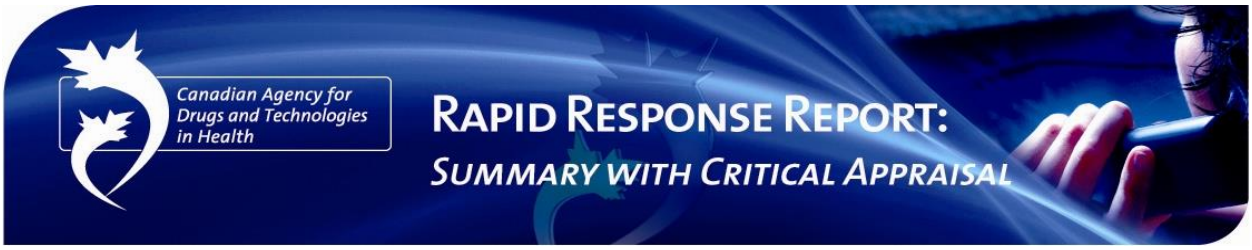



**TITLE: Vitamin D for the Treatment or Prevention of Multiple Sclerosis: A Review of the Clinical Effectiveness**

**DATE:** 10 March 2016

## CONTEXT AND POLICY ISSUES

Multiple sclerosis (MS) is a chronic, relapsing, inflammatory condition of the central nervous system (CNS). It is characterized by destruction of myelin and subsequent deposits of scar tissue, and results in debilitating physical and cognitive deficits, as well as a substantial burden on quality of life. Although treatment advances have led to improved longevity, overall MS-attributed mortality rates have not changed over time.[1] Further, recent surveillance indicates that the prevalence of MS is increasing, particularly in women.[2,3] It is the most common demyelinating condition of the CNS affecting an estimated 2.5 million people worldwide,[4-6] and an estimated 100 thousand in Canada.[7] The prevalence of MS in Canada is one of the highest rates in the world, and nine times higher that of the global average.[4,8-10] This is reflected in substantial direct and indirect healthcare costs,[11] with lifetime costs to MS sufferers in Canada likely exceeding 1.5 million dollars.[12,13]

Diagnosis of MS is commonly made using McDonald criteria, which aims to determine the presence of demyelinating lesions.[14] Alternative criteria are available, and the combination of clinical assessment, neurological examination (including nerve conduction studies), medical imaging, and spinal fluid analysis may be implemented.[15] Disease presentation varies substantially depending on the MS phenotype (i.e., relapsing-remitting [RRMS], primary progressive, secondary progressive, progressive-relapsing, clinically isolated syndrome) in terms of symptoms, pace, and progression.[16] The most common phenotype is RRMS, which is associated with alternating bouts of relapse and remission. Progressive MS is characterized by consistently worsening disability. Disability is often quantified using the Kurtzke Expanded Disability Status Scale (EDSS), which is a standardized measure that considers neurological and functional aspects of the disease.[17] In Canada, several disease-modifying agents are currently approved for use in MS including interferon (IFN)-β.[11] Treatment effectiveness is difficult to ascertain in MS due to fluctuations in symptoms and frequent relapse and remission periods. Treatments aim to maximize recovery from relapses, prevent fatigue and infection, and postpone bedridden stages of disease as no proven treatments exist for changing the course of

MS. Physical therapy may address functional disabilities, and pharmaceuticals may address spasticity and immunological symptoms.

The precise cause of MS remains unclear. Minor familial tendencies, geographic susceptibility, and viral infections (e.g., Epstein-Barr) have been highlighted as potential triggers.[18] Multiple sclerosis is more common in young adults, women, smokers, individuals who have had Epstein Barr virus, obese individuals, and individuals who live farther from the equator.[3,19-21] Latitude is inherently tied to sun exposure, and consequently, vitamin D status has also been speculated as a potential determinant of MS risk as well as a potential therapeutic option.[22,23]

Vitamin D is an essential fat-soluble vitamin obtained through exposure to sunlight as well as dietary sources such as animal protein, fish liver oil, and fortified dairy and cereal products. Adults are recommended to consume 600 international units (IU) of vitamin D per day to maintain adequate vitamin D status, which is defined as serum 25 hydroxyvitamin D (25(OH)D) concentrations greater than 75 nmol/L.[24] Deficiency is accepted to occur below 30 nmol/L, though there is considerable debate about these cut-offs.[24] Vitamin D deficiency manifests as osteomalacia in adults, which may lead to osteoporosis, falls and fractures. It has also been associated with an increased risk of certain cancers, autoimmune diseases, cardiovascular disease, and infectious diseases.[25] Conversely, excessive intake can lead to hypervitaminosis D; elevated serum calcium and phosphorus and the calcification of soft tissues.[26] Thus, an upper limit of 4000 IU is set for adults, despite persistent uncertainty surrounding long-term impacts of high consumption.[27] Vitamin D affects gene transcription through interaction with the vitamin D receptor (VDR) on cell membranes. Most of these genes are related to mineral metabolism, reflecting vitamin D's role in bone mineral homeostasis, but it also performs other functions including roles in cell differentiation, proliferation, and growth. Specific to the immune system, vitamin D assumes paracrine hormone functions that support the maintenance of immunity, and reduce inflammation. The exact mechanism of vitamin D's potential therapeutic role in MS remains unclear, but hypotheses suggest it may be tied to the purported immunologic benefits, and reduced breakdown of nervous system tissue.[28]

Patients with MS have been shown to have lower 25(OH)D levels than healthy controls and vitamin D adequacy has been associated with reduced risk of developing MS and a reduced risk of relapse.[29-31] There is evidence that vitamin D supplementation raises 25(OH)D levels in patients with MS,[32-34] but it is unclear whether this leads to any direct clinical benefits. Questions remain regarding whether vitamin D supplementation can help prevent MS, and also whether it has a therapeutic role in modifying disease activity

This report will review evidence investigating the uncertainty surrounding the clinical effectiveness of vitamin D supplementation for both the prevention and treatment of MS in adults.

## RESEARCH QUESTIONS

1. What is the clinical effectiveness of vitamin D supplementation for the prevention of multiple sclerosis?

2. What is the clinical effectiveness of high versus low dose vitamin D supplementation for the prevention of multiple sclerosis?

3. What is the clinical effectiveness of vitamin D supplementation for the treatment of multiple sclerosis?

4. What is the clinical effectiveness of high versus low dose vitamin D supplementation for the treatment of multiple sclerosis?

## KEY FINDINGS

Four systematic reviews, eight randomized controlled trials, and three non-randomized studies were identified regarding the clinical effectiveness of vitamin D supplementation for the prevention or treatment of multiple sclerosis. Due to substantial heterogeneity between studies, the evidence for most clinical outcomes was limited and often conflicting. Very limited evidence suggests a potential benefit of vitamin D supplementation for the prevention of MS, but this needs to be verified by future studies. Results of treatment of MS with vitamin D were inconsistent, with most evidence suggesting no effect on disability scores, and relapse rates. There were both positive and negative results for immunologic factors, imaging studies, and functional outcomes. Safety data suggests that high dose vitamin D is well tolerated and associated with minimal risk.

## METHODS

### Literature Search Methods

A limited literature search was conducted on key resources including PubMed, The Cochrane Library, University of York Centre for Reviews and Dissemination (CRD) databases, Canadian and major international health technology agencies, as well as a focused Internet search. No filters were applied to limit the retrieval by study type. Where possible, retrieval was limited to the human population. The search was also limited to English language documents published between January 1, 2011 and February 9, 2016.

Rapid Response reports are organized so that the evidence for each research question is presented separately.

### Selection Criteria and Methods

One reviewer screened citations and selected studies. In the first level of screening, titles and abstracts were reviewed and potentially relevant articles were retrieved and assessed for inclusion. The final selection of full-text articles was based on the inclusion criteria presented in Table 1.

| Table 1: Selection Criteria | |
|---|---|
| **Population** | Q1 and 2: Adults at high risk of developing multiple sclerosis<br>Q2 and 4: Adults with multiple sclerosis |
| **Intervention** | Q1 and 3: Vitamin D2 or D3 supplementation (any dose) with or without other MS therapy (e.g., interferon β)<br>Q2 and 4: Vitamin D2 or D3 supplementation > 1000 IU[a] per day with or without other MS therapy |
| **Comparator** | Q1 and 3: No vitamin D supplementation with or without other MS therapy<br>Q2 and 4: Vitamin D2 or D3 supplementation ≤ 1000 IU per day with or without other MS therapy |
| **Outcomes** | Clinical benefits including delayed disease progression, relapse rates, interleukin-17 levels, systemic inflammation, reduction of symptoms and long-term disability, improved quality of life;<br>Harms (e.g., hypervitaminosis D, hypercalcemia) |
| **Study Designs** | Health technology assessments, systematic reviews, meta-analyses, randomized controlled trials, non-randomized studies |

[a]1000 IU was designated as the cut-off for high versus low dose supplements based on what was observed in the literature for studies that reported on high versus low dose supplementation[35-38]

### Exclusion Criteria

Articles were excluded if they did not meet the selection criteria outlined in Table 1, they were duplicate publications, or were published prior to 2011. Health Technology Assessment reports, systematic reviews (SRs), and meta-analyses were excluded if there was incomplete reporting of methods or if they were superseded by more recent and/or rigorous review or an update. Randomized controlled trials (RCTs) and non-randomized studies (NRSs) were excluded if they were described within an included SR.

### Critical Appraisal of Individual Studies

Key methodological aspects specific to each study design were appraised. The included SRs were critically appraised using 'A Measurement Tool to Assess Systematic Reviews' (AMSTAR) criteria.[39] The methods used when conducting the literature search, study selection, quality assessment, data extraction, and for summarizing the data were assessed. Primary clinical studies (RCTs and NRSs) were critically appraised using the Downs and Black checklist.[40] reporting quality, external validity, internal validity in terms of bias and confounding, and power were assessed. Summary scores were not calculated for the included studies; rather, a review of the strengths and limitations of each included study were described narratively.

## SUMMARY OF EVIDENCE

### Quantity of Research Available

A total of 579 citations were identified in the literature search. Following screening of titles and abstracts, 545 citations were excluded and 34 potentially relevant reports from the electronic search were retrieved for full-text review. No potentially relevant publications were retrieved from the grey literature search. Of these potentially relevant articles, 19 publications were excluded. Two SRs were excluded, one due to an inappropriate population,[41] and one due to an inappropriate intervention.[42] Eight RCTs were excluded due to inclusion in a SR included in this

report.[34,35,43-48] One secondary analysis of an RCT was excluded due to irrelevant outcomes.[49] Two reports were excluded as they were trial protocols,[50,51] and six NRSs were excluded — three due to no comparator,[52-54] one due to an inappropriate comparator,[55] one due to no intervention,[56] and one due to an inappropriate population.[57] After exclusion, 15 publications[36,37,58-70] met the inclusion criteria and were included in this report. Appendix 1 describes the PRISMA flowchart of the study selection.

Additional references of potential interest, including on-going clinical trials, are provided in Appendix 5.

## Summary of Study Characteristics

Detailed study characteristics are presented by study type in Appendix 2.

Four SRs[58-61] (one with meta-analysis[60]), eight RCTs[36,37,49,62-67] (including one reported in two separate publications[36,49] and one secondary analysis of an RCT included in several SRs[62]), and three NRSs[68-70] were identified regarding the clinical effectiveness of vitamin D supplementation for MS.

