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Vitamin D Deficiency in the Development and Pathology of Multiple Sclerosis

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Abstract
BACKGROUND: With the strikingly longitudinal distribution of multiple sclerosis prevalence and incidence throughout the planet, it has been suggested that past sun exposure may be implicated in the development of MS. Namely, vitamin D synthesized from sun exposure has been the main focus of this burgeoning area of research. It has been hypothesized that low levels of vitamin D increase the risk of developing multiple sclerosis.

METHODS: A systematic review of the most current literature pertaining to vitamin D and the development and pathology of MS was performed using MEDLINE-Ovid, All EBM Reviews and CINAHL. Search terms utilized were “vitamin D” and “multiple sclerosis” combined with the “and” command. A modified validity score (0-9) was assigned to the articles in order to objectively measure the validity of the overall results of each paper.

RESULTS: Nine studies met inclusion criteria, and were subsequently utilized in the review. Four studies were case-control type, three were cross-sectional studies and two were large cohorts.

CONCLUSION: Based on this systematic review, it appears that there is an inverse relationship between circulating vitamin D levels and risk of developing multiple sclerosis. Moreover, it appears that vitamin D not only acts as a disease determinant, but also a disease modifier, as is evidenced by its correlation with relapses and in its newly discovered specific immunomodulatory properties.

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Vitamin D Deficiency in the Development and Pathology of Multiple Sclerosis

By: Christopher J Newhouse

A Clinical Graduate Project Submitted to the Faculty of the
School of Physician Assistant Studies
Pacific University
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Clinical Graduate Project Coordinators: Rob Rosenow PharmD, OD & Annjanette Sommers MS, PAC
Biography

Chris grew up in the suburbs of Minneapolis and later moved with his family to Brainerd, Minnesota where he went to high school. He spent his time working and playing on the lakes that pepper central Minnesota. After completing high school, Chris attended the University of Wisconsin-Madison where he majored in Biology, worked as an EMT on a private ambulance service and co-founded the school’s first, and only, pre-physician assistant student organization. After graduating in 2005, he moved to Minneapolis and took a job as an E.R. technician at an area hospital. After spending a year in the emergency department, he accepted a full time position in the O.R. at Abbott Northwestern Hospital in Minneapolis. It was during this year that Chris began applying for PA school, and was accepted at Pacific University of Oregon. He will soon be traveling cross-country yet again to begin his job at St. Luke’s Medical Center Emergency Department, the busiest E.R. in Milwaukee, WI.
Abstract

BACKGROUND: With the strikingly longitudinal distribution of multiple sclerosis prevalence and incidence throughout the planet, it has been suggested that past sun exposure may be implicated in the development of MS. Namely, vitamin D synthesized from sun exposure has been the main focus of this burgeoning area of research. It has been hypothesized that low levels of vitamin D increase the risk of developing multiple sclerosis.

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KEYWORDS: vitamin D; multiple sclerosis
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To my fellow classmates: Thank you for becoming not only my new friends, but an extended family to a fellow who was far from home, family and friends.

To Oregon: Thank you for being the best place I’ve had the fortune of living. I’ll be back.

To My Family: Thank you for your endless encouragement and belief in me even when I didn’t believe in myself.
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List of Abbreviations

1,25 (OH)₂ vitamin D: .................................................................Calcitriol
25 (OH) vitamin D: .................................................................Calcidiol
EAE: .............................................................Experimental Autoimmune Encephalomyelitis
EDSS: .................................................................Expanded Disability Status Scale
IDO: ...............................................................Indoleamine 2,3, Dioxygenase
MRI: .................................................................Magnetic Resonance Imaging
MS: ..............................................................Multiple Sclerosis
NHS: .................................................................Nurses’ Health Study
PBMC: ............................................................Peripheral Blood Mononuclear Cells
PPMS: ............................................................Primary Progressive Multiple Sclerosis
PTH: ...............................................................Parathyroid Hormone
RRMS: ...........................................................Relapsing-Remitting Multiple Sclerosis
SPMS: ............................................................Secondary Progressive Multiple Sclerosis
TSH: ...............................................................Thyroid Stimulating Hormone
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Appendix A: Sample of the MVS scoring criteria
Introduction

Multiple sclerosis (MS) is a fairly common disorder which is classified as a demyelinating disease of the central nervous system. It affects women more frequently than men, with a male to female ratio of nearly 1:4. In total, MS affects around 350,000 United States citizens and an estimated 2.5 million people worldwide. The age of onset ranges from 20 – 40, with a peak near 30. It is classified as either relapsing-remitting, primary progressive, secondary progressive or primary-relapsing. Relapsing-remitting is by far the most common (affecting 85% of the MS population) and, as the name implies, it is the mildest form which is characterized by periods of exacerbation and remission.

