lastly, we will not deal here with the well known effects on multiple sclerosis prevalence of migrations that occurred during the first two decades of life from a region of high prevalence to a region of low prevalence, or inversely, since such effects appear to result obligatorily from the action of environmental risk factors, which most likely include climatic factors and/or a role of past infections (Gale and Martyn, 1995; Hammond *et al.*, 2000; Ascherio and Munger, 2007*a*, *b*; Handel *et al*., 2010; McDowell *et al*., 2010); in particular, there appears to be a beneficial climatic effect for young adults who have migrated (after the age of 15–20 years) from a high-latitude

region (of high multiple sclerosis prevalence) to a sunnier, lower-latitude region (of low multiple sclerosis prevalence). In conclusion, latitude globally influences the risk for multiple sclerosis (Fig. 3, link A–D), but other intermediate factors might be involved between latitude and this risk.

Effect of exposure to sun on the risk of multiple sclerosis

The first studies in this field were based on questionnaires, i.e. the amount of time spent outdoors during holidays and weekends during the first two decades of life in patients with multiple sclerosis and control subjects. The risk of multiple sclerosis was significantly lower in those subjects who spent the most time outdoors during their youth (Acheson *et al.*, 1960; van der Mei *et al.*, 2003; Kampman *et al.*, 2007; Dwyer *et al.*, 2008; Sloka *et al.*, 2008), including within pairs of monozygotic twins (Islam *et al.*, 2007). These results are also supported by studies of skin actinic activity, measured on the back of the hand and reflecting total accumulated exposure to sun; the subjects who had the highest level of actinic activity also had the lowest multiple sclerosis risk (van der Mei *et al.*, 2003; Lucas *et al.*, 2008).

In a second type of study, there were very strong correlations between multiple sclerosis prevalence in the different States of the USA or multiple sclerosis prevalence in nine large-scale areas of North America and the corresponding mean annual amounts of UV in these areas (Beretich and Beretich, 2009). In recent preliminary results based on a meta-analysis performed on 52 studies from various countries around the world, a very highly significant link ($P<10^{-8}$) existed between multiple sclerosis prevalence and the annual amount of UV in the different countries, this link being moreover 20 times more significant than that existing between multiple sclerosis prevalence and simple latitude (Sloka *et al.*, 2009). In France, sunshine maps show large climate areas analogous to those of the main zones of multiple sclerosis prevalence identified in farmers by Vukusic *et al.* (2007) (Ebers, 2008, 2009; Handel *et al.*, 2010). This analysis has been extended here, using a method similar to that reported by Beretich and Beretich (2009) i.e. crossing figures for regional multiple sclerosis prevalence in French farmers with those of the average annual global regional solar irradiation determined from climatic maps provided by a European Environmental Institute (Suri *et al.*, 2007) (Fig. 4A); the correlation is highly significant (Pearson test, $r=-0.812$, $P=0.0000041$) (Fig. 4B). Moreover, it should be noted that this correlation between regional multiple sclerosis prevalence and regional sunshine (Fig. 3, link B–D) appears to be more significant than that existing between regional multiple sclerosis prevalence and mean regional latitude (Pearson's test: $r=0.688$, $P=0.00042$) (Fig. 3, link A–D), which is confirmed using a linear regression model ($P<0.004$). Thus, in this example of France as well world-wide (Sloka *et al.*, 2009), the risk of multiple sclerosis appears to be more influenced by sun exposure than simply by latitude. Furthermore, at identical latitudes, the risk of multiple sclerosis is lower in the sunniest regions (van Amerogen *et al.*, 2004; van der Mei *et al.*, 2007*a*, *b*), in particular in high-altitude regions compared to lowland regions (Kurtzke, 1967). These multiple and diverse studies consistently support the hypothesis that exposure to sun influences the risk of multiple sclerosis (Fig. 3, link B–D).