*Overlap Amongst Primary Studies in Systematic Reviews*

There was some overlap among the studies included in the four SRs.[58-61] Seven studies were common to at least two SRs. The rest were unique to a single SR. Discrepancies occurred due to the search timeframes and types of studies included. Two SRs included only RCTs[60,61] and two included both RCTs and NRSs.[58,59]

**Table 2. Overlap Amongst Primary Studies Included in Systematic Reviews[a]**

| Primary Study First Author, Publication Year | Systematic Review First Author, Publication Year | | | |
|---|---|---|---|---|
| | Autier, 2014[58] | Ganesh, 2013[59] | James, 2013[60] | Pozuelo-Moyano, 2013[61] |
| Amezcua 2012 | | • | | |
| Burton 2010 | • | | • | |
| Demirkaya 2009 | | • | | |
| Disanto 2011 | | • | | |
| Embry 2000 | | • | | |
| Gelfand 2011 | | • | | |
| Grau-Lopez 2012 | | • | | |
| Holmoy 2009 | | • | | |
| **Norwegian Vitamin D Study**<br>Kampman 2012[44]<br>Steffensen 2011[34] | • | | • | • |
| Kimball, 2011[45] | • | | | |
| Langer-Gould 2011 | | • | | |
| Loken-Amsrud 2012 | | • | | |
| Mosayebi 2011[46] | • | | | • |
| Mowry 2010 | | • | | |
| Mowry 2012 | | • | | |
| Munger 2006 | • | | | |
| Neau 2011 | | • | | |
| Pakdaman 2012 | | • | | |

| Table 2. Overlap Amongst Primary Studies Included in Systematic Reviews[a] | | | | |
|---|---|---|---|---|
| | Systematic Review First Author, Publication Year | | | |
| Primary Study First Author, Publication Year | Autier, 2014[58] | Ganesh, 2013[59] | James, 2013[60] | Pozuelo-Moyano, 2013[61] |
| Rose 2012 | | • | | |
| Runia 2012 | • | • | | |
| Scott 2012 | | • | | |
| Shahbeigi 2012 | | • | | |
| Shayganeedjad 2012[47] | | | • | • |
| Simpson 2010 | | • | | |
| Smolders 2008 | | • | | |
| Soilu 2005 | | • | | |
| Soilu-Hanninen 2008 | | • | | |
| **Finnish Vitamin D Study**<br>Soilu-Hanninen 2012[48]<br>Åivo 2012[43] | • | | • | • |
| Stein 2011[35] | | | • | • |
| Stewart 2012 | | • | | |
| Tostmann 2010 | • | | | |
| van der Mei 2007 | | • | | |
| Vogelzangs 2012 | • | | | |
| Weinstock Guttman 2011 | | • | | |
| Wium-Andersen, 2013 | • | | | |

[a]Not all listed studies meet the inclusion criteria of this report as some systematic reviews had more broad inclusion criteria; how ever, all studies listed focus on multiple sclerosis patients

*Prevention of Multiple Sclerosis*

Two primary studies[64,68] investigated the effectiveness of vitamin D supplementation for the prevention of MS.

*Study Design*

The RCT was double blind and placebo controlled.[64] The NRS was a retrospective case-control study.[68]

*Country of Origin*

The RCT was conducted in Iran[64] and the NRS was conducted in Norway.[68]

*Patient Population*

The RCT was conducted in optic neuritis patients deemed at risk of developing MS.[64] The NRS included patients who were diagnosed with MS for less than 10 years based on McDonald criteria, and age and sex matched controls randomly selected from a population-based registry (36% participation rate).[68] Both studies were conducted in adults.

*Interventions*

The RCT assessed vitamin D3 at a dose of 50 000 IU per week for 12 months,[64] whereas the NRS assessed varying supplemental doses of vitamin D as determined through self-reported cod liver oil consumption during childhood, adolescence and adulthood.[68]

*Comparators*

Both studies compared vitamin D supplementation to no vitamin D supplementation,[68] or placebo.[64]

*Outcomes*

The RCT[64] investigated the effect of supplementation on conversion to MS and changes in magnetic resonance imaging studies investigating various structural lesions.[64] The NRS[68] investigated the effect of supplementation on the risk of developing MS.

*Treatment of Multiple Sclerosis*

*Study Design*

The majority of studies reviewed in this report focused on treatment of patients already diagnosed with MS. Four systematic reviews,[44,58-60] seven RCTs,[36,37,49,62,63,65-67] and two NRSs[69,70] were identified regarding the clinical effectiveness of vitamin D supplementation for the treatment of MS. The RCTs were primarily double-blind, with the exception of one open-label study.[65] The non-randomized studies included a cross-sectional survey,[69] and a controlled before and after study.[70]

*Country of Origin*

The SRs were conducted by study authors based in France,[58] Canada,[59] the United Kingdom,[60] and Spain.[61] The RCTs were conducted in Israel,[36,49] the United States,[37] Norway,[66] Finland,[62] and Iran.[63-65,67] The NRSs were conducted in Australia,[69] and Iran.[70]

*Patient Population*

All SRs[58-61] included adult patients with MS, in some cases specified as RRMS, determined by a variety of methods including McDonald criteria. One SR[58] was designed to evaluate the role of vitamin D supplementation in a variety of health conditions, but for the purpose of this review, only information relevant to MS was assessed. The clinical populations in the reviews included a combination of patients identified as having RRMS and patients with an unspecified MS phenotype. The RCTs included patients with RRMS,[36,37,49,62,63,66,67] and unspecified MS phenotypes.[65] In most RCTs MS status was determined using McDonald criteria,[14] though some studies failed to specify their diagnostic process.[37,65,66] The NRSs included patients with RRMS,[70] or unspecified MS phenotype,[69] as determined by a medical doctor[69,70] and McDonald criteria.[70] The age of patients in all treatment studies and reviews ranged approximately from early adulthood to middle age.

*Interventions*

All SRs investigated the use of vitamin D supplements of various doses (2800 IU per day to 40 000 IU per day) and formulation (i.e., vitamin D2, D3, and calcitriol) and with[59-61] or without[58] concomitant therapy and calcium supplements. The RCTs all investigated the use of doses of vitamin D supplements (doses ranging from 2000 IU per day [usually delivered weekly] to 10 000 IU per day) for a variety of durations (12 weeks to approximately 2 years). Most studies reported concomitant immunomodulatory therapy, often interferon-β. One NRS assessed a range of vitamin D supplement doses (i.e., 1 to 5000 IU),[69] and the other assessed treatment with 50 000 IU per week. Follow-up was unspecified by one study[69] and the other reported a treatment duration of 6 months.[70]

*Comparators*

Of the SRs, three systematic reviews compared vitamin D to no vitamin D treatment[58-61] and one additionally compared high versus low dose vitamin D.[60] Five RCTs reported comparing vitamin D to no vitamin D or placebo.[62,63,65-67] Two RCTs reported comparing high dose (4730 IU per day[36] or 10 400 IU per day[37]) vitamin D to low dose (800 IU per day)[36,37] vitamin D.[36,37,49] Both NRSs[69,70] compared vitamin D supplementation to no vitamin D supplementation. Concomitant therapy was always consistent across intervention and comparator groups.

*Outcomes*

All studies assessed the effect of vitamin D supplementation on 25(OH)D levels. While this outcome is not directly relevant to the research questions, it is essential for interpretation of the results and is reported where available. A variety of clinical and surrogate outcomes were assessed, including disease course, activity or progression,[58,59,61,66] risk of relapse,[36,37,58-61,65] disability scores,[36,58,59,65,69] functional measurements,[61] cognitive functioning,[61] quality of life,[36,61,69] radiological measures,[59] immunologic activity and inflammation,[37,59,62,63,66,67,70] bone mineral density,[66] flu-like symptoms,[36] and neuroimaging parameters.[61] In addition, safety outcomes were assessed by some studies, but some only reported on serum calcium levels.[36,37,58,60-65]

**Summary of Critical Appraisal**

Specific strengths and limitations of the identified evidence are presented in Appendix 3.

*Systematic Reviews*

None of the SRs provided a study protocol so it was unclear whether all design aspects were established a priori. This prevents the assessment of selective reporting of outcomes and data mining. Two SRs conducted duplicate screening[59,61] one of which conducted duplicate extraction.[59] Two SRs[58,60] did not report the number of reviewers at each stage, increasing the likelihood of potential bias in study selection and errors in data abstraction. All SRs performed a comprehensive literature search on multiple databases. Three SRs[59,60] performed a thorough grey literature search to identify additional publications and unpublished studies. One SR only searched personal files and reference lists,[61] and one[58] only searched reference lists, increasing the likelihood of overlooking relevant evidence. One[58] SR provided a list of included and excluded studies in a supplementary appendix; however, study characteristics were not provided. The other three SRs[59-61] only provided a list of included studies and study

characteristics. The absence of a list of excluded studies makes it difficult to determine whether there was any bias in study selection. Two SRs[59,61] used formal quality assessment tools to assess study quality, while one only assessed quality informally,[60] and one did not perform any quality assessment.[58] Two SRs considered quality in the formulation of conclusions,[60,61] one mentioned quality only briefly,[59] and one did not mention quality at all.[58] Three SRs[58,59,61] did not pool studies and summarized findings narratively, but only one of these reviews provided their rationale for not pooling studies (heterogeneity of dosing and outcome measures).[61] One SR[60] formally assessed publication bias both visually and using Egger's test. An attempt to minimize publication bias was made by one SR that pursued and included unpublished data,[59] while the other SRs[58,61] did not report an assessment or method of attenuating publication bias. All SRs reported that study authors had no conflict of interest.

*Randomized Controlled Trials*

*Reporting*

All studies reported hypotheses or objectives upfront.[36,37,62-67] All but one study[37] clearly described outcome measures. Patient characteristics were described by all studies; however, several studies[36,37,63-65,67] did not report on relevant characteristics including but not limited to ethnicity or skin color, body mass index (BMI), smoking status, and vitamin D intake from food or supplementation (in addition to that provided in study intervention). Without information about these potential determinants of vitamin D status, it is unclear whether any factors confounded the relationship between vitamin D supplementation and the outcomes of interest. All studies clearly described the intervention and comparators. Distribution of potential confounders was presented by all studies excluding one;[65] however, owing to the exclusion of several relevant patient characteristics mentioned above, this information was often limited. Findings were clearly described by all studies; however, some did not present direct group comparisons (i.e., they only reported changes within treatment groups) or change from baseline values for some but not all outcomes. In these cases, it was not possible to assess or report the effect of vitamin D supplementation against the study comparators for all outcomes of interest. No studies comprehensively reported on adverse events. Some studies reported on limited harm outcomes,[36,37,62,64,65] while some failed to disclose any.[63,66,67] Potential harms of excessive vitamin D intake are well described in the literature,[71] so it is unclear why standard outcomes such as hypervitaminosis D and hypercalcemia were not explored. All studies reported on losses to follow-up. Characteristics of patients lost to follow-up were reported by two studies.[65,66] The remainder may have reported reasons for loss to follow-up, but did not disclose patient characteristics. It is unclear whether there were potentially meaningful differences between those who discontinued treatment and those who finished the trials. All except one study[65] reported actual probability values.

*External Validity*

No studies reported random sampling techniques, but some studies included subjects who were consecutively enrolled upon diagnosis of MS.[36,62,64-66] The other studies had unclear sampling procedures and it is not possible to determine whether patients are representative of the broader population.[37,63,67] No studies commented on whether there were differences between patients who were willing to participate and those who declined enrolment. One study[63] was conducted in a setting (staff, places, and facilities) that is representative of the care most patients would receive. All other studies had either an unclear setting or were conducted in

specialty facilities where patients may receive a higher standard of care not generalizable to the broader MS population.

*Internal Validity – Bias*

All except one open-label study[65] blinded patients to treatment. Two studies did not blind outcome assessors, increasing the risk of ascertainment bias, particularly for subjective measurements.[63,65] In two cases[36,62] it was unclear whether secondary analyses were planned a priori, and data dredging cannot be ruled out. Two studies adjusted for different lengths of follow-up or did not require adjustment due to complete follow-up data being available for all participants.[65,67] All studies used appropriate statistical tests based on data distribution. Some studies assessed patient compliance through pill counting, verbal confirmation, or using 25(OH)D levels as a surrogate for adherence to the study protocol.[37,62,63,67] The other four studies either failed to report on compliance rates or did not assess compliance.[36,64-66] The main outcome measures were accurate in all cases; however, some studies assessed biomarkers or surrogate outcomes and did not assess meaningful clinical endpoints such as relapse rates, physical changes as assessed by imaging studies, or disability scores. Relative validity and reliability of various assays used for some clinical measurements are discussed in Limitations section.

*Internal Validity - Confounding*

Patients in all study groups were recruited from the same source population in most cases.[36,62,64-67] In two cases the source population was unclear.[37,63] Three studies did not report on whether patients were recruited over the same time period.[37,62,67] The rest[36,63-66] recruited patients over the same time period, but it was unclear whether the distribution of recruitment across seasons was equal between groups when recruitment periods were longer. Vitamin D status is affected by season, especially in countries farther from the equator, as is immune function. All studies except one[67] employed acceptable randomization techniques. In the case of four studies[62,63,65,67] it was unclear whether random allocation was concealed. One[66] study adequately adjusted for confounding. In many cases, due to the limited reporting on relevant baseline values, it was unclear whether all confounders were accounted for. One study accounted for losses to follow-up in analysis.[65] The others reported drop-out rates, but did not employ any analytical techniques to explore the impact of drop-outs on the main study findings.