Frustrating both clinicians and patients alike, is the fact that the pathogenesis of MS is still not completely understood. Moreover, recent research has uncovered evidence that seems to suggest that each subtype may be a result of a slightly different, and distinct, disease process.\(^3\) This fact only serves to further complicate the issue of understanding the pathology and subsequent treatment of MS. What is known, however, is that multiple sclerosis seems to result from a number of different factors, including genetic susceptibility, history of Epstein-Barr virus (EBV) infection, environmental factors\(^1\) and ultimately a continued dysfunctional immune response. This being said, MS can be generally viewed as an autoimmune condition whereby T cells attack specific myelin bands in the central nervous system.\(^9\) Multiple plaques may be seen on an MRI of the brain, where the white matter is being attacked by CD4+ T cells, and a host of monocytes and macrophages.
Despite the possible differences in etiologies, each subtype can produce a multitude of similar symptoms that can be highly debilitating to the patient. Some of the most common include generalized weakness, blurred vision, muscle spasticity, paresthesias in a non-dermatomal pattern, impaired coordination, ataxia, bladder dysfunction, bowel dysfunction and sensitivity to heat. These symptoms, among others, may come and go (as in relapsing-remitting MS), or begin and persist throughout the disease course (as in primary progressive MS).

What is very striking is that the incidence of multiple sclerosis at the equator is nearly zero, while the incidence in the United States, collectively, is around 1:1000. In fact, it is not only the U.S. that suffers such a high incidence of this central nervous system disorder, but all countries throughout the world at latitudes roughly equal to the U.S. More specifically, there appears to be a gradual increase in incidence and prevalence of all types of MS as the north and south poles are approached from the equator. This pattern is quite consistent throughout the entire planet; with increasing latitude comes increasing risk of developing MS. With this observation in place, it has been asserted that perhaps reduced exposure to sunlight is an important environmental factor in the development of multiple sclerosis.

With sunlight being implicated in the development of multiple sclerosis, naturally Vitamin D follows. This is because the vast majority of vitamin D is not acquired through dietary means, but synthesized by the skin secondary to ultraviolet radiation from the sun. In fact, full body exposure to the peak summer sun for 10-15 minutes will synthesize and release about 20,000 units of vitamin D3, or about 50 times the recommended daily dose. Specifically, it is the UVB rays that cause the skin to generate
Vitamin D₃ from 7-dehydrocholesterol.¹⁷ There exists an ample amount of 7-dehydrocholesterol in the stratum basale layer of the hypodermis, where it is endogenously produced. It is this stockpile of vitamin D₃ precursor, that is readily converted to pre-vitamin D₃ when exposed to sunlight. Pre-vitamin D₃ is then converted to vitamin D₃ (cholecalciferol), which is the form most often taken in dietary supplements. It is this form that is then converted, by the liver, to the first of two principle metabolites found in the human body; 25(OH) vitamin D (calcidiol). It is calcidiol that is often measured to assess for vitamin D deficiency, despite the fact that it is not regarded as the biologically active form of vitamin D. The product of calcidiol hydroxylation (via 1α-hydroxylase) by the kidneys is the second principle metabolite, and the biologically active form of vitamin D; 1,25(OH)₂ vitamin D (calcitriol).¹³

Vitamin D is most widely known as a regulator of calcium absorption and subsequent utilization by facilitating the absorption of dietary calcium, and is therefore linked to osteoporosis and osteopenia. In recent years it has been proven to also have immunomodulatory properties, some of which are just being discovered.²⁻³⁻⁷ Vitamin D deficiency has been linked to many disease processes including MS. What is still unknown is exactly how vitamin D is involved in the pathogenesis of these diseases, if at all, for, simple correlation does not necessarily equal causation.

It is the aim of this study to investigate the correlation between vitamin D deficiency and multiple sclerosis. Furthermore, an effort will be made to uncover any causality which may exist between the lack of vitamin D and the development and severity of MS. In order to accomplish this, and to address the hypothesis, focus will be placed on the most recent, pertinent, literature available.
It is the hypothesis of this paper that there exists a significant inverse relationship between vitamin D levels and the risk of developing MS. Moreover, the active role played by vitamin D in immune system modulation possesses significance in multiple sclerosis pathology.

The implications of this possible link between vitamin D and multiple sclerosis could be very important. With such a high proportion of the world’s population being affected, no definitive cure and only mildly effective symptomatic treatments, it would be a monumental advancement if a link were to be discovered and later developed into a new form of prevention and even treatment. If it were as simple as high dose vitamin D replacement, it would be supremely cost effective and available to nearly every MS patient worldwide. It could decrease the current MS burden markedly, and possibly eliminate multiple sclerosis in future generations.

**Methods**

In order to gather all of the most pertinent articles relating to the chosen topic, an exhaustive literature search was undertaken making use of MEDLINE-Ovid, CINAHL, and All EBM Reviews. The search terms utilized were, “Vitamin D” and “Multiple Sclerosis.” These terms were searched separately as keywords, then combined to form a single search using the “and” command. The literature search for articles was limited to the English language, human populations and must have been published between 2004 and 2009. Furthermore, systematic reviews were excluded along with meta-analyses.
After narrowing the search as described, articles were further investigated and excluded if they did not directly address the interaction between vitamin D and the development and pathology of multiple sclerosis.