Effect of vitamin D status on the risk of multiple sclerosis

In some studies, oral intake of vitamin D in the form of diverse vitamin supplements (Munger *et al.*, 2004) or oily fish (Kampmann *et al.*, 2007; Kampmann and Brustad, 2008) was found to be linked with a lower risk of multiple sclerosis. However, it cannot be ruled out that associated factors existed in these studies. Of greater significance, since it was based on the serum level of vitamin D itself, was a study performed in young American soldiers who had given at least two serum samples a few years before the onset of any neurological symptoms during their military service (Munger *et al.*, 2006). Those with levels of vitamin D in the highest quintile (i.e. between 99 and 152 nmol/l) had a significantly lower risk of multiple sclerosis than those with the lowest levels of vitamin D (i.e. between 15 and 63 nmol/l) ($P<0.01$).

Crossing the figures for regional multiple sclerosis prevalence in French farmers (Vukusic *et al.*, 2007) with those of the mean serum levels of vitamin D reported by Chapuy *et al.* (1996) in normal adults of nine French regions (Fig. 4A), a significant correlation was found (Pearson's test, $r=-0.832$, $P=0.0054$) (Fig. 4C), which suggests that an indirect link may exist between these two variables involving populations living in analogous regions (Pierrot-Deseilligny, 2009) (Fig. 3, link C–D). However, further epidemiological studies are now required to correlate regional multiple sclerosis prevalence with regional serum levels of vitamin D in patients with multiple sclerosis. Lastly, other epidemiological results may be cited here: the risk of multiple sclerosis is lower for births in autumn (mainly in November) and higher for births in spring (mainly in May) (Templer *et al.*, 1992; Willer *et al.*, 2005; Sotgiu *et al.*, 2006; Bayes *et al.*, 2009; Fernandes de Abreu *et al.*, 2009; Ramagopalan *et al.*, 2009*b*), which is also correlated with the presence of a familial risk factor (Stogiu *et al.*, 2006) or with the phenotype HLA-DRB1 (Ramagopalan *et al.*, 2009*b*). These results may be related to the vitamin D status of pregnant women (Willer *et al.*, 2005; Salzer *et al.*, 2010), since 25(OH)D serum levels are at their highest in autumn and their lowest in spring (Handel *et al.*, 2010). Accordingly, various results suggest that vitamin D status also influences the risk of multiple sclerosis (Fig. 3, link C–D).

Epidemiological synthesis of the climatic risk of multiple sclerosis

It should be noted that among the three links connecting environmental factors to the risk of multiple sclerosis (Fig. 3, vertical links A–D, B–D and C–D), the last one (link C–D) does not yet appear to be as strong as the other two. However, two types of indirect arguments reinforce the likelihood of a link between vitamin D status and the risk of multiple sclerosis. First, there is a very solid connection between latitude and exposure to the sun (Fig. 3, link A–B), which is a geographical reality observed all around

the globe, including in France, between mean regional latitude and mean regional sunshine (Pearson's test: $r = -0.889$, $P = 3.17 \times 10^{-8}$). Furthermore, the connection between exposure to the sun and vitamin D status is also strong (Fig. 3, link B–C), both at the individual level, which is elementary physiology (Armas *et al.*, 2007) and at the population scale (Chapuy *et al.*, 1996). There are also direct correlations between latitude and serum level of vitamin D (i.e. vitamin D status) (Fig. 3, link A–C) in France (Chapuy *et al.*, 1996) and on a world scale (Hagenau *et al.*, 2009). Therefore, the chain of links existing between the three environmental factors (Fig. 3, horizontal chain A–B–C)—i.e. latitude, exposure to sun and vitamin D status—appears to be strong, representing a whole set of arguments converging on the same final factor, namely vitamin D status, which thus indirectly reinforces its subsequent link with the risk of multiple sclerosis (Fig. 3, link C′–D).