*Power*

Some studies reported sample size or power calculations; however, with the exception of one study[37] all were underpowered or had unclear power to detect differences in clinical outcomes.

*Non-Randomized Studies*

*Reporting*

All studies reported clear objectives or hypotheses upfront.[68-70] The main outcomes, characteristics of included patients, and interventions of interest were clearly stated in all cases. One study did not report the distribution of potential confounders.[70] One study presented insufficient primary data to support study results, and interpretation of main findings was difficult.[69] This study[69] also failed to consistently provide estimates of random variability for the main outcomes. None of the studies reported on all relevant adverse events. One study

described characteristics of patients lost to follow-up,[69] the others did not.[68,70] Two studies reported actual probability values[69,70] and the other reported them categorically.[68]

*External Validity*

One study sampled patients who are likely representative of Norwegians with MS in the community as they were drawn from a database of patients with confirmed MS.[68] One study may only be representative of patients who are highly engaged as they were recruited through online advertising using MS content.[69] One study included patients treated at MS clinics, who may receive a higher standard of care.[70] Differences between willing and unwilling participants were unclear in all cases. None of the studies contained sufficient information to judge whether the context of care was representative of that received by the broader MS population.

*Internal Validity – Bias*

No studies blinded patients or outcome assessors to the intervention, increasing the risk of ascertainment bias. Unplanned analyses could not be ruled out for two studies.[68,70] No adjustment for potential differences in recall periods or length-of follow-up was conducted. The statistical tests used to assess the main outcomes were appropriate. No studies assessed patient compliance with the intervention. As supplemental intake was self-reported in some cases, the potential for recall bias is high. All studies included accurate outcome measures.

*Internal Validity – Confounding*

Two studies ensured recruitment of participants from the same source populations.[68,70] All studies recruited patients over the same time period. There was no randomization of study subjects by any study, increasing the risk of selection bias. Two studies conducted regression analysis to investigate potential confounding factors in the relationship between vitamin D supplementation and MS-related outcomes.[68,70] Any losses-to-follow-up, variable recall periods, and drop-outs were not taken into accounts in analysis.

*Power*

Two studies recruited large sample sizes, which were likely sufficient to ensure adequately powered analyses;[68,69] however, no studies disclosed a sample size or power calculation.

**Summary of Findings**

A detailed summary of findings is presented in Appendix 4.

All treatment and prevention studies observed a significant increase in 25(OH)D levels following vitamin D supplementation.

Where results from narrative SRs are reported, all reported trial results are summarized. For example, where a single trial's results are shared for a specified outcome, only the results from one study were reported (and therefore assumed to be measured and available) within the SR. This does not account for potential selective outcome reporting in the original SRs.

*PREVENTION*

*What is the clinical effectiveness of vitamin D supplementation for the prevention of multiple sclerosis?*

*Multiple Sclerosis Risk*

One NRS[68] conducted in MS patients in Norway reported that cod liver oil consumption during adolescence but not childhood or adulthood showed a significant inverse association with MS risk when controlling for sun exposure, infectious mononucleosis, smoking, body size, oily fish consumption and education. When various subgroups of escalating doses of vitamin D (contained in cod liver oil) were considered, doses in the range of 0 to 200, 201 to 400, and 691 to 800 IU were significantly associated with a reduced odds of developing MS. Doses in the range of 401-600 and >800 IU trended towards a reduced odds of developing MS but were not statistically significant. The authors report a dose-response relationship but this is not reflected in the data.

*Clinical Symptoms Indicative of Conversion to Multiple Sclerosis*

One RCT[64] conducted in Iran reported significantly fewer cases of a second demyelinating attack suggestive of potential MS conversion in the vitamin D-treated group (50 000 IU weekly) versus placebo after 12 months of treatment in patients with optic neuritis at risk for MS.

*Imaging Studies*

One RCT[64] conducted in Iran reported that there was a significantly lower incidence of various lesions (including black holes, cortical, juxtacortical, corpus callosal, new gadolinium-enhanced, and new T2) in the vitamin D group (50 000 IU weekly) versus placebo in patients with optic neuritis at risk for MS. Conversely there was no difference in other lesions (including periventricular or brain stem plaques).[64]

*Adverse Events*

One RCT[64] conducted in Iran reported no incidence of hypercalcemia or vitamin D toxicity in patients with optic neuritis treated with vitamin D (50 000 IU weekly) or placebo.

*What is the clinical effectiveness of high versus low dose vitamin D supplementation for the prevention of multiple sclerosis?*

No relevant evidence was identified regarding the clinical effectiveness of high versus low dose vitamin D supplementation for the prevention of MS; therefore, no summary can be provided.

*TREATMENT*

*What is the clinical effectiveness of vitamin D supplementation for the treatment of multiple sclerosis?*

*Disease Activity*

One SR[61] reported narratively that one trial in patients with MS observed no changes in disease activity when vitamin D (20 000 IU per week) was given for 96 weeks.[44]

*Relapse*

One SR of six trials[58] reported narratively that no studies had results suggestive of a benefit of vitamin D supplementation for reducing relapse rates. Another SR[60] reported that there was no significant difference in the pooled odds of relapse between high dose vitamin D supplements and low dose or placebo, including in subgroups of patients treated for at least a year, comparisons only to placebo, and studies only providing vitamin D3. Other SRs[59,61] reported narratively that some individual trials in patients with unspecified MS phenotypes or RRMS observed no differences in relapse rates following treatment with 20 000 IU per week or 300 000 IU per month.[46,47]

One RCT[65] conducted in Iran reported that the rate of relapse in pregnant women was significantly reduced in both vitamin D treated (50 000 IU per week) and routine care groups, but to a greater extent in the vitamin D group, over 6 months.

One NRS[69] conducted in MS patients in Australia reported that ordinal regression models controlled for various potential confounders did not find an association between vitamin D supplementation and reduced relapse rate.

*Disability*

One narrative SR of six trials[58] reported that no studies had results suggestive of a benefit of vitamin D supplementation for improving disability scores. Another SR[59] reported narratively that one trial in MS patients demonstrated no significant difference in EDSS scores after 6 months of treatment with 300 000 IU of vitamin D per month.[46] Conversely, they reported that another trial in RRMS patients showed a trend towards reduced disability accumulation after one year of supplementation with 20 000 IU per week.[48,59] One SR[61] reported narratively that one trial in RRMS patients observed no significant change in EDSS in patients who received low dose calcitriol (0.25 µg) for one year versus placebo.[47]

One RCT[65] conducted in Iran reported that EDSS scores were significantly lower in pregnant patients supplemented with vitamin D (50 000 IU per week) versus patients who received routine care at 6 months.

One NRS[69] conducted in MS patients in Australia reported that vitamin D supplementation was not associated with reduced odds of moderate or high disability (versus no or mild disability) in logistic regression models controlled for potential confounders.

*Immunologic Outcomes*

Two SRs[59,61] narratively reported mixed results. Individual trials reported that anti-inflammatory cytokine levels were increased and peripheral blood mononuclear cell (PBMC) proliferative responses were suppressed, but that there was no significant change in disease-associated PBMC responses. These reviews also reported that one trial in MS patients observed reductions in T-cell proliferation after treatment with an escalating dose of vitamin D.[46]

One RCT[63] conducted in RRMS patients in Iran reported no difference in interleukin (IL)-10 levels after 3 months between vitamin D supplemented (50 000 IU every five days) and placebo groups; however, in multiple linear regression models adjusted for age, sex, and EDSS scores, there was a positive association between vitamin D consumption and the log of IL-10 measures.[63] Another RCT[66] conducted in RRMS patients in Norway reported no difference in the change from baseline values of various serum markers of inflammation between vitamin D-treated patients (20 000 IU per week plus 500 mg of calcium per day) and those who received placebo. The proportion of patients with increased IL-17 levels after 12 weeks of treatment was not significantly different between treatment (50 000 IU every five days) and control groups in one RCT conducted in RRMS patients in Iran;[67] however, in the Norwegian trial, vitamin D supplementation showed a significant positive correlation with the log of IL-17 measures when adjusted for EDSS score.[66]

One NRS[70] conducted in RRMS patients in Iran reported that the rise in Epstein-Barr Virus antibodies was significantly lower in the vitamin D-treated group (50 000 IU per week) versus no vitamin D treatment. At six months, levels of virus capsid antigen (VCA) immunoglobulin G (IgG) and Epstein-Barr virus nuclear antigen-1 IgG antibodies were significantly lower in the vitamin D-treated group. A greater proportion of patients in the vitamin D-treated group displayed a decline in anti-VCA and anti-Epstein-Barr virus nuclear antigen-1 antibody titers.[70]

*Imaging Studies*

Two SRs[59,61] narratively reported on the same trial, which observed fewer new MRI lesions after a year of supplementation in patients with RRMS who received 20 000 IU weekly.[48] These effects were more pronounced in a secondary analysis of patients with at least one relapse using the preceding year or enhancing T1 lesions at baseline.[43] However, another trial in patients with MS showed no difference in MRI lesions after 6 months of treatment with 300 000 IU per month.[46]

*Functional Outcomes*

One SR[59] reported narratively that one trial in patients with RRMS demonstrated improved tandem walk time after one year of weekly supplementation with 20 000 IU.[48] Another SR[61] reported narratively that one trial[44] in patients with MS demonstrated no changes in fatigue severity scores after 96 weeks of high-dose vitamin D treatment (20 000 IU weekly).

*Quality of Life*

One NRS[69] conducted in MS patients in Australia reported that controlling for potential confounders, vitamin D supplementation was associated with an improved quality of life as measured by the MSQOL-54.

*Safety*

In general, high dose (short-term use of up to 40 000 IU per day) vitamin D supplementation appears to be well-tolerated versus placebo or routine care.

One SR[60] reported that high dose vitamin D supplementation (2000 to 40 000 IU per day) was not associated with an increase in serious adverse events, nephrolithiasis, renal dysfunction, or hypercalcemia. Low rates of mild gastrointestinal symptoms were reported.[60] Another SR[61]

reported narratively that three trials observed no serious adverse events beyond mild gastrointestinal symptoms, fever, fatigue and headache in patients with unspecified MS phenotypes or RRMS.[44,47,48]

Several RCTs reported on adverse events. Overall there was no concern about, or difference in, the rates of hypercalcemia[62,63,67] or respiratory tract infections in RRMS patients treated with 20 000 weekly to 50 000 IU every five days.[62] One RCT[65] reported that there was no incidence of urinary dysfunction, symptomatic nephrolithiasis, or disturbances of cardiac rhythm frequency in pregnant women with MS treated with vitamin D (50 000 IU per week) for six months.

One NRS reported that there was no incidence of vitamin D intoxication during high dose vitamin D supplementation (50 000 IU per week) for 6 months in patients with RRMS.[70]

*What is the clinical effectiveness of high versus low dose vitamin D supplementation for the treatment of multiple sclerosis?*

*Clinical Symptoms*

One RCT[36] conducted in RRMS patients in Israel reported no significant changes in flu-like symptoms in high (4370 IU per day) or low (800 IU per day) dose vitamin D groups, and that there was insufficient power to detect a difference between groups.

*Relapse*

One SR[60] reported that there was no significant difference in the pooled odds of relapse between high dose (2000 to 40 000 IU per day) vitamin D supplements and low dose or placebo (0 to 1000 IU per day).

One RCT[36] conducted in Israel reported a higher increase in relapse rate in the high-dose (4370 IU per day) group versus the low-dose (800 IU per day) group that was not statistically significant. One RCT[37] conducted in the United States demonstrated an identical occurrence of relapse in high (10 400 IU per day) and low (800 IU per day) dose vitamin D groups after six months of treatment.

*Disability*

One SR[61] reported narratively that a single trial in RRMS patients measured and observed higher exit EDSS scores in high dose (6000 IU per day) versus low dose (1000 IU per day) vitamin D2 supplementation groups.[35]

*Immunologic Outcomes*

One RCT[37] conducted in RRMS patients in the United States reported a significantly greater negative change in pro-inflammatory IL-17$^{+}$CD4$^{+}$ T cells in the high dose (10 400 IU) versus low dose (800 IU) vitamin D group. There were no significant differences in the levels of IFN-γ, effector memory, central memory, naïve, CD161, CD85J positive CD4 T cells, or 51 cytokines.