There appeared to be two distinct types of articles remaining after further narrowing the search in this manner. First were articles which focused on the risk of developing MS as associated with vitamin D deficiency, and examined the possible pathological explanation, and second were those assessing the use of vitamin D as a treatment in patients with established MS. While the latter are of great interest and relevance to MS research, they addressed a slightly different topic with a much different PICO (population, intervention, comparison and outcome) than the former, and were subsequently excluded.

The remaining articles were then critically appraised and assessed as to their validity and reliability in relation to the clinical question. The Jadad score, which is a commonly used set of criteria to assess the validity of certain types of articles, could not be applied to the remaining population of works, as they were not randomized controlled trials. It became necessary to create a new validity grading scale, to more formally assess the group of articles accrued. This modified validity scale (MVS) can be viewed in appendix A. Each article was given a numeric score according to this set of criteria. This score, ranging from 0-9, would then represent how much weight should be given to the article in supporting the hypothesis.
Results

In all, nine studies met inclusion criteria. Furthermore, all nine were homogeneous enough to allow for comparison between them where appropriate. Four of the studies were case-control type, two were large cohorts and three were cross-sectional type studies. In compliance with inclusion criteria, the studies were limited to the last five years, and all focused on vitamin D deficiency as it relates to the risk of developing multiple sclerosis. Some went on to investigate the possible immunological link between vitamin D and the pathological processes of MS. Collectively, the studies demonstrated an increased risk for the development of MS with deficient levels of vitamin D. Those which investigated the role of vitamin D in the immunological response with regard to MS, showed a significant correlation between vitamin D and clinical disease activity, and one even touched on specific cellular interactions within the immune system.

The case-control study, *Immunomodulatory effects of Vitamin D in multiple sclerosis* (2009), investigated not only the relationship between vitamin D levels and multiple sclerosis, but went on to explore the specific immunomodulatory effects of vitamin D on the human body. The investigators began by selecting 132 Hispanic patients with diagnosed MS and subdividing them into three separate groups: relapsing-remitting multiple sclerosis (RRMS) in remission, relapsing-remitting multiple sclerosis (RRMS) in acute exacerbation, and primary progressive multiple sclerosis (PPMS). They then selected 60 healthy control subjects from the same geographical location (Buenos Aires, latitude 34.5°, longitude 58°) and matched them to experimental groups with respect to race, age, place of residence and gender. The control group was also
subdivided into two separate groups which differed only in age. This was done to better match for age between the relapsing-remitting and the primary progressive groups. As is the case in the general MS population, patients in this study who suffered from PPMS were significantly older than those with RRMS.

The researchers began by measuring $25(\text{OH})$ vitamin D and $1,25(\text{OH})_2$ vitamin D levels in each of the participants. They found that both $25(\text{OH})$ vitamin D and $1,25(\text{OH})_2$ vitamin D levels were significantly lower in relapsing-remitting subjects during both remission and acute exacerbation when compared to the control groups. Moreover, they found significantly lower levels of both vitamin D metabolites in patients during exacerbation than when in remission. However, they found no significant difference when comparing vitamin D levels of primary progressive MS patients with healthy controls. Overall, these results indicated to the researchers that both forms of vitamin D are significantly lower in RRMS, particularly during an acute exacerbation. They also provide some evidence toward the growing hypothesis that PPMS and RRMS are of two distinct pathologies, being that vitamin D levels in PPMS did not differ from healthy controls.

The authors then cultured peripheral blood mononuclear cells (PBMC) taken from both control and experimental groups. They grew out CD4+ T cells \textit{ex vivo}, as well as dendritic cells to mimic the myelinated cells which are attacked in MS. They were able to then introduce $1,25(\text{OH})_2$ vitamin D to the cultured cells and compare the results to controls using $25,26(\text{OH})$ vitamin D and $24,25(\text{OH})$ vitamin D. They found that CD 4+ cells and MBP-peptide specific T cell proliferation was inhibited by $1,25(\text{OH})_2$ vitamin D, but not by the two vitamin D controls. The percentage of inhibition was found to be
dependent on the concentration; higher concentrations equaled greater inhibition.

Furthermore, this inhibition was consistent between MS and control groups.

The scientists were also able to investigate T cell conversion of 25(OH) vitamin D to 1,25(OH)\(_2\) vitamin D in the periphery. It has recently been shown that the hydroxylation of calcidiol not only occurs in the kidneys, but also in different tissues throughout the body, via 1\(\alpha\)-hydroxylase. The authors studied this by looking at expression of 1\(\alpha\)-hydroxylase mRNA by CD4\(^+\) T cells, which were found to constitutively express this mRNA while in a resting state, and were also significantly up-regulated when activated. They also found that the addition of [\(^3\)H]-25(OH) vitamin D was converted to [\(^3\)H]-1,25(OH)\(_2\) vitamin D with a significant rise in 1\(\alpha\)-hydroxylase activity (8-10 fold after 24 hours of incubation, and 25-33 fold after 48 hours).