The second type of indirect arguments supporting the existence of this last link (Fig. 3, link C–D) stem from the current absence of a consistent, truly documented alternative hypothesis to that of vitamin D. For the first link (Fig. 3, link A–D), latitude is so general a factor as to suggest that another as yet unknown intermediate factor might exist to account for the risk of multiple sclerosis, a factor termed 'X' here (Fig. 3). Several factors such as urbanization, Western lifestyle or viral infections have been proposed as a potential intermediate factor 'X' between latitude and the risk of multiple sclerosis. However, the first two factors are barely dissociable from the lack of sunshine and the third one cannot alone explain all the environmental risk factors (see above the first chapter on the different environmental risk factors and also the summarized epidemiological results of migrations on the risk of multiple sclerosis). Furthermore, given the strength of the link that exists between latitude and exposure to the sun, it seems much more likely that the main factor 'X' is in fact exposure to the sun, as also suggested by the results observed in France (Fig. 3, link A–B) and at the world level (Sloka *et al*., 2009). Concerning another possible but as yet unknown intermediate factor between exposure to the sun and the risk of multiple sclerosis (Fig. 3, link B–D), old and still rather vague hypotheses about general immunological effects of sunshine itself, through UVB (Loser and Beissert, 2009), or even of mere sunlight (via the eyes and vision) (Mehta, 2010) have thus far not succeeded in ruling out an associated role of vitamin D. Be that as it may, the UVB-vitamin D immunological hypothesis and another immunological mechanism potentially resulting only from UVB, for which there are no currently available original human data, are not in fact mutually exclusive, with, therefore, possibly two parallel immunological effects originating from sunshine-UVB action. Moreover, these two immunological effects resulting from sunshine might both involve the active metabolite of vitamin D i.e. 1,25(OH)2D, which (i) for the classical stimulation of vitamin D by UVB, is produced at the end of successive transformations (in the skin, liver and kidney); and (ii) in the case of the specific UVB mechanism, could be elicited directly within the skin (Lehman *et al*., 2001) and initiate another, parallel immunosuppressive mechanism subsequently involving the draining lymph nodes and the general immune system (Gorman *et al*., 2007; Loser and Beissert, 2009 and see the Experimental results section above). It remains to be determined whether this second mechanism depends solely on UVB or may also be influenced by the general vitamin D status. In temperate and Nordic countries, besides the role of sunshine, other confounders may exist such as Western lifestyle or diet but, as mentioned above, the former is barely dissociable from the question of lack of sunshine and the latter does not appear to play a major role in the total vitamin D supply and requirements. Therefore, the absence of a genuine consistent alternative hypothesis to explain the effects of both latitude and exposure to the sun on the risk of multiple sclerosis also indirectly reinforces, at least for the time being, the existence of a link between vitamin D status and this risk (Fig. 3, link C″–D).

Taken together, these multiple results and direct or indirect arguments suggest that a chain of influence may exist between latitude, exposure to the sun, vitamin D status and the risk of multiple sclerosis (Fig. 3, chain A–B–C–D) and that sunlight influences not only the vitamin D status in the general population but probably also, mainly through this intermediate factor, the risk of multiple sclerosis in this population (Fig. 3, chain A′–B–C–D). Vitamin D appears in fact to be the best candidate for the last link in this chain since it is located precisely at the interface between the organism, in which it permanently circulates, and the environment, which obviously influences it.

Immunological aspects

The immunology of multiple sclerosis is complex and only partly known. The effect of vitamin D on the immune response in general could be an enhancement of innate immunity coupled with multifaceted regulation of adaptive immunity (Adorini and Penna, 2008). Macrophages and activated T and B lymphocyte cells contain vitamin D receptors and vitamin D appears to control activation of human T cells (von Essen *et al*., 2010). Furthermore, whereas it is acknowledged that regulatory T lymphocyte cells and cytokines play major roles in autoimmunity (Bettini and Vignali, 2009), 1,25(OH)2D inhibits *in vitro* the production of inflammatory cytokines and promotes the development of regulatory T lymphocyte cells expressing cytotoxic T lymphocyte antigen 4 (CTLA-4) and forkhead box P3 (FoxP3), resulting in an anti-inflammatory effect (Jeffery *et al.,* 2009). This confirms different beneficial immunological effects previously reported using 1,25(OH)2D *in vitro* (Lyakh *et al*., 2005).