*Adverse Events*

One RCT[36] conducted in Israel reported that serum calcium levels remained stable and within normal range in both high (4370 IU) and low (800 IU) dose groups of patients with RRMS throughout the follow-up period. High and low dose vitamin D was well tolerated with no reports of withdrawals due to adverse events, or possible vitamin D-related adverse events.[36]

**Limitations**

*Confounding by Determinants of Vitamin D Status*

Factors such as season, age, latitude, adiposity, physical activity, smoking, and diet can influence the relationship between vitamin D and health outcomes. Some studies, particularly NRSs and open-label RCTs, failed to control for these factors or assess baseline imbalance, so it is unclear whether these factors contributed to a blunted or exaggerated effect of vitamin D on MS related outcomes. Benefit or lack of benefit observed may to some extent conflate true treatment effects with differences in outcomes attributable to variations in baseline risk.

*Baseline Vitamin D Status*

Having sufficient vitamin D status may influence the response to vitamin D supplementation. That is, if an individual already has sufficient vitamin D status, they may not benefit to the same extent as someone with vitamin D insufficiency. Studies that included a greater proportion of individuals with vitamin D sufficiency, or intentionally excluded patients with the lowest vitamin D status may have been less likely to observe a benefit of treatment.

*Comparison of Change from Baseline Values*

Change from baseline scores may be more appropriate to reduce potential between-person variability when assessing an intervention like vitamin D supplementation that has many potential confounders. Despite many studies having baseline and end line values, many chose to only assess differences between groups at each time point. As a result, potential confounding of baseline imbalances (known and unknown) may have affected end line comparisons.

*Validity of Outcome Measures*

Although most studies employed the most frequently used and accurate outcome measures available to quantify MS treatment response, they are not without limitations.

*Vitamin D Status Assessment*

The most commonly used methods of quantification of 25(OH)D levels are the DiaSorin Liaison and liquid chromatography tandem mass spectrometry (LC-MS/MS) assays, though other methods such as alternative chemiluminescence immunoassays, radioimmunoassays, and high-performance liquid chromatography methods are in use. It has been observed that patients are significantly more likely to be classified as having insufficient vitamin D status using the DiaSorin Liaison assay versus the LC-MS/MS assay due to the presence of 25(OH) vitamin D2 molecule.[72] Thus, studies that employed the DiaSorin method may report lower baseline and end line vitamin D status than those that use the LC-MS/MS method. This may influence the observed impact of supplementation on vitamin D status, which was used in some cases as a

covariate in multivariate analyses. Further, results of studies using different quantification methods may not be comparable, which is of particular concern when results are pooled as it may increase non-statistical heterogeneity.[73]

*Disability Scales*

The EDSS and the Multiple Sclerosis Functional Composite (MSFC) measure are the most frequently used instruments to assess disability status and monitor disease progression in MS studies. While these are surrogate outcomes compared to imaging studies, the EDSS is widely accepted as a relevant clinical endpoint, whereas, the MSFC may be more appropriate as a secondary outcome due to the absence of a visual dimension and low acceptance by patients. The EDSS has limited inter-and intra-rater reliability so adequate training of staff and consistency in outcome assessors is important. Few studies commented on these aspects of operationalizing the EDSS so it is unclear whether it was scored using a valid and consistent approach. The MSFC contains additional components such as upper limb function and cognitive skills, which may provide valuable information not conveyed by the EDSS. However, a learning effect – meaning patients score higher as they learn about the tests, has been observed, and physicians may find the scale difficult to interpret.[74]

*Lack of Long-Term Follow-Up*

The longest study duration for the studies included in this review was less than two years. As MS is a progressive disease, this may be insufficient to observe clinical endpoints such as severe physical or cognitive morbidity, or mortality. Further, in the case of the single prevention trial, 12 months of observation is likely insufficient to properly observe rates of MS conversion.[64] Trials of longer duration are needed.

*Limited Adverse Event Reporting*

Vitamin D is a fat-soluble vitamin, meaning that it is concentrated in tissue rather than excreted when excess is consumed. Vitamin D doses below 10 000 IU/day are noted to be safe and effective for maintaining sufficient vitamin D status; however, in the context of therapeutic doses safety is less clear. Doses equal to or above 50 000 IU per day have been associated with toxicity.[71] It should be noted that there is not a clear consensus on the dose at which risk outweighs benefit, with most experts suggesting that the toxicity threshold lies between 10 000 and 40 000 IU per day and serum 25(OH)D levels of 500 to 600 nmol/L. Some studies included in this review lie within that threshold. Potential adverse outcomes associated with excessive intake and hypervitaminosis D include hypercalcemia and hypercalciuria leading to soft tissue and vascular calcification,[75] and associations with impaired gastrointestinal side-effects, renal function, cardiovascular risk, falls and fractures, cancer, and all-cause mortality.[26,71] Further evidence is needed to determine whether high-dose supplementation precipitates undesirable outcomes in MS patients.

*Type of Multiple Sclerosis Unclear*

While some studies specified the stage of MS — usually relapsing remitting, which is the most common — many studies did not disclose distribution of types of MS or indicate restriction to one type of MS. Failure to specify the MS phenotype limits the ability to interpret generalizability of findings to the appropriate patient populations, and does not allow for assessment of comparative effectiveness across MS phenotypes. For instance, an effective treatment in RRMS

may not have the same outcome in secondary progressive MS and if so a distinction should be made. It has been noted that immunomodulatory strategies used for RRMS have not proven effective when applied in progressive MS,[76] suggesting cause for caution when generalizing results to the greater MS population.

## CONCLUSIONS AND IMPLICATIONS FOR DECISION OR POLICY MAKING

Four SRs,[58-61] eight RCTs,[36,37,62-67] and three NRSs[68-70] were identified regarding the clinical effectiveness of vitamin D supplementation for MS.

Collectively, the evidence is mixed partially owing to substantial clinical and methodological heterogeneity across studies, small sample sizes and inadequate statistical power, insufficient follow-up duration, inconsistent outcome measures, and insufficient accounting for potential confounders.

For prevention, limited evidence from one NRS[68] and one RCT[64] suggests potential benefits of cod liver oil supplementation during adolescence on MS risk[68] as well as a reduced risk of second demyelinating attacks and lower occurrence of various lesions as visualized by MRI,[64] with no apparent risks of supplementation in patients with optic neuritis.[64]

For treatment with vitamin D versus no vitamin D, there was no evidence to suggest a benefit for disease activity.[44] Most studies and reviews reported no change in relapse rates.[58-61,69] One study in pregnant women suggested a benefit of vitamin D on relapse rates.[65] A similar pattern was observed for disability. Several SRs and one NRS reported no effect of vitamin D on reducing disability,[58,61,69] or mixed results,[59] while the study in pregnant women showed a potential benefit.[65] The effect on immunological outcomes was mixed, with some studies and reviews reporting mixed results,[59,61,63,67] namely, two studies reported an association between vitamin D supplementation and levels of anti-inflammatory cytokines[63,67] one study reported reduced antibodies and titers to the Epstein-Barr virus,[70] and another trial showed no difference in serum markers of inflammation.[66] Limited evidence from two trials summarized within two SRs[59,61] was mixed for outcomes of imaging studies, with one study showing fewer new MRI lesions, and the other trial showing no effect. This trend persisted for functional outcomes, with one study summarized in an SR showing improved tandem walk time, and one study showing no change in fatigue severity scores. One NRS[69] reported improved quality of life, and no major short-term safety issues were identified.[60-63,65,67,70]

For treatment with high dose vitamin D versus low dose vitamin D, evidence presented in one SR[61] from one trial reported higher relapse rates in high dose patients, while evidence from another SR[60] suggested no difference in the odds of relapse, and one trial[37] suggested no differences in relapse rates between groups. Flu-like symptoms were not different between groups as summarized by one SR.[36] A single study summarized in a SR[61] reported increased disability scale scores in the high dose group, and one study reported mixed effects on inflammatory factors with most factors showing no change with high or low dose treatment. High and low dose treatment was well tolerated, and no concerning adverse events were reported.

Current guidelines from the National Institute for Health and Care Excellence on the management of MS in adults state that vitamin D should not be offered solely for the purpose of treating MS.[76] However, MS patients should maintain sufficient intake of vitamin D to ensure adequate vitamin D status.

Several large-scale, methodologically rigorous trials are underway (Appendix 5). Completion of these trials, as well as increased awareness regarding potential subgroup effects including but not limited to vitamin D dose, form of vitamin D, duration of treatment, background therapies, MS phenotype, age and duration of disease, and country of residence may help to clarify some of the uncertainty surrounding the effectiveness of vitamin D supplementation for the treatment of MS.

Based on the evidence contained in this review, the role of high-dose vitamin D supplementation in MS treatment and prevention remains unclear.

**PREPARED BY:**

Canadian Agency for Drugs and Technologies in Health
Tel: 1-866-898-8439
www.cadth.ca

## REFERENCES


1. Warren SA, Janzen W, Warren KG, Svenson LW, Schopflocher DP. Multiple Sclerosis Mortality Rates in Canada, 1975-2009. Can J Neurol Sci. 2016 Jan;43(1):134-41.

2. Kingwell E, Zhu F, Marrie RA, Fisk JD, Wolfson C, Warren S, et al. High incidence and increasing prevalence of multiple sclerosis in British Columbia, Canada: findings from over two decades (1991-2010). J Neurol [Internet]. 2015 Oct [cited 2016 Feb 25];262(10):2352-63. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4608995

3. Orton SM, Herrera BM, Yee IM, Valdar W, Ramagopalan SV, Sadovnick AD, et al. Sex ratio of multiple sclerosis in Canada: a longitudinal study. Lancet Neurol. 2006 Nov;5(11):932-6.

4. Atlas of MS 2013: mapping multiple sclerosis around the world [Internet]. London (GB): Multiple Sclerosis International Federation; 2013. 28 p. [cited 2016 Feb 25]. Available from: http://www.msif.org/wp-content/uploads/2014/09/Atlas-of-MS.pdf

5. Atlas multiple sclerosis resources in the world 2008 [Internet]. Geneva (SW): World Health Organization; 2008. [cited 2016 Mar 2]. Available from: http://www.who.int/mental_health/neurology/Atlas_MS_WEB.pdf

6. Milo R, Kahana E. Multiple sclerosis: geoepidemiology, genetics and the environment. Autoimmun Rev. 2010 Mar;9(5):A387-A394.

7. Poppe AY, Wolfson C, Zhu B. Prevalence of multiple sclerosis in Canada: a systematic review. Can J Neurol Sci. 2008 Nov;35(5):593-601.

8. CIHI [Internet]. Ottawa: Canadian Institute for Health Information. Multiple sclerosis; 2014 [cited 2016 Feb 25]. Available from: https://www.cihi.ca/en/types-of-care/specialized-services/multiple-sclerosis

9. CANSIM [Internet]. Ottawa: Statistics Canada. Table 105-1300. Neurological conditions, by age group and sex, household population aged 0 and over, 2010-2011; 2012 Sep 18 [cited 2016 Feb 25]. Available from: http://www5.statcan.gc.ca/cansim/a26?lang=eng&retrLang=eng&id=1051300&paSer=&pattern=&stByVal=1&p1=1&p2=31&tabMode=dataTable&csid

10. Evans C, Beland SG, Kulaga S, Wolfson C, Kingwell E, Marriott J, et al. Incidence and prevalence of multiple sclerosis in the Americas: a systematic review. Neuroepidemiology. 2013;40(3):195-210.

11. Dorman E, Kansal AR, Sarda S. The budget impact of introducing delayed-release dimethyl fumarate for treatment of relapse-remitting multiple sclerosis in Canada. J Med Econ. 2015 Dec;18(12):1085-91.

12. Karampampa K, Gustavsson A, Miltenburger C, Kindundu CM, Selchen DH. Treatment experience, burden, and unmet needs (TRIBUNE) in multiple sclerosis: the costs and utilities of MS patients in Canada. J Popul Ther Clin Pharmacol. 2012;19(1):e11-e25.

13. Burden of illness of multiple sclerosis: Part I: Cost of illness. The Canadian Burden of Illness Study Group. Can J Neurol Sci. 1998 Feb;25(1):23-30.

14. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol [Internet]. 2011 Feb [cited 2016 Mar 2];69(2):292-302. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3084507

15. National Multiple Sclerosis Society (U.S.) [Internet]. New York: National MS Society. Diagnosing MS; 2016 [cited 2016 Mar 3]. Available from: http://www.nationalmssociety.org/Symptoms-Diagnosis/Diagnosing-MS

16. Files DK, Jausurawong T, Katrajian R, Danoff R. Multiple sclerosis. Prim Care. 2015 Jun;42(2):159-75.

17. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. 1983 Nov;33(11):1444-52.