Collectively, this demonstrated that CD4\(^+\) T cells were capable of metabolizing calcidiol to calcitriol, and as a result, would inhibit CD4\(^+\) T cell proliferation due to the increased levels of calcitriol.

The authors went on to investigate the effect of vitamin D on T cell function. To accomplish this, they looked at the products of T cells; cytokines. They looked at IFN-\(\gamma\), IL-4, IL-6, IL-10 and IL-17 to assess T cell function in the absence and presence of calcitriol in both control, and MS cell lines. What they found was that the calcitriol led to decreased numbers of IL-6 and IL-17 producing T cells, and an increase in IL-10 producing T cells in both control and MS groups. This particular experiment confirmed the fact that IL-10 is a positive autocrine factor that acts directly on the T cells and enhances the action of calcitriol, thereby creating a type of positive feedback loop. It was also demonstrated that there was a down regulation in IL-17 and IL-6, which is of great
importance because IL-6 has been shown to be a critical factor in Th17 production, which is thought to be a important T helper cell, responsible for tissue injury in autoimmune disease.

To further understand the immunological role of vitamin D, the team examined IDO (indoleamine 2,3, dioxygenase) mediated tryptophan catabolism in relation to CD4+CD25+ FoxP3+ T cells. What they found was that both CD4+ T cells and dendritic cells of MS patients and controls displayed IDO mRNA presence, and that these levels were significantly increased after exposure to 1,25(OH)2 vitamin D. Furthermore, the calcitriol significantly increased CD4+CD25+FoxP3+ regulatory T cell percentages.

Overall, this study found those with RRMS to have significantly lower vitamin D levels than matched controls. Moreover, levels were lower during exacerbation of RRMS. They also found that in PPMS, vitamin D levels were the same as matched controls. They went on to find a body of evidence suggesting that vitamin D, specifically the 1,25(OH)2 vitamin D metabolite, is an integral part of the immunomodulatory system.

In 25-Hydroxyvitamin D levels in serum at the onset of multiple sclerosis (2005), the authors evaluated vitamin D levels in newly diagnosed MS patients, and compared them to healthy controls. The controls were not matched for age, sex or race. Serum vitamin D levels were drawn in all patients in the same manner and processed using the same equipment. It was found was that there was no difference between controls and MS patients with regards to vitamin D levels, except in the summer months, when there was a statistically significant difference. MS patients suffered from vitamin D deficiency in the summer, while controls did not. Furthermore, the scientists noted a significant difference in vitamin D levels between MS patients whose blood was drawn during a period of
remission versus those who were experiencing a relapse. Patients in relapse at the time of vitamin D assessment were found to be significantly lower in circulating vitamin D than the MS patients who were in remission.

In *Serum 25-Hydroxyvitamin D Levels and Risk of Multiple Sclerosis* (2006), the investigators made use of a prospective, nested, case-control study. It was their sole aim to assess whether or not 25(OH) vitamin D levels were associated with the risk of developing MS. They made use of a population of 7 million U.S. military personnel who had serum samples stored with the Department of Defense Serum Repository. They identified 257 MS cases diagnosed between 1992 and 2004 which were then included in the study. Each MS case was assigned two controls which were matched by age, sex, race and dates of blood collection. The results of their analysis showed an overall significant trend of increasing risk for MS with decreasing 25(OH) vitamin D levels for Caucasians. In fact, with each 50-nmol increase in serum vitamin D levels, there was a 41% risk reduction. However, this was found to be race specific, among African-Americans and Hispanics, there was not a significant trend.

*Assessment of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D3 concentrations in male and female multiple sclerosis patients and control volunteers* (2007) also investigated the possible link between the two major vitamin D metabolites and multiple sclerosis. Specifically, this group sought to uncover whether or not 1,25(OH)_{2} vitamin D was correlated with MS development and its clinical activity. In this cross-sectional study, 29 MS patients were compared with 22 age and sex matched, healthy control subjects. Serum samples were drawn and tested for both vitamin D metabolites, as well as parathyroid hormone (PTH). They found no significant
differences between MS patients and controls with regard to vitamin D levels and PTH. They did note a significantly higher vitamin D level in women as a whole when compared to men as a whole, despite finding no significant difference between MS women and control women. This led them to conclude that there may be different vitamin D requirements as it pertains to sex.