Three major independent immunological studies involving vitamin D in patients with multiple sclerosis were published in 2009, with analogous conclusions. In the first study (132 patients with multiple sclerosis and 53 controls), it was suggested that CD4+ T cell proliferation was inhibited by 1,25(OH)2D and that more cells adopted a regulatory T lymphocyte phenotype (Correale *et al*., 2009). The second study ($n = 26$) showed that the number of regulatory T lymphocyte cells was correlated with the serum levels of 25(OH)D and 1,25(OH)2D (Royal *et al*., 2009). In the third study ($n = 29$), there was no correlation between the 25(OH)D serum levels and the number of regulatory lymphocyte cells, but the inhibitory activity *in vitro* of these cells on the (aggressive) Th1 lymphocytes was correlated with the serum level of 25(OH)D, with an additional beneficial effect in interferon-β users

(Smolders *et al.*, 2009*b*), without correlation with 1,25(OH)2D, parathyroid hormone and calcium (Smolders *et al.*, 2010). Moreover, similar results appear to have emerged from two other studies (Sloka *et al.*, 2009; Burton *et al.*, 2010) and tumour growth factor-β1, a cytokine produced by several cell types, including the regulatory T lymphocyte cells, was found to be increased in patients supplemented with 1000 IU/day of vitamin D for 6 months (Mahon *et al.*, 2003). Further studies in patients with multiple sclerosis will probably provide more details on the immunological effects of vitamin D, but the results already reported confirm some experimental findings observed in EAE and show that this vitamin, like interferon-β, influences the number and/or activity of the regulatory T lymphocyte cells. This action could represent a direct intervention in the pathogenesis of the disease and strongly suggests that vitamin D plays an immunmodulatory role in multiple sclerosis.