18. Laplaud DA, Confavreux C. [Etiology of multiple sclerosis]. Rev Prat. 2006 Jun 30;56(12):1306-12.

19. Spelman T, Gray O, Trojano M, Petersen T, Izquierdo G, Lugaresi A, et al. Seasonal variation of relapse rate in multiple sclerosis is latitude dependent. Ann Neurol. 2014 Dec;76(6):880-90.

20. Niino M, Miyazaki Y, Fukazawa T, Kikuchi S. [Vitamin D and latitude as environmental factors in multiple sclerosis]. Nihon Rinsho. 2014 Nov;72(11):1924-9.

21. Mouhieddine TH, Darwish H, Fawaz L, Yamout B, Tamim H, Khoury SJ. Risk factors for multiple sclerosis and associations with anti-EBV antibody titers. Clin Immunol. 2015 May;158(1):59-66.

22. Beretich BD, Beretich TM. Explaining multiple sclerosis prevalence by ultraviolet exposure: a geospatial analysis. Mult Scler. 2009 Aug;15(8):891-8.

23. Orton SM, Wald L, Confavreux C, Vukusic S, Krohn JP, Ramagopalan SV, et al. Association of UV radiation with multiple sclerosis prevalence and sex ratio in France. Neurology [Internet]. 2011 Feb 1 [cited 2016 Mar 2];76(5):425-31. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3034408

24. Drezner MK. Patient information: vitamin D deficiency (beyond the basics). In: Post TW, editor. UpToDate [Internet]. Waltham (MA): UpToDate; 2015 Jun 9 [cited 2016 Mar 3]. Available from: www.uptodate.com Subscription required.

25. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr [Internet]. 2008 Apr [cited 2016 Mar 2];87(4):1080S-6S. Available from: http://ajcn.nutrition.org/content/87/4/1080S.full.pdf+html

26. Vitamin D toxicity associated with different vitamin D dosing regimens: safety [Internet]. Ottawa: CADTH; 2014 Dec 11. [cited 2016 Mar 2]. (Rapid response report: summary of abstracts). Available from: https://www.cadth.ca/media/pdf/htis/dec-2014/RB0777%20Vitamin%20D%20Toxicity%20Final.pdf

27. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, Ross AC, Taylor CL, Yaktine AL, Del Valle HB. Dietary reference intakes for calcium and vitamin D [Internet]. Washington (DC): National Academies Press (US); 2011. Tolerable upper intake levels: calcium and vitamin D. [cited 2016 Mar 3]. (The National Academies Collection: Reports funded by National Institutes of Health). Available from: http://www.ncbi.nlm.nih.gov/books/NBK56070/pdf/Bookshelf_NBK56070.pdf

28. Raghuwanshi A, Joshi SS, Christakos S. Vitamin D and multiple sclerosis. J Cell Biochem [Internet]. 2008 Oct 1 [cited 2016 Apr 3];105(2):338-43. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2859312

29. Lucas RM, Ponsonby AL, Dear K, Valery PC, Pender MP, Taylor BV, et al. Sun exposure and vitamin D are independent risk factors for CNS demyelination. Neurology. 2011 Feb 8;76(6):540-8.

30. McDowell TY, Amr S, Culpepper WJ, Langenberg P, Royal W, Bever C, et al. Sun exposure, vitamin D and age at disease onset in relapsing multiple sclerosis. Neuroepidemiology. 2011;36(1):39-45.

31. Duan S, Lv Z, Fan X, Wang L, Han F, Wang H, et al. Vitamin D status and the risk of multiple sclerosis: a systematic review and meta-analysis. Neurosci Lett. 2014 Jun 6;570:108-13.

32. Hiremath GS, Cettomai D, Baynes M, Ratchford JN, Newsome S, Harrison D, et al. Vitamin D status and effect of low-dose cholecalciferol and high-dose ergocalciferol supplementation in multiple sclerosis. Mult Scler. 2009 Jun;15(6):735-40.

33. Steffensen L, Brustad M, Skeie L, Jorgensen Y, Figenschau M, Kampman M. Vitamin D status in individuals with multiple sclerosis at baseline and after supplementation with 20,000 IU cholecalciferol a week for 48 weeks [Internet]. Poster presented at: 25th Congress of the European Committee for the Treatment and Research in Multiple Sclerosis (ECTRIMS); 2009 Aug; Düsseldorf (DE). [cited 2016 Mar 2]. Available from: http://registration.akm.ch/einsicht.php?XNABSTRACT_ID=94079&XNSPRACHE_ID=2&XNKONGRESS_ID=106&XNMASKEN_ID=900

34. Steffensen LH, Jorgensen L, Straume B, Mellgren SI, Kampman MT. Can vitamin $D_3$ supplementation prevent bone loss in persons with MS? A placebo-controlled trial. J Neurol [Internet]. 2011 Sep [cited 2016 Feb 16];258(9):1624-31. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3165120

35. Stein MS, Liu Y, Gray OM, Baker JE, Kolbe SC, Ditchfield MR, et al. A randomized trial of high-dose vitamin D2 in relapsing-remitting multiple sclerosis. Neurology. 2011 Oct 25;77(17):1611-8.

36. Golan D, Halhal B, Glass-Marmor L, Staun-Ram E, Rozenberg O, Lavi I, et al. Vitamin D supplementation for patients with multiple sclerosis treated with interferon-beta: a randomized controlled trial assessing the effect on flu-like symptoms and immunomodulatory properties. BMC Neurol [Internet]. 2013 [cited 2016 Feb 16];13:60. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3691658

37. Sotirchos ES, Bhargava P, Eckstein C, Van HK, Baynes M, Ntranos A, et al. Safety and immunologic effects of high- vs low-dose cholecalciferol in multiple sclerosis. Neurology. 2016 Jan 26;86(4):382-90.

38. Bhargava P, Cassard S, Steele SU, Azevedo C, Pelletier D, Sugar EA, et al. The vitamin D to ameliorate multiple sclerosis (VIDAMS) trial: study design for a multicenter, randomized, double-blind controlled trial of vitamin D in multiple sclerosis. Contemp Clin Trials. 2014 Nov;39(2):288-93.

39. Shea BJ, Grimshaw JM, Wells GA, Boers M, Andersson N, Hamel C, et al. Development of AMSTAR: a measurement tool to assess the methodological quality of systematic reviews. BMC Med Res Methodol [Internet]. 2007 [cited 2016 Mar 9];7:10. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1810543/pdf/1471-2288-7-10.pdf

40. Downs SH, Black N. The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. J Epidemiol Community Health [Internet]. 1998 Jun [cited 2016 Mar 9];52(6):377-84. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1756728/pdf/v052p00377.pdf

41. Antico A, Tampoia M, Tozzoli R, Bizzaro N. Can supplementation with vitamin D reduce the risk or modify the course of autoimmune diseases? A systematic review of the literature. Autoimmun Rev. 2012 Dec;12(2):127-36.

42. Farinotti M, Vacchi L, Simi S, Di Pietrantonj C, Brait L, Filippini G. Dietary interventions for multiple sclerosis. Cochrane Database Syst Rev. 2012;12:CD004192.

43. Aivo J, Lindsröm BM, Soilu-Hanninen M. A randomised, double-blind, placebo-controlled trial with vitamin D3 in MS: subgroup analysis of patients with baseline disease activity despite Interferon treatment. Mult Scler Int [Internet]. 2012 [cited 2016 Feb 16];2012:802796. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3420140

44. Kampman MT, Steffensen LH, Mellgren SI, Jorgensen L. Effect of vitamin $D_3$ supplementation on relapses, disease progression, and measures of function in persons with multiple sclerosis: exploratory outcomes from a double-blind randomised controlled trial. Mult Scler. 2012 Aug;18(8):1144-51.

45. Kimball S, Vieth R, Dosch HM, Bar-Or A, Cheung R, Gagne D, et al. Cholecalciferol plus calcium suppresses abnormal PBMC reactivity in patients with multiple sclerosis. J Clin Endocrinol Metab [Internet]. 2011 Sep [cited 2016 Feb 16];96(9):2826-34. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3417163

46. Mosayebi G, Ghazavi A, Ghasami K, Jand Y, Kokhaei P. Therapeutic effect of vitamin D3 in multiple sclerosis patients. Immunol Invest. 2011;40(6):627-39.

47. Shaygannejad V, Janghorbani M, Ashtari F, Dehghan H. Effects of adjunct low-dose vitamin d on relapsing-remitting multiple sclerosis progression: preliminary findings of a randomized placebo-controlled trial. Mult Scler Int [Internet]. 2012 [cited 2016 Feb 16];2012:452541. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3337486

48. Soilu-Hanninen M, Aivo J, Lindstrom BM, Elovaara I, Sumelahti ML, Farkkila M, et al. A randomised, double blind, placebo controlled trial with vitamin D3 as an add on treatment to interferon beta-1b in patients with multiple sclerosis. J Neurol Neurosurg Psychiatry. 2012 May;83(5):565-71.

49. Golan D, Staun-Ram E, Glass-Marmor L, Lavi I, Rozenberg O, Dishon S, et al. The influence of vitamin D supplementation on melatonin status in patients with multiple sclerosis. Brain Behav Immun. 2013 Aug;32:180-5.

50. Smolders J, Hupperts R, Barkhof F, Grimaldi LM, Holmoy T, Killestein J, et al. Efficacy of vitamin $D_3$ as add-on therapy in patients with relapsing-remitting multiple sclerosis receiving subcutaneous interferon beta-1a: a Phase II, multicenter, double-blind, randomized, placebo-controlled trial. J Neurol Sci. 2011 Dec 15;311(1-2):44-9.

51. O'Connell K, Kelly S, Kinsella K, Jordan S, Kenny O, Murphy D, et al. Dose-related effects of vitamin D on immune responses in patients with clinically isolated syndrome and healthy control participants: study protocol for an exploratory randomized double- blind placebo-controlled trial. Trials [Internet]. 2013 [cited 2016 Feb 16];14:272. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3844318

52. Pierrot-Deseilligny C, Rivaud-Pechoux S, Clerson P, de Paz R, Souberbielle JC. Relationship between 25-OH-D serum level and relapse rate in multiple sclerosis patients before and after vitamin D supplementation. Ther Adv Neurol Disord [Internet]. 2012 Jul [cited 2016 Feb 16];5(4):187-98. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3388527

53. Knippenberg S, Smolders J, Thewissen M, Peelen E, Tervaert JW, Hupperts R, et al. Effect of vitamin $D_3$ supplementation on peripheral B cell differentiation and isotype switching in patients with multiple sclerosis. Mult Scler. 2011 Dec;17(12):1418-23.

54. Naghavi Gargari B, Behmanesh M, Shirvani Farsani Z, Pahlevan Kakhki M, Azimi AR. Vitamin D supplementation up-regulates IL-6 and IL-17A gene expression in multiple sclerosis patients. Int Immunopharmacol. 2015 Sep;28(1):414-9.

55. Farsani ZS, Behmanesh M, Sahraian MA. Interleukin-10 but not transforming growth factor-beta1 gene expression is up-regulated by vitamin D treatment in multiple sclerosis patients. J Neurol Sci. 2015 Mar 15;350(1-2):18-23.

56. Runia TF, Neuteboom RF, de Groot CJ, de Rijke YB, Hintzen RQ. The influence of vitamin D on postpartum relapse and quality of life in pregnant multiple sclerosis patients. Eur J Neurol. 2015 Mar;22(3):479-84.

57. Allen AC, Kelly S, Basdeo SA, Kinsella K, Mulready KJ, Mills KH, et al. A pilot study of the immunological effects of high-dose vitamin D in healthy volunteers. Mult Scler. 2012 Dec;18(12):1797-800.

58. Autier P, Boniol M, Pizot C, Mullie P. Vitamin D status and ill health: a systematic review. Lancet Diabetes Endocrinol. 2014 Jan;2(1):76-89.

59. Ganesh A, Apel S, Metz L, Patten S. The case for vitamin D supplementation in multiple sclerosis. Mult Scler Relat Disord. 2013 Oct;2(4):281-306.

60. James E, Dobson R, Kuhle J, Baker D, Giovannoni G, Ramagopalan SV. The effect of vitamin D-related interventions on multiple sclerosis relapses: a meta-analysis. Mult Scler. 2013 Oct;19(12):1571-9.