In *Vitamin D levels in people with multiple sclerosis and community controls in Tasmania, Australia* (2005), the researchers recruited MS patients from Tasmania, as well as controls. In total, there were 136 MS patients, and 272 controls matched for age and sex in this cross-sectional, case-control study. Not only did they measure vitamin D via serum samples, but they also measured approximate sun exposure time using a “time in sun” survey questionnaire. They also matched this subjective information with objective signs of sun exposure by obtaining silicon casts of subjects’ hands and inspecting for actinic damage. Furthermore, they utilized a spectrophotometer on the upper inner arm and buttock to assess skin phenotype, and measured the average intake of vitamin D through dietary means using a specific questionnaire geared towards vitamin D.

In combining these factors, they were able to show that disability level in MS was strongly associated with lower 25(OH) vitamin D levels as well as reduced sun exposure. Cases with a higher disability score (EDSS) were more likely to have vitamin D insufficiency than matched controls.

The authors of *Higher levels of 25-hydroxyvitamin D are associated with a lower incidence of multiple sclerosis only in women* sought to study the role of vitamin D metabolism in MS. They utilized two blood samples (summer and winter) from 103 MS
patients and 110 healthy control subjects to assess calcidiol and calcitriol levels. They found that both the control group and the experimental group had significantly higher levels of both vitamin D metabolites in the summer months versus the winter months. They also found that there seemed to be a link between MS and 25(OH) vitamin D levels, but only in women. They calculated a reduction in MS risk of 19% for every 10-nmol/L rise in serum 25(OH) vitamin D levels. Furthermore, they found a significant correlation between winter serum vitamin D levels and summer EDSS scores. This suggested to the researchers that the lower serum vitamin D levels in the winter months caused the higher disability scores (EDSS) in the summer. They attributed the inverse relationship of EDSS score and vitamin D levels to lag time in vitamin D uptake, metabolism and effect on the immune system. However, this relationship was found only in women, which led the researchers to conclude that the protective effect of 25(OH) vitamin D was exclusive to women.

In a large prospective cohort study of female nurses, *Vitamin D intake and incidence of multiple sclerosis* (2004), the designers made use of a very large number of women to investigate the link between vitamin D and MS. In fact, two cohorts were used to select study subjects. They were the Nurses’ Health Study, comprised of 92,253 women, and the Nurses’ Health Study II, made up of 95,310 women. The original NHS followed women from 1980 to 2000, and the NHS II followed the participants from 1991 to 2001. In total, 173 cases of MS were diagnosed during the study timeframe, which were then used for comparison to healthy controls. The study designers not only measured vitamin D intake from diet, but a multitude of other nutrients, as well as other possibly relevant pieces of information such as smoking history and latitude of
birthplace. Of course, in addition to the above, serum vitamin D levels were measured. With all the information in place, they were able to construct quintiles in regards to total vitamin D intake. What they found was that the total vitamin D intake, at baseline, was inversely correlated with risk of MS. In comparing the lowest quintile to the highest quintile, there was a risk reduction of 0.67 in regards to developing multiple sclerosis, resulting in a finding that women who used supplemental vitamin D (which came largely from multi-vitamins) had a 40% lower risk of developing MS than women who did not.

M Soili-Hanninen and his colleagues investigated the role of vitamin D in MS in the paper, *A longitudinal study of serum 25-hydroxyvitamin D and intact parathyroid hormone levels indicate the importance of vitamin D and calcium homeostasis regulation in multiple sclerosis* (2008). In it, the correlation between vitamin D metabolism and MS activity was studied. In order to accomplish this, they measured 25(OH) vitamin D, PTH, calcium, phosphate, magnesium, chloride, alkaline phosphatase, albumin and TSH in 23 patients with MS, and 23 healthy controls. These samples were obtained via venipuncture at three month intervals for one year, and at times of clinically defined relapse in the patients with multiple sclerosis. To further assess multiple sclerosis progression, and the possible correlation between it and vitamin D levels, the group also observed the demyelinating lesions secondary to MS, by utilizing MRI of the brain during periods of relapse.

What they discovered was that vitamin D deficiency was common in both groups, (affecting approximately half of the controls and MS patients sometime during the year) but that 25(OH) vitamin D serum levels were significantly lower, and PTH levels significantly higher, in the MS group during acute exacerbations when compared to
periods of remission. They found that in each case of relapse, the PTH was greater than 20 ng/L, and may have actually been a more reliable marker for MS relapse than 25(OH) vitamin D. With the correlation between low calcidiol and increased MS clinical activity in place, as well as no appreciable difference between controls and MS groups with regard to overall vitamin D levels, the authors suggested that vitamin D may actually be functioning as a disease modifier in multiple sclerosis patients, rather than just a disease determinant in the general population. They go on to admit that this relationship has one of two explanations: either higher levels of circulating vitamin D reduce the risk of MS exacerbation, or multiple sclerosis relapse reduces serum vitamin D levels. However, the team goes on to state that they found a trend, which did not reach significance, (possibly secondary to the small population of participants) showing a correlation between increasing 25(OH) vitamin D levels and lower incidence of relapse, as well as less EDSS progression. This trend, though not statistically significant, reinforces their hypothesis that higher calcidiol levels reduce the risk of MS relapse, rather than MS relapse depleting vitamin D levels. They felt able to reach this conclusion despite the fact that there seemed to be no correlation between vitamin D levels and MRI parameters for relapse.