Serum levels of vitamin D in multiple sclerosis

Significance of low serum levels of vitamin D

The serum levels of vitamin D are reported to be low, whatever the assays, in most patients with a relapsing-remitting form of multiple sclerosis, including at the initial stage of the disease (Soilu-Haninnen *et al.*, 2005; Barnes *et al.*, 2007; van der Mei *et al.*, 2007*a*; Kragt *et al.*, 2008; Smolders *et al.*, 2008*b*; Soilu-Haninnen *et al.*, 2008; Correale *et al.*, 2009; Pierrot-Deseilligny, 2009; Steffensen *et al.*, 2010; Mowry *et al.*, 2010). The mean serum levels were generally between 50 and 65 nmol/l and the levels were lower in winter than in summer, but other particularities were mentioned in some of these studies. Where a control group existed, the serum levels were significantly lower in patients than in controls in some studies (Soilu-Hänninen *et al.*, 2005; van der Mei *et al.*, 2007*b*; Correale *et al.*, 2009), but not in others (Barnes *et al.*, 2007; Kragt *et al.*, 2008; Soilu-Hänninen *et al.*, 2008). Inverse correlations between the 25(OH)D serum levels and the relapse rate or the degree of disability (Expanded Disability Status Scale) were sometimes observed (Barnes *et al.*, 2007; Van der Mei *et al.*, 2007*a*; Smolders *et al.*, 2008*b*; Mowry *et al.*, 2010). It should be noted that in the study by Mowry *et al.* (2010), performed in 110 children (mean age: 15 years; follow-up: 30 months) with a clinically isolated syndrome or paediatric-onset multiple sclerosis, an elevation of 25 nmol/l of the 25(OH)D serum level was associated with a 34% decrease in relapse rate, which suggests a protective role of vitamin D. The 25(OH)D serum levels could also be lower during relapses than between relapses, with higher serum levels of parathyroid hormone during relapses (Soilu-Hänninen *et al.*, 2008). These particularities will, however, require confirmatory studies and the rest of the discussion will focus on the significance of the simple decrease in the 25(OH)D serum level observed in patients with multiple sclerosis. Firstly, this decrease in the 25(OH)D serum level observed in most patients with multiple sclerosis is of crucial importance if one hypothesizes that hypovitaminosis D partly contributes to the risk of multiple sclerosis. Secondly, such a decrease is not constant since it is observed in 60–95% of patients with multiple sclerosis, depending on the study and the cut-off. If one accepts that the 25(OH)D serum level may remain durably normal in a few patients, even in winter, this fact does not in itself preclude a partial global effect of hypovitaminosis D on the risk of multiple sclerosis at the scale of a population. Indeed, the risk factors for multiple sclerosis are numerous (see above and Fig. 1) and it may be that for a few individuals with multiple sclerosis, with for example a highly unfavourable genetic disposition (including for the metabolism and effects of vitamin D) and the presence of other deleterious environmental risk factors (past infections, smoking, etc.), the vitamin D status remains apparently normal and hypovitaminosis D *per se* is not required in order to trigger the disease. Thirdly, hypovitaminosis D may seem banal since it is not specific to multiple sclerosis and may be found, when looked for, in all kinds of pathologies as well as in most sections of the general population. This last point may explain why 25(OH)D serum levels in patients with multiple sclerosis are not very different from those of controls in some studies. However, patients with multiple sclerosis could radically differ from normal subjects in that, in addition to hypovitaminosis D, they have multiple other risk factors that normal subjects do not have (e.g. genetic disposition and past infections), all these factors likely interacting together to trigger the disease (see above and Fig. 1). It should be noted, moreover, that in order to suspect a possible role of hypovitaminosis D in the pathogenesis of a given affection, there must also be a rationale and various studies involving vitamin D upstream in the same pathology, such as those that already exist in multiple sclerosis (see the preceding chapters of this paper and Fig. 1) but not, at least for the time being, in other neurological pathologies. In summary, our vitamin D hypothesis in multiple sclerosis could explain (i) the existence of a few multiple sclerosis cases with apparently normal vitamin D status, other environmental and genetic risk factors then likely being determinant for them; and (ii) the fact that both patients with multiple sclerosis and normal subjects often have a similar low vitamin D status, since all the risk factors—including hypovitaminosis D, other environmental factors and particular genetics—may interact together in patients with multiple sclerosis, but probably not in normal subjects, finally triggering the disease.

Relation to clinical stages

In multiple sclerosis, hypovitaminosis D is observed throughout the course of the disease, including at the moment of the first relapses or even in a clinically isolated syndrome, in which a majority of patients are already in a state of insufficiency (Soilu-Hänninen *et al.*, 2005; Hanwell *et al.*, 2009; Hiremath *et al.*, 2009; Pierrot-Deseilligny, 2009; Mowry *et al.*, 2010; Fig. 5). Moreover, vitamin D status cannot be predicted for a given patient since a severe deficiency may be observed in people who are young, not yet disabled and apparently in good general health. It should also be emphasized that many relatively young ambulatory patients already have both marked osteoporosis and a chronic vitamin D insufficiency (Marrie *et al.*, 2009; Sioka *et al.*, 2009; Steffensen *et al.*, 2010). Therefore, in an optimal preventive perspective, it