61. Pozuelo-Moyano B, Benito-León J, Mitchell AJ, Hernández-Gallego J. A systematic review of randomized, double-blind, placebo-controlled trials examining the clinical efficacy of vitamin D in multiple sclerosis. Neuroepidemiology [Internet]. 2013 [cited 2016 Feb 16];40(3):147-53. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3649517

62. Aivo J, Hänninen A, Ilonen J, Soilu-Hänninen M. Vitamin D3 administration to MS patients leads to increased serum levels of latency activated peptide (LAP) of TGF-beta. J Neuroimmunol. 2015 Mar 15;280:12-5.

63. Ashtari F, Toghianifar N, Zarkesh-Esfahani SH, Mansourian M. Short-term effect of high-dose vitamin D on the level of interleukin 10 in patients with multiple sclerosis: a randomized, double-blind, placebo-controlled clinical trial. Neuroimmunomodulation. 2015;22(6):400-4.

64. Derakhshandi H, Etemadifar M, Feizi A, Abtahi SH, Minagar A, Abtahi MA, et al. Preventive effect of vitamin D3 supplementation on conversion of optic neuritis to clinically definite multiple sclerosis: a double blind, randomized, placebo-controlled pilot clinical trial. Acta Neurol Belg. 2013 Sep;113(3):257-63.

65. Etemadifar M, Janghorbani M. Efficacy of high-dose vitamin D3 supplementation in vitamin D deficient pregnant women with multiple sclerosis: Preliminary findings of a randomized-controlled trial. Iran J Neurol [Internet]. 2015 Apr 4 [cited 2016 Feb 16];14(2):67-73. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4449396

66. Rosjo E, Steffensen LH, Jorgensen L, Lindstrom JC, Saltyte BJ, Michelsen AE, et al. Vitamin D supplementation and systemic inflammation in relapsing-remitting multiple sclerosis. J Neurol. 2015 Dec;262(12):2713-21.

67. Toghianifar N, Ashtari F, Zarkesh-Esfahani SH, Mansourian M. Effect of high dose vitamin D intake on interleukin-17 levels in multiple sclerosis: a randomized, double-blind, placebo-controlled clinical trial. J Neuroimmunol. 2015 Aug 15;285:125-8.

68. Cortese M, Riise T, Bjornevik K, Holmoy T, Kampman MT, Magalhaes S, et al. Timing of use of cod liver oil, a vitamin D source, and multiple sclerosis risk: The EnvIMS study. Mult Scler [Internet]. 2015 Dec [cited 2016 Feb 16];21(14):1856-64. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4657387

69. Jelinek GA, Marck CH, Weiland TJ, Pereira N, van der Meer DM, Hadgkiss EJ. Latitude, sun exposure and vitamin D supplementation: associations with quality of life and disease outcomes in a large international cohort of people with multiple sclerosis. BMC Neurol [Internet]. 2015 [cited 2016 Feb 16];15:132. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4525738

70. Najafipoor A, Roghanian R, Zarkesh-Esfahani SH, Bouzari M, Etemadifar M. The beneficial effects of vitamin D3 on reducing antibody titers against Epstein-Barr virus in multiple sclerosis patients. Cell Immunol. 2015 Mar;294(1):9-12.

71. Tolerable upper intake levels: calcium and vitamin D. In: Ross CA, Taylor CL, Yaktine AL, Del Valle HB, editors. Dietary reference intakes for calcium and vitamin D [Internet]. Washington (DC): National Academies Press (US); 2011 [cited 2016 Mar 2]. Chapter 6. p. 403-55. Available from: https://fnic.nal.usda.gov/sites/fnic.nal.usda.gov/files/uploads/TolerableUpperIntakeLevels.pdf

72. de Koning L, Al-Turkmani MR, Berg AH, Shkreta A, Law T, Kellogg MD. Variation in clinical vitamin D status by DiaSorin Liaison and LC-MS/MS in the presence of elevated 25-OH vitamin D2. Clin Chim Acta. 2013 Jan 16;415:54-8.

73. Enko D, Fridrich L, Rezanka E, Stolba R, Ernst J, Wendler I, et al. 25-hydroxy-Vitamin D status: limitations in comparison and clinical interpretation of serum-levels across different assay methods. Clin Lab. 2014;60(9):1541-50.

74. Meyer-Moock S, Feng YS, Maeurer M, Dippel FW, Kohlmann T. Systematic literature review and validity evaluation of the Expanded Disability Status Scale (EDSS) and the Multiple Sclerosis Functional Composite (MSFC) in patients with multiple sclerosis. BMC Neurol [Internet]. 2014 [cited 2016 Feb 3];14:58. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3986942

75. Methods and results from the AHRQ-Ottawa evidence-based report on effectiveness and safety of vitamin D in relation to bone health. In: Ross CA, Taylor CL, Yaktine AL, Del Valle HB, editors. Dietary reference intakes for calcium and vitamin D [Internet]. Washington (DC): National Academies Press (US); 2011 [cited 2016 Mar 2]. Chapter C. p. 539-722. Available from: https://fnic.nal.usda.gov/sites/fnic.nal.usda.gov/files/uploads/AppendixC.pdf

76. Multiple sclerosis in adults: management [Internet]. London: National Institute for Health and Care Excellence; 2014 Oct 8. [cited 2016 Mar 4]. (NICE guidelines CG186). Available from: https://www.nice.org.uk/guidance/cg186

77. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US); 2000 -. Identifier NCT01005095, The effects of interferon beta combined with vitamin D on relapsing remitting multiple sclerosis patients; 2012 Sep 2 [cited 2016 Feb 25]. Available from: https://clinicaltrials.gov/ct2/show/NCT01005095

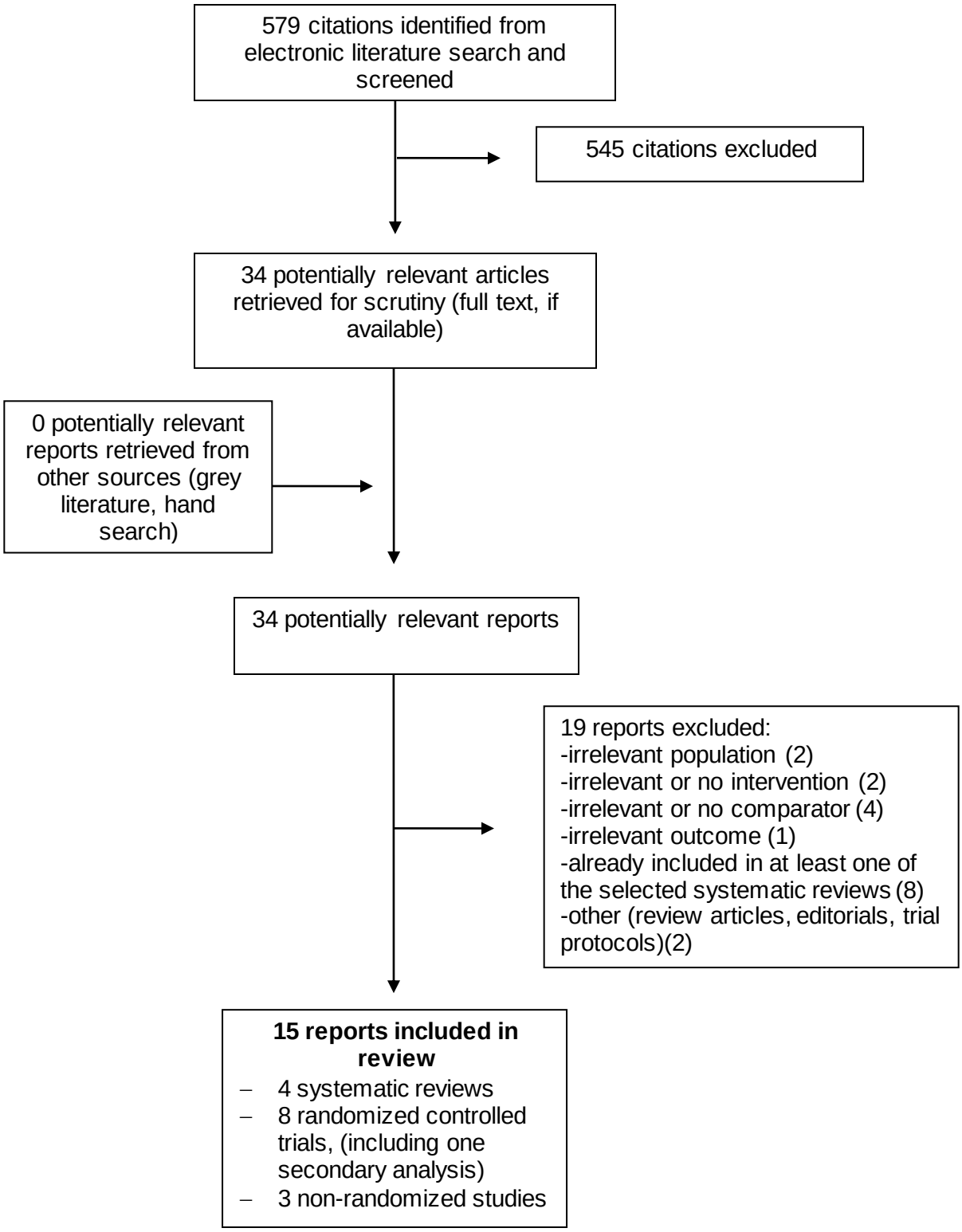
78. Hadgkiss EJ, Jelinek GA, Weiland TJ, Pereira NG, Marck CH, van der Meer DM. Methodology of an international study of people with multiple sclerosis recruited through web 2.0 platforms: demographics, lifestyle, and disease characteristics. Neurol Res Int [Internet]. 2013 [cited 2016 Mar 3];2013:580596. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3649686

79. Burton JM, Kimball S, Vieth R, Bar-Or A, Dosch HM, Cheung R, et al. A phase I/II dose-escalation trial of vitamin D3 and calcium in multiple sclerosis. Neurology [Internet]. 2010 Jun 8 [cited 2016 Mar 9];74(23):1852-9. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2882221

80. Merck KGaA. Supplementation of VigantOL® Oil versus placebo as add-on in patients with relapsing remitting multiple sclerosis receiving Rebif® treatment (SOLAR). 2011 Jan 26 [cited 2015 Mar 4; last updated 2015 Jun 3]. In: ClinicalTrials.gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT01285401 Identifier: NCT01285401.

81. Dorr J, Ohlraun S, Skarabis H, Paul F. Efficacy of vitamin D supplementation in multiple sclerosis (EVIDIMS Trial): study protocol for a randomized controlled trial. Trials [Internet]. 2012 [cited 2016 Mar 3];13:15. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3298796

**APPENDIX 1: Selection of Included Studies**



## APPENDIX 2: Characteristics of Included Publications

To convert vitamin D supplementation dose from **µg** to **IU** multiply by 40 — to convert from **IU** to **µg** divide by 40
To convert serum 25(OH)D levels from **ng/mL** to **nmol/L** multiply by 2.5 — to convert from **nmol/L** to **ng/mL** divide by 2.5

**Table A1:** Characteristics of Included Systematic Reviews and Meta-Analyses

| First Author, Publication Year, Country; Search dates, Databases | Types and numbers of primary studies included | Population Characteristics[c] | Intervention | Comparator(s) | Clinical Outcomes, Length of Follow-Up |
|---|---|---|---|---|---|
| Autier, 2014, France[58,a]<br><br>Inception to December 2012 PubMed and Embase | Prospective and nested-case control studies, n = 0[b] RCTs, n = 6<br><br>Excluded cross-sectional and case-control studies to avoid reverse causation bias | Patients (≥18 years) with MS | Vitamin D2 or Vitamin D3 (71 to 800 µg/day) without concomitant therapy with the exception of calcium supplements | Placebo (control group unspecified in some cases) | Disease course, risk of relapse, disability |
| Ganesh, 2013, Canada[59];<br><br>Inception to October 2012 Medline, Embase, and Pubmed | Any excluding case reports, expert opinions, or reviews, including unpublished studies, conference abstracts, ongoing studies, and terminated studies | Patients diagnosed with MS | Vitamin D supplementation | Placebo or alternative comparator | MS disease activity (clinical severity [relapse risk, disability, radiological measures of MS], and immunological activity [levels, proportions, or action of various inflammatory markers]) |
| James, 2013, United Kingdom[60];<br><br>MEDLINE 1950 to September 2012, EMBASE 1980 to September 2012, Cochrane Library and Google Scholar in September 2012 | RCTs, n = 5 | Adult patients with MS | Any dose of vitamin D (D3, D2, or calcitriol) supplement, m = 129 | Low dose vitamin D (n = 35) or placebo (n = 90) | Relapse, 26 to 96 week follow-up;<br><br>25(OH)D levels;<br><br>Safety;<br><br>Dropouts |

| Table A1: Characteristics of Included Systematic Reviews and Meta-Analyses | | | | | |
|---|---|---|---|---|---|
| **First Author, Publication Year, Country; Search dates, Databases** | **Types and numbers of primary studies included** | **Population Characteristics[c]** | **Intervention** | **Comparator(s)** | **Clinical Outcomes, Length of Follow-Up** |
| Pozuelo-Moyano, 2013, Spain[61];<br><br>PubMed/Medline and Cochrane Central Register of Controlled Trials in August 2012 | Double-blind placebo-controlled RCTs, n = 5 | Patients with MS determined by various methods including McDonald criteria | Vitamin D supplementation (20000 IU weekly for one year to 300000 IU/month for six months) | Placebo | Disease progression (EDSS) or MS functional composite; relapse rate, proportion of relapse-free patients, cognitive functioning, health-related quality of life, neuroimaging parameters; Adverse effects |