In the cross-sectional study, Association of vitamin D metabolite levels with relapse rate and disability in multiple sclerosis (2008), J Smolders and his fellow investigators aimed to explore the possible link between low circulating serum levels of calcitriol and calcidiol and increased disability and severity of MS. The researchers recruited 267 patients with confirmed multiple sclerosis, further identifying them as relapsing-remitting, primary progressive, secondary progressive or unknown. Each was
enrolled from the University Hospital outpatient clinic from 2005-2007 and lived in the southern area of the Netherlands (latitude 50-51°). The study did not make use of matched, healthy controls, but instead compared each MS subgroup to each other, as well as to assigned “normal” vitamin D levels. The investigators measured both 25(OH) vitamin D and 1,25(OH)\textsubscript{2} vitamin D levels a single time in each study volunteer. They also monitored MS clinical activity (number of relapses and overall disability levels) using the EDSS scoring criteria, and then calculated a one-year relapse rate for each subject.

Overall, the results of the study showed that circulating levels of both vitamin D metabolites were significantly lower in the progressive forms of MS when compared to RRMS. Furthermore, they found no significant association between vitamin D metabolites and relapse rate. However, when comparing RRMS patients who suffered relapses to those who did not, the relative risk of remaining relapse free in the previous two years increased by 51% for each 10 nmol/L increase of 25(OH) vitamin D. They also noted that both vitamin D metabolites were lower in patients with one or more relapses than those without any relapses. What also surfaced was the fact that high levels of both calcidiol and calcitriol were associated with a high chance of remaining relapse free. As a consequence, it was concluded that the raw 25(OH) vitamin D levels correlated negatively with EDSS scores in the entire study population.
When taken together, the evidence from each of the above articles is striking. It suggests that vitamin D plays an integral role in multiple sclerosis development and pathology. With the exception of one article, \textit{(Assessment of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D3 concentrations in male and female multiple sclerosis patients and control volunteers)} all of the research demonstrated a significant link between vitamin D and either the development, pathology or both, of multiple sclerosis. Not only does it seem to be “protective,” (or preventative) as has been asserted previously,\footnote{2} but it seems to have an active, and helpful, role in the dysfunctional immunologic response of multiple sclerosis.\footnote{3}

Each of the articles (except the Barnes study) outlined the benefit of higher circulating levels of vitamin D, and many highlighted a link between relapse and lower levels of vitamin D, thereby reinforcing the presumed correlation between vitamin D and the pathological process of MS. \textit{Immunomodulatory effects of Vitamin D in multiple sclerosis} (2009) supplied detailed evidence to further support a link between vitamin D and MS. The authors of this paper went into depth in exploring not simply the correlation, but the specific immunological and biochemical reactions that are a direct result of the presence of vitamin D. They were then able to hypothesize why vitamin D levels were correlated to relapses in, and the pathogenesis of MS.

In a series of experiments, Correale and his colleagues demonstrated that 1,25 (OH)$_2$ vitamin D exerts its immunomodulatory effects on the immune system. By adding vitamin D to T cells from participants, they were able to demonstrate the inhibition of
CD4+ T cells, as well as local conversion of 25 (OH) vitamin D into 1,25 (OH)2 vitamin D by the T cells themselves. They were also able to show that production of certain cytokines was significantly altered by addition of calcitriol. IL-10 was increased, which solidified its role as a positive autocrine factor; a regulator of the immune system. IL-6 and IL-17 production was significantly decreased in response to calcitriol. This is of great importance because IL-6 is a critical factor in the production of Th17, which is an important cell type in the autoimmune response, specifically, one responsible for tissue destruction. If IL-6 is down-regulated by 1,25(OH)2 vitamin D, then Th17 should also be down-regulated as a consequence. In turn, this could result in a decrease in tissue damage, which may manifest as a decrease in MS relapse rate and disability.

The team went on to investigate the activity of calcitriol on tryptophan metabolism in CD4+CD25+ FoxP3+ T cells. It has been previously shown that the IDO induced tryptophan catabolism suppresses T cell responses, and promotes tolerance in autoimmune, pregnancy and allergic mediated inflammation.\textsuperscript{11,16} Simply stated, it dampens the immune response, which could be potentially important in multiple sclerosis treatment. By demonstrating that mRNA of IDO was significantly increased after addition of calcitriol, the researchers compiled further evidence that vitamin D is an important immunomodulator. It directly, and significantly, increased the presence of IDO mRNA in CD4+CD25+ FoxP3+ T cells, which have been shown to be vital in the inhibition of autoimmune diseases and graft rejection (specifically CD4+ and CD25+ T cells).