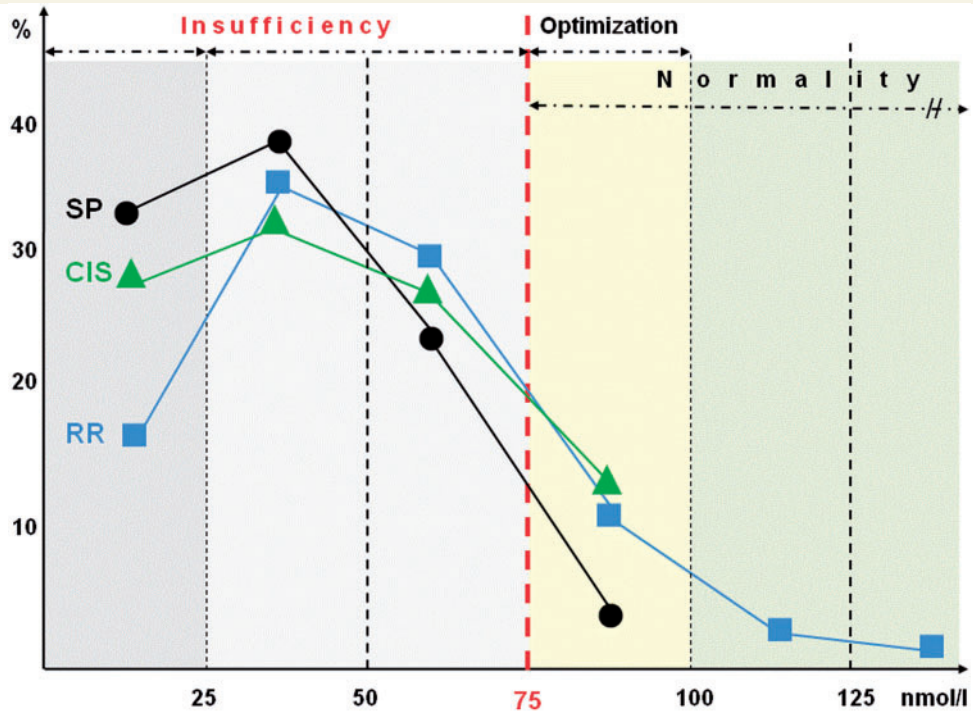

Figure 5 Serum levels of 25(OH)D in a Paris cohort of patients with multiple sclerosis. A total of 325 consecutive outpatients with a relapsing-remitting form (RR, $n = 202$), secondary-progressive form (SP, $n = 91$) of multiple sclerosis or a clinically isolated syndrome (CIS, $n = 32$) were referred to Salpêtrière hospital between 1 June 2008 and 31 May 2009, most of the patients with multiple sclerosis being treated with immunomodulator or immunosuppressive therapies, but none being supplemented with vitamin D at the time of titration: 222 female and 103 male; mean age: 41 years in relapsing-remitting form, 51 years in secondary progressive form and 36 years in clinically isolated syndrome; mean Expanded Disability Status Scale: 3.1 in relapsing-remitting, 5.6 in secondary progressive and 0.9 in clinically isolated syndrome; mean duration of disease: 6.9 years in relapsing-remitting, 14.6 years in secondary progressive and 0.4 year in clinically isolated syndrome; mean serum level of vitamin D: 50 nmol/l in relapsing-remitting, 39 nmol/l in secondary progressive and 45 nmol/l in clinically isolated syndrome. The sex ratio and the proportion of serum samples collected during the two half-years (i.e. June–November and December–May) were analogous in the three groups of patients. The results of serum levels of vitamin D were divided into quantiles of 25 nmol/l and the percentage of patients in each quantile is shown. Note that the international norm for 25(OH)D serum level commonly accepted at present is over 75 nmol/l and that most patients in the three forms of multiple sclerosis were in a state of insufficiency (< 75 nmol/l), very few reaching the currently recommended level of 100 nmol/l.