[a]This systematic review investigated the effect of vitamin D status and supplementation on various health conditions, including MS. Only the studies and analyses focused on multiple sclerosis are reported.
[b]No intervention; therefore, irrelevant to this report
[c]A combination of relapsing-remitting and unspecified MS phenotypes were included in all reviews
25(OH)D = 25 hydroxyvitamin D; EDSS = Expanded Disability Status Scale; MS = multiple sclerosis; NR = not reported; RCT = randomized controlled trial

| Table A2: Characteristics of Included Randomized Controlled Trials | | | | | |
|---|---|---|---|---|---|
| **First Author, Publication Year, Country, Study Name** | **Study Design** | **Patient Characteristics** | **Intervention(s)** | **Comparator(s)** | **Clinical Outcomes, Length of Follow-Up** |
| ***TREATMENT*** | | | | | |
| *Vitamin D versus No Vitamin D* | | | | | |
| Äivo, 2015, Finland[62b] | Double blind RCT | Patients (18 to 55 years) with RRMS according to McDonald criteria and interferon- β use for at least three months, EDSS score ≤ 5, not pregnant and serum 25(OH)D ≤ 85 nmol/L for 12 months | Vitamin D3 20 000 IU as cholecalciferol in arachis oil (Dekristol) once weekly for 12 months plus interferon-β | Identical placebo capsules plus interferon-β | Inflammatory cytokines (IFN- γ, IL-17A, IL-2, IL-10, IL-9, IL-22, IL-6, IL-13, IL-4, IL5, IL-1β, TNF-alpha),<br><br>1 year |
| Ashtari, 2015, Iran[63] | Double blind RCT | Patients (18 to 55 years) with RRMS based on McDonald criteria[14], EDSS score <4 and no relapse 30 days before inclusion, no pregnancy | Vitamin D3 50000 IU every 5 days for 12 weeks plus interferon-β | Interferon-β and no restrictions on vitamin D supplementation | Serum IL-10 at 3 months; calcium and 25(OH)D concentrations,<br><br>12 weeks |

| Table A2: Characteristics of Included Randomized Controlled Trials | | | | | |
|---|---|---|---|---|---|
| **First Author, Publication Year, Country, Study Name** | **Study Design** | **Patient Characteristics** | **Intervention(s)** | **Comparator(s)** | **Clinical Outcomes, Length of Follow-Up** |
| Etemadifar, 2015, Iran[65] | Open-label RCT (single center) | Pregnant women (20 to 40 years) with 'clinically definite'[a] MS (phenotype unspecified), n = 52 eligible, n – 15 completed | Vitamin D3 50000 IU per week from 12 to 16 weeks gestation till delivery, patients remained on any current medications though they were unspecified | Routine care from 12 to 16 weeks gestation till delivery, patients remained on any current medications though they were unspecified | Serum 25(OH)D levels; EDSS score; number of relapse events during pregnancy and within 6 months after delivery; Adverse events |
| Røsjø, 2015, Norway[66] | Double blind placebo controlled RCT | Patients with RRMS (18 to 50 years) with EDSS score ≤ 4.5 | Vitamin D3 20000 IU per week plus calcium 500 mg/day and no restrictions on concomitant regular vitamin D supplementation or immunomodulatory treatment (i.e., IFN-β, glatiramer acetate, or natalizumab) for 96 weeks | Placebo capsules and no restrictions on concomitant regular vitamin D supplementation or immunomodulatory treatment (i.e., IFN-β, glatiramer acetate, or natalizumab) for 96 weeks | 11 serum markers of inflammation, bone mineral density, clinical disease activity, disease progression, 25(OH)D,<br><br>96 weeks |
| Toghianifar, 2015, Iran[67] | Double blind placebo controlled RCT | Patients with RRMS according to McDonald criteria, n = 94 | Vitamin D3 50000 IU every 5 days for 12 weeks plus interferon-β treatment | Placebo plus interferon-β treatment | Serum IL-17 levels,<br><br>12 weeks |
| *High-Dose versus Low Dose Vitamin D* | | | | | |
| Sotirchos, 2016, United States[37] | Double blind RCT | Patients (18 to 55 years) with RRMS (unclear how this was determined), n = 40 | Vitamin D3 10000 IU for 6 months plus multivitamin containing 400 IU of vitamin D3 and 1000 mg of calcium with (89%) or without (11%) immunomodulatory therapy | Vitamin D3 400 IU for 6 months plus multivitamin containing 400 IU of vitamin D3 and 1000 mg of calcium with immunomodulatory therapy | Baseline, 3 and 6-month measurements of 25-OH-D levels, adverse events, relapses, IL-17$^+$ CD4$^+$ T cells, central memory CD4$^+$ T cells, naïve CD4$^+$ T cells |

**Table A2**: Characteristics of Included Randomized Controlled Trials

| First Author, Publication Year, Country, Study Name | | Study Design | Patient Characteristics | Intervention(s) | Comparator(s) | Clinical Outcomes, Length of Follow-Up |
|---|---|---|---|---|---|---|
| Carmel Medical Centre Trial in Israel[77c] | Golan, 2013, Israel[36] | Double blind RCT | Patients with RRMS according to McDonald criteria, n = 45 | Vitamin D3 4370 IU plus interferon-β treatment for 1 year, n = 24 | Vitamin D3 800 IU plus interferon-β treatment for 1 year, n = 21 | Flu-like symptoms assessed monthly;<br><br>25-OH-D levels, calcium, PTH, cytokine levels (IL-17, IL-10, and IFN-γ)<br><br>EDSS, relapses, adverse events and quality of life,<br><br>1 year |
| ***PREVENTION*** | | | | | | |
| Derakhshandi, 2013, Iran[64] | | Double-blind parallel group RCT | Patients (20 to 40 years) with optic neuritis at risk of developing MS, n = 30 | Vitamin D3 50 000 IU weekly for 12 months until upper limit of 100 ng/mL achieved then maintained at lower dose | Placebo weekly for 12 months | Optic neuritis conversion to MS;<br><br>Imaging studies: Changes in the number of T1-hypointense and T2-hyperintense brain MRI lesions at various sites, the number of new T2 and gadolinium-enhancing lesions,<br><br>12 months |

[a]MRI, clinical or laboratory-supported diagnosis of definite MS, stable neurological functioning for at least 1-monyh prior to entry and an EDSS score ≤ 6, serum 25(OH)D < 20 ng/mL and a willingness to continue current medications
[b]Substudy of Finnish Vitamin D Study[48]
[c]Trial terminated due to low odds for proving primary hypothesis as indicated by interim analysis[77]
25(OH)D = 25 hydroxyvitamin D; EDSS = expanded disability status scale; IL = interleukin; 0MS = multiple sclerosis; RCT = randomized controlled trial; RRMS = relapsing remitting MS

**Table A3:** Characteristics of Included Non-Randomized Studies

| First Author, Publication Year, Country, Study Name | Study Design | Patient Characteristics | Covariates | Intervention(s) | Comparator(s) | Clinical Outcomes, | Length of Follow-Up |
|---|---|---|---|---|---|---|---|
| *PREVENTION* | | | | | | | |
| *Vitamin D versus No Vitamin D* | | | | | | | |
| *Environmental Factors in Multiple Sclerosis Study*<br><br>Cortese, 2015, Norway[68] | Multicenter retrospective case-control study | Adult patients diagnosed with MS for < 10 years (based on McDonald criteria), n = 2670 | Sun exposure, dietary intake of vitamin D, surrogate for BMI, infectious mononucleosis, smoking, education, family history of MS | Self-reported cod liver oil consumption (vitamin D dose specified, 5 mL = 400 IU) during childhood, adolescence and early adulthood, n = 1479 (n = 518 (54.4%) cases, and n = 960 (55.8%) controls) (unclear if patients taking any other medication) | No cod liver oil (vitamin D dose specified) during childhood, adolescence and early adulthood, n = 1192 (unclear if patients taking any other medication) | Risk of MS | No follow-up, observation period from childhood until middle-age |
| *TREATMENT* | | | | | | | |
| *Vitamin D versus No Vitamin D* | | | | | | | |
| Jelinek, 2015, Australia[69a] | Cross-sectional survey | Adults (≥18 years) diagnosed with MS by a medical doctor, n = 2133 | Deliberate sun exposure, latitude, gender, age, disability, physical activity, fish consumption | Vitamin D supplementation (1 to >5000 IU), n = 1803 (unclear if patients taking any other medication) | No vitamin D supplementation, n = 399 (unclear if patients taking any other medication) | Health-related quality of life (MSQOL-54); level of disability (Patient Determined Disease Steps scale), relapse rate | No follow-up asked to recall behavior over up to 5 years |
| Najafipoor, 2015, Iran[70] | Controlled before and after study | Patients with RRMS approved by neurologists according to the revised McDonald criteria, n = 40 | No covariates investigated | Vitamin D3 50000 IU per week for 6 months, with or without disease modifying treatment (interferon-β), n = 27 | No vitamin D3 supplementation, with or without disease modifying treatment (interferon-β), n = 13 | 25(OH)D levels, incremental Epstein-Barr virus antibody titers | 6 months |

[a]Additional study details contained in published protocol[78]
25(OH)D = 25 hydroxyvitamin D; IU = international units; MS = multiple sclerosis; MSQOL-54 = Multiple Sclerosis Quality of Life-54 Instrument; RRMS = relapsing remitting MS

## APPENDIX 3: Critical Appraisal of Included Publications

**Table A4:** Strengths and Limitations of Systematic Reviews and Meta-Analyses using AMSTAR[39]

| Strengths | Limitations |
|---|---|
| Autier, 2014[58a] | |
| • Comprehensive literature search performed on multiple databases<br>• List of included and excluded studies provided in supplementary appendix<br>• Study authors declared no conflict of interest<br>• Study quality considered in the discussion section | • No reference to a priori protocol<br>• Unclear number of authors involved in study selection and data extraction<br>• Limited grey literature search (reference lists only)<br>• Unclear if unpublished data was searched for or included<br>• Many characteristics of included studies not reported (e.g., comparators and specific vitamin D doses)<br>• No formal quality assessment<br>• Explicit discussion of quality not made in formulation of conclusions<br>• No pooling of studies was performed<br>• Narrative synthesis of findings was limited and lacked context<br>• Likelihood of publication bias not assessed |
| Ganesh, 2013[59] | |
| • Duplicate study selection and data extraction conducted<br>• Comprehensive literature search performed on multiple databases<br>• Grey literature search included reference list searching, searching for unpublished trials and conference proceedings<br>• List of included studies and study characteristics provided<br>• Scientific quality of primary studies assessed using the Cochrane Risk of Bias tool<br>• Publication bias not assessed, but unpublished data included in the review<br>• Study authors declared no conflict of interest | • Review protocol mentioned but not referenced<br>• List of excluded studies not provided<br>• Quality of studies only briefly mentioned in formulation of conclusions<br>• Studies summarized narratively, no pooling of results and no explanation given for lack of pooling<br>• |
| James, 2013[60] | |
| • Comprehensive literature search performed<br>• Grey literature search included screening for unpublished trials on multiple trial registration repositories, hand searching of reference lists<br>• List of included studies and study characteristics provided<br>• Study quality (determined through informal methods) considered in the formulation of conclusions<br>• Heterogeneity was assessed using the $I^2$ statistic<br>• Publication bias assessed visually using a funnel plot and statistically using Eggers test<br>• Authors declared no conflict of interest | • No reference to a priori review protocol<br>• Unclear number of authors involved in study selection and data extraction<br>• List of excluded studies not provided<br>• No formal assessment of study quality |
| Pozuelo-Moyano, 2013[61] | |
| • Two authors were involved in study selection<br>• Comprehensive search performed on multiple databases<br>• Grey literature search included reference list searches of included articles as well as vitamin D in MS studies known by the reviewers<br>• List of included studies and study characteristics provided | • No reference to a priori review protocol<br>• Unclear number of authors involved in data extraction<br>• List of excluded studies not provided<br>• No formal assessment of publication bias; no apparent search for unpublished data |