With the exception of the 2007 M.S. Barnes study, all the articles supported the hypothesis presented by this paper. The discordant results of the Barnes study may be
due to a number of factors. First, the study made use of a small sample size (29 MS patients and 22 controls), which decreases the power of the article and makes attainment of statistical significance between values very difficult. Furthermore, the design itself may have led to its divergence from the other articles. The cross-sectional study design only incorporated a single vitamin D level for each study participant. Moreover, the sample was taken in late February through early March, which according to a previous study, is a point in time where there may be no notable difference between controls and MS patients in regards to vitamin D levels. This same study demonstrated that, while vitamin D status did not differ in the winter months, it was significantly different in the summer months. Furthermore, it found statistically significant differences in vitamin D levels when comparing MS patients in remission with those experiencing a relapse. The Barnes article did not assess either of these two variables, which may have led to the eventual incongruent conclusion.

There was no incongruence, however, between articles that investigated vitamin D levels at times of relapse or remission. Each study found that, during an acute exacerbation of RRMS, there were significantly lower levels of vitamin D. The 2008 M Soilu-Hanninen paper went so far as to try to prove, qualitatively, that it was not the disease activity causing the vitamin D stores to be low, but rather the low levels of vitamin D causing the relapse. They concluded that, in fact, that this was the case. It was in this indirect fashion that the authors were able to provide more evidence that vitamin D is an active immunological molecule that functions in the MS disease process. Not only might the vitamin D metabolites, specifically 1,25(OH)₂ vitamin D, be an important
immunomodulator in the primary prevention of MS, but also in the prevention of relapse and progression as well.

Another inconsistency worth noting in the articles reviewed can be found within the 2009 article by JJ Kragt. They found that higher levels of 25(OH) vitamin D were associated with a lower risk of MS, but only in women. This is not in agreement with the remainder of the articles reviewed. The explanation might be found in the design of the study, particularly in the control group. First, the selection of the control group was far from random, “Patients were asked to bring a healthy control with them when possible, preferably their partner because this would result in roughly matching for age and simultaneous enrollment of patients and controls.” When a control was not included in the study by the MS patient referral, a hospital employee volunteer was taken. This method of control group recruitment is far from random, and therefore negatively impacted the validity of the study. Furthermore, it has been shown that it is necessary to match controls for age, place of residence and especially sex, (as the rest of the studies reviewed did) for these factors will change the vitamin D levels in normal, healthy adults. This study did not match for sex, in fact, in its ideal design, each MS patient would bring in their partner, which would be of the opposite sex nearly 100% of the time. Moreover, the effect of this poorly selected control group can be seen in their Table 1, where it shows a statistically significant difference between the control and MS groups with regards to sex (p value = 0.001) at the onset of the study. This alone could account for their results which were inconsistent with those of the other articles reviewed. However, this anomaly was not the only statistically significant difference between controls and MS patients at the beginning of the study. There was also a significant difference in regards
to the use of supplements containing vitamin D, and a significant difference with regards to birthplace. Both of these factors are well known determinants for both vitamin D status\textsuperscript{5,18} and risk of MS.

When taken together, the faulty design by which controls were recruited, as well as the resultant significant differences between the control group and the multiple sclerosis group detract from the validity of the study’s results. If the control and MS groups had not been significantly different, especially in regards to sex, one would be able to accept the results with more confidence. But as it stands, one must be highly skeptical of the conclusion drawn by the authors of this article, especially in light of the other literature which contradicts it.

The limitations of this systematic review are those placed upon it by the review articles themselves. The quality and quantity of the papers which make up a review article are of the utmost importance, and in this case, were of an acceptable quality. With minor exceptions, each article was performed in such a way that allowed for a respectable validity score as shown in table II, which consequently adds to the validity and reliability of this review article. However, what must be taken into account is that this MVS has not been analyzed to assess its own validity in rating research articles as the Jadad score has.

While there was enough high quality, focused literature related to this paper’s hypothesis, there cannot be a definitive conclusion drawn as it relates to vitamin D in the treatment of MS. Though it seems that vitamin D is implicated in the pathology, and pathogenesis of MS, the papers reviewed in this article did not address the issue of vitamin D as a treatment for multiple sclerosis. Therefore, conclusions drawn from this paper must be limited to the role of vitamin D in the pathogenesis and immunological
response in multiple sclerosis. There are a small number of articles in the literature which focus on the treatment of MS using vitamin D, though they did not meet the inclusion criteria.

An animal study performed at the UW-Madison using EAE, (an animal model for MS) displayed the most powerful evidence yet for vitamin D. Specifically, 1,25(OH)2 vitamin D was shown to prevent the EAE from causing paralytic symptoms in mice when given before inoculation with the disease. Furthermore, given after infection with EAE, 1,25(OH)2 vitamin D was able to slow and stop the progression of paralysis when compared to controls. What must be taken into account, of course, is the fact that this was an animal study, and may not translate similarly in a human model. In humans, the studies using vitamin D as a treatment have only, thus far, focused on its general safety and efficacy in raising vitamin D levels, but have failed to place any focus on vitamin D’s effect on multiple sclerosis disease progression.