appears important to determine the vitamin D status of all patients, regardless of their appearance and the stage of the disease. This systematic measurement could also be extended to subjects with a radiologically isolated syndrome, siblings of patients with multiple sclerosis and even young adults who have had late infectious mononucleosis, i.e. categories of subjects in whom the risk of multiple sclerosis is potentially higher than in the general population. However, it is also true that the more advanced the disease, the greater the vitamin D insufficiency. Thus, the serum level of patients with a secondary progressive form of multiple sclerosis is generally very low, around 40 nmol/l (Nieves *et al*., 1994; Ozgocmen *et al*., 2005; Smolders *et al*., 2008*b*; Fig. 5), whereas parathyroid hormone serum levels may be high (Nieves *et al*., 1994). From a pathogenic point of view, three different but associated factors may contribute to worsening an initial hypovitaminosis D in the course of multiple sclerosis: (i) sensitivity to heat (Uthoff symptom), including to sun, which increases symptoms when the external or internal temperature increases, may lead patients to avoid sunshine as early as the beginning of the disease; (ii) a little later in the disease, disability may limit the amount of time patients spend outdoors and consequently their exposure to the sun; and (iii) lastly, with age, the synthesis of vitamin D is less efficient, which also constitutes an aggravating factor. Therefore, in advanced forms of multiple sclerosis, hypovitaminosis D could partly be the consequence of sensitivity to heat (Simmons *et al*., 2004), disability (Van der Mei *et al*., 2007*a*) and age, independently of being one of the possible mechanisms worsening the neurological status. Accordingly, whatever the various factors contributing to the hypovitaminosis D observed in multiple sclerosis, the lack of vitamin D is widespread in this affection and should therefore be systematically detected, with a view to simple supplementation for patients in a state of insufficiency to improve their general health (Myrh, 2009; Pierrot-Deseilligny, 2009). The distinct question of an additional, specifically neurological curative effect of vitamin D in multiple sclerosis will not be resolved until the results of reliable therapeutic trials become available, i.e. in several years time. Be that as it may, it should be noted that this question of a possible neurological curative effect of vitamin D in multiple sclerosis may be only partly linked to that of a role of hypovitaminosis D as a risk factor before the start of the disease.

Conclusions

We have successively reviewed the physiological, experimental, epidemiological, immunological and biological arguments supporting a role of hypovitaminosis D in the risk of multiple sclerosis. The specific contributions of these different fields may be differentiated. The first and last groups of studies—i.e. the general physiological data and the serum level of vitamin D in multiple sclerosis—are both necessary but not sufficient in the discussion on the involvement of hypovitaminosis D in the risk of multiple sclerosis. For the physiological basis, it is for example essential to know that the basic circulating immunity cells contain receptors specific to vitamin D, but this is not sufficient to involve multiple sclerosis itself. Likewise, for the biological basis, the observation that hypovitaminosis D is widespread in patients with multiple sclerosis, in particular even at the earliest stages of the disease, is crucial for its possible involvement as a risk factor, but we have seen that such an insufficiency is not absolutely constant and, in addition, is far from specific to this disease. So, the truly significant results for implicating hypovitaminosis D in the risk of multiple sclerosis i.e. which could be both necessary and sufficient, are those of the other types of studies reviewed above. The experimental results are both necessary and sufficient for mice, but they cannot be extrapolated to humans. The epidemiological results already form a solid whole, but the relative fragility of the last link (vitamin D status and risk of multiple sclerosis), even after reinforcement with indirect arguments, may still appear to be insufficiently convincing. Lastly, the immunological results are consistent but may still be considered too recent and not yet sufficiently detailed. Therefore, although the importance of each of these different steps may still be questioned (Ascherio *et al*., 2010), the fact remains that they all contribute to implicating hypovitaminosis D in the risk of multiple sclerosis and that these different approaches, precisely by the consistency of their implications or conclusions, have already allowed us to reach a global level of evidence that should be considered important. However, further research is needed to confirm the involvement of hypovitaminosis D as a risk factor for multiple sclerosis and to determine whether vitamin D treatment may influence the course of the disease.

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