Conclusion

Despite the small, but growing, number of articles available that evaluate the efficacy and safety of vitamin D treatment for multiple sclerosis, there is no current human study published that demonstrates the efficacy of vitamin D treatment for MS in reducing or preventing relapses or disease progression. The possible ramifications of research of this type could be drastic. There is a fair amount of literature currently available (much of it within the last decade) that addresses the very question posed by
this paper. It first must be understood what role, if any, vitamin D plays in the pathology of multiple sclerosis before it can be used as a treatment.

The medical community, as of late, has recently begun to acknowledge the importance of vitamin D deficiency for a variety of reasons. A continually growing number of clinicians are measuring vitamin D levels, as it is the most common vitamin deficiency in the United States. With this rise in awareness it seems reasonable to assume that more research will continue to surface with respect to treatment of multiple sclerosis with vitamin D.


Table I: Pertinent Data

<table>
<thead>
<tr>
<th>Author &amp; Year</th>
<th>Population</th>
<th>Outcome</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.A.F. van der Mei et al.  (2007)</td>
<td>Patients &lt;60 with diagnosed MS</td>
<td>Increasing EDSS was inversely associated with vitamin D levels</td>
<td>Unaffected, in the same area, matched for sex and age</td>
</tr>
<tr>
<td>M Soilu-Hanninen et al. (2008)</td>
<td>Patients diagnosed by both clinical and MRI findings.</td>
<td>There is an inverse relationship between 25(OH) D and MS clinical activity (relapse vs. remission)</td>
<td>Unaffected people living in the same area</td>
</tr>
<tr>
<td>Jorge Correale et al. (2009)</td>
<td>Patients with RRMS and PPMS</td>
<td>MS population had lower 1,25 D and 25 D than controls; levels were lower during relapse than during remission; 1,25(OH)2 D acted as an immunomodulator</td>
<td>Unaffected age and sex matched people from the same geographical area</td>
</tr>
<tr>
<td>K.L Munger et al. (2004)</td>
<td>cohort of female nurses who were MS free at the beginning of the study.</td>
<td>Those women with higher Vitamin D levels had about a 40% less chance in developing MS.</td>
<td>The unaffected in the cohort served as controls</td>
</tr>
<tr>
<td>Kragt JJ et al. (2009)</td>
<td>Affected MS patients and unmatched controls</td>
<td>Inverse relationship between 25 vitamin D and MS, only in women, not men</td>
<td>Unaffected volunteer</td>
</tr>
<tr>
<td>J Smolders et al. (2008)</td>
<td>MS patients with RRMS SPMS and PPMS</td>
<td>Serum 25(OH) D levels were associated, inversely, with both relapse rate and disability in MS patients</td>
<td>Comparison between MS groups</td>
</tr>
<tr>
<td>Munger Kassandra L et al. (2006)</td>
<td>cohort of military personnel, MS free at time of enrollment</td>
<td>Inverse relationship between vitamin D levels and MS development</td>
<td>Unaffected personnel in the database</td>
</tr>
<tr>
<td>Barnes MS et al. (2007)</td>
<td>29 patients with diagnosed MS</td>
<td>They found that in women with MS, vitamin D levels were higher.</td>
<td>Unaffected age and sex matched</td>
</tr>
<tr>
<td>Soilu-Hanninen M et al (2005)</td>
<td>40 MS patients at the time of diagnosis</td>
<td>Differences in D only in summer; difference between RRMS patients in relapse vs. remission</td>
<td>26 non-MS pts.</td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------------</td>
<td>-------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Sample size of at least 100?</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Well defined inclusion criteria for MS?</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Acceptable control subjects? (matched for age, sex, residence, race)</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Subjects similar at the beginning of the study? If not, accounted for in somehow?</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Outside forms of vitamin D accounted for?</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Researchers blinded to control vs MS patients?</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Reliable, repeatable measure of vitamin D?</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Controls selected in a randomized fashion?</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>At least two vitamin D levels drawn?</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>5</td>
<td>5</td>
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</table>
Appendix A: Sample of MVS criteria

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Allowable Points</th>
<th>Points Given</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size of at least 100 subjects?</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Well defined inclusion criteria for multiple sclerosis?</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Were there acceptable control subjects? (appropriately matched for age, sex, race and geographical site)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Were the subjects similar at the beginning of the study? If not, was this accounted for in some way?</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Were outside forms of vitamin D level interference cited and accounted for? (dietary Vitamin D, time spent in sun, etc.)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Were researchers blinded to controls versus MS patients?</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Reliable and repeatable measure of Vitamin D?</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Were controls initially selected in a randomized fashion?</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>At least two vitamin D levels drawn?</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>