**Table A4:** Strengths and Limitations of Systematic Reviews and Meta-Analyses using AMSTAR[39]

| Strengths | Limitations |
|---|---|
| • Quality of studies assessed using the Jadad checklist<br>• Quality considered in formulation of conclusions<br>• Pooling of results deemed inappropriate due to heterogeneity in dosing and outcome measures<br>• Funding sources acknowledged and no conflict of interest noted | |

[a]Note that this review assessed the effectiveness of vitamin D in various health conditions. The observations presented apply specifically to the information summarized for MS, where possible. Reporting quality was variable across disease conditions.
MS = multiple sclerosis

**Table A5:** Strengths and Limitations of Randomized Controlled Trials using Downs and Black[40]

| Strengths | Limitations |
|---|---|
| ***Treatment***<br>*Vitamin D versus No Vitamin D* | |
| Äivo, *2015*[62] | |
| *Reporting*<br>• Study objectives stated in the methods section<br>• Main outcomes to be measured clearly described in methods section<br>• Patient characteristics clearly described in primary study<br>• Interventions of interest clearly described in primary study<br>• Distribution of principal confounders clearly described<br>• Main study findings clearly described<br>• Estimates of random variability presented for main outcomes<br>• Several relevant adverse events assessed<br>• Reasons for drop-outs reported<br>• Actual probability values reported<br>*External Validity*<br>• Patients representative of population of patients treated at outpatient polyclinics of various hospitals across Finland over an unspecified time period<br>• Staff, places and facilities likely representative of context of care for patients across Finland<br>*Internal Validity – Bias*<br>• Investigator, patients, and staff blinded to treatment allocation<br>• Statistical tests used to assess the main outcomes were appropriate<br>• Compliance inferred through pill counting and measurement of changes in 25(OH)D levels<br>• Main outcome measures accurate<br>*Internal Validity – Confounding*<br>• Patients in different groups recruited from the same population<br>• Randomization conducted using software generating randomly permuted blocks for within center randomization | *Reporting*<br>• Limited patient characteristics presented in secondary analysis, several relevant baseline characteristics such as smoking and skin color not disclosed<br>• Not all potential adverse events reported<br>• Characteristics of patients for whom relevant measurements were unavailable were unclear<br>*External Validity*<br>• No disclosure of differences between willing and unwilling participants<br>*Internal Validity – Bias*<br>• Unclear if the analysis was pre-planned<br>• No adjustment made for different length of follow-up<br>*Internal Validity – Confounding*<br>• Unclear whether all patients were recruited over the same timeframe<br>• Randomization lists were concealed from patients and study staff<br>• No intention to treat analysis conducted, potentially inadequate adjustment for confounding<br>• Losses to follow-up not taken into account<br>*Power*<br>• Authors state that due to the small sample size the trial was not powered to detect differences in clinical outcomes |

**Table A5:** Strengths and Limitations of Randomized Controlled Trials using Downs and Black[40]

| Strengths | Limitations |
|---|---|
| Ashtari, 2015[63] | |
| *Reporting*<br>• Hypothesis clearly stated in the introduction section<br>• Main outcomes and demographic characteristics stated in the introduction and methods sections<br>• Limited baseline patient characteristics described<br>• Intervention of interest clearly described<br>• Distribution of principal confounders provided<br>• Main study findings clearly described<br>• Estimates of random variability provided for main outcomes<br>• Actual probability values reported<br>*External Validity*<br>• Staff, places and facilities where patients were treated likely representative of the treatment patients will receive (outpatient treatment)<br>*Internal Validity – Bias*<br>• Patients and laboratory staff blinded to treatment allocation<br>• No unplanned analyses were apparent<br>• Statistical tests used to assess the main outcomes were appropriate and specific to non-normal and normal data<br>• Compliance was adequate and assessed by observing changes in 25(OH)D levels<br>• Main outcome measures accurate<br>*Internal Validity – Confounding*<br>• All participants recruited over the same period of time<br>• Study subjects randomized to intervention groups<br>• Patients and laboratory staff blinded to treatment allocation until recruitment was complete<br>• Losses to follow-up reported | *Reporting*<br>• Several key baseline characteristics (i.e., BMI, skin color, smoking status, vitamin D supplementation) not disclosed<br>• Several potential confounders not assessed<br>• Rate of adverse events associated with the intervention not reported<br>• Patients who did not attend final follow-up visits were eliminated from the study and their characteristics were not described – it was simply disclosed that they did not have any symptoms or medical problems or medication side effects<br>*External Validity*<br>• Patient sampling method unclear<br>• Differences between willing and unwilling participants unclear<br>*Internal Validity – Bias*<br>• Unclear whether outcome assessors (e.g., interpreters of lab results) were blinded<br>• No adjustment for difference lengths of follow-up that occurred due to premature exit from the trial<br>*Internal Validity – Confounding*<br>• Unclear whether treatment allocation was concealed from staff physician<br>• Intention to treat analysis was not conducted despite dropouts<br>• EDSS score differed between treatment groups, but was not adjusted for in analyses<br>• Unclear whether volume of patients lost to follow-up is large enough to affect main findings<br>*Power*<br>• No sample size or power calculation disclosed |
| Etemadifor, 2015[65] | |
| *Reporting*<br>• Hypothesis clearly stated in introduction section<br>• Main outcome measures summarized in the methods section<br>• Limited baseline patient characteristics presented<br>• Intervention of interest clearly described<br>• Main findings of the study clearly described<br>• Estimates of random variability reported for main outcomes<br>• Limited adverse event reporting<br>• No patients lost to follow-up within the 6 month observation period<br>*External Validity*<br>• Subjects representative of patients with clinically definite MS with intention to become pregnant who were treated at outpatient clinics of a University Hospital in Iran; recruited consecutively<br>*Internal Validity – Bias*<br>• No losses to follow-up; therefore no adjustment required<br>• No unplanned analyses were apparent<br>• Statistical tests used to assess the main outcomes | *Reporting*<br>• Several key baseline characteristics (i.e., BMI, skin color, smoking status, vitamin D supplementation) not disclosed<br>• Distribution of potential confounders not provided<br>• Several potential adverse events (e.g., hypercalcemia) not reported<br>• p-values reported categorically, rather than by exact value<br>*External Validity*<br>• Unclear whether subjects prepared to participate were representative of the entire recruitment pool<br>• Staff, places and facilities where patients were treated only representative of outpatient MS clinics; patients may receive higher standard of care<br>*Internal Validity – Bias*<br>• Study authors reported that "patients who…failed to attend for follow-up visits…were also excluded" suggesting possible follow-up exclusions that were not reported<br>• No blinding of study subjects or outcome assessors (open label) |

**Table A5:** Strengths and Limitations of Randomized Controlled Trials using Downs and Black[40]

| Strengths | Limitations |
|---|---|
| were appropriate<br>• Compliance assessed by inquiring about missed doses and counting unused supplements<br>• Main outcome measures accurate[74]<br>*Internal Validity – Confounding*<br>• Patients in different intervention groups were recruited from the same hospital population<br>• Patients were recruited over an 18 month period<br>• Patients randomized according to a computer-generated list<br>• No losses to follow-up; no adjustment required | • Outcome of compliance assessment not reported<br>*Internal Validity – Confounding*<br>• Seasonal differences in time of recruitment of patients were not taken into account<br>• Concealment of random allocation unclear<br>• Adjustment for potential confounders was insufficient<br>*Power*<br>• Study authors stated that the trial was not powered to properly address clinical outcomes |
| Røsjø, 2015[66] | |
| *Reporting*<br>• Study aims and reference made to a priori study protocol made in introduction and methods section<br>• Main outcome measures summarized in methods section<br>• Characteristics of patients included in the study clearly described<br>• Intervention of interest clearly described<br>• Main findings of the study clearly described<br>• Estimates of random variability provided for main outcomes<br>• The number of patients missing from analysis reported<br>• Actual probability values reported<br>*External Validity*<br>• Patients representative of adult patients with RRMS<br>*Internal Validity – Bias*<br>• Patients and assessors blinded to allocation throughout the study<br>• No apparent data dredging<br>• Statistical tests used to assess the main outcomes were appropriate<br>• Main outcome measures accurate<br>*Internal Validity – Confounding*<br>• Patients in all treatment groups recruited from the same population<br>• Patients in all treatment groups recruited over the same time period of 25 months<br>• Patients were randomized and allocation was concealed from study personnel<br>• Adjusted analysis was performed for potential confounding factors that could affect the relationship between vitamin D supplementation and inflammation | *Reporting*<br>• No reporting on adverse events<br>*External Validity*<br>• No disclosure of differences between willing and unwilling participants<br>*Internal Validity – Bias*<br>• No adjustment for dropouts<br>• Compliance with supplementation protocol not assessed<br>*Internal Validity – Confounding*<br>• A small number of patients were lost to follow-up, but this was not taken into account in analysis<br>*Power*<br>• No power or sample size calculation disclosed |
| Toghianifar, 2015[67] | |
| *Reporting*<br>• Study aim described clearly in introduction<br>• Main outcomes described clearly in introduction and methods section<br>• Limited baseline patient characteristics presented<br>• Intervention of interest clearly described<br>• Distribution of potential confounders presented<br>• Main findings clearly described<br>• Estimates of random variability provided alongside | *Reporting*<br>• Several key baseline characteristics (i.e., BMI, skin color, smoking status, vitamin D supplementation) not disclosed<br>• No adverse events apart from aggregate serum calcium levels (not rate of hypercalcemia) reported<br>• Losses to follow-up reported but it was only commented that "They did not have any symptom or medical problem or medication side effects"[67]<br>*External Validity* |

**Table A5:** Strengths and Limitations of Randomized Controlled Trials using Downs and Black[40]

| Strengths | Limitations |
|---|---|
| main outcomes<br>• Actual probability values reported for main outcomes<br>*Internal Validity – Bias*<br>• Patient and laboratory staff were blinded to intervention<br>• No apparent data dredging<br>• Length of follow-up the same for all study groups<br>• Statistical tests used to assess the main outcomes were appropriate<br>• Compliance assessed by observing serum 25(OH)D levels over course of intervention<br>• Main outcome measures accurate<br>*Internal Validity – Confounding*<br>• Patients in all study groups recruited from the same population<br>• Patients were randomized to intervention groups<br>*Power*<br>• Authors declared that 80% power was established for all analyses | • Insufficient information about source population provided to judge how representative subjects were<br>• No disclosure of differences between willing and unwilling participants<br>• No information provided regarding staff, places, and facilities where patients were treated<br>*Internal Validity – Bias*<br>• Timeframe for recruitment of study participants is unclear<br>*Internal Validity – Confounding*<br>• Concealment of random allocation from study physicians, personnel and patients unclear<br>• Minimal adjustment for confounding conducted using multivariate regression with EDSS measures as a covariate; many other potential confounders not considered<br>• Losses to follow-up not taken into account in analysis<br>*Power*<br>• Unclear whether dropouts critically affected study power; no sample size calculation disclosed |
| ***High-Dose versus Low Dose Vitamin D*** | |
| Sotirchos, 2016[37] | |
| *Reporting*<br>• Study objectives, a study protocol and trial registration including a priori study design were reported in the introduction and methods sections<br>• Baseline patient characteristics reported<br>• Interventions of interest clearly described<br>• Distribution of potential confounders clearly described<br>• Estimates of random variability provided for main outcomes<br>• Most important adverse events captured<br>• Actual probability values reported<br>*Internal Validity – Bias*<br>• Patients and outcome assessors blinded to study intervention dose, though they were aware of the study intervention<br>• Patients interviewed by telephone monthly between visits to assess compliance<br>• No apparent data dredging<br>• Statistical tests used to assess main outcomes were appropriate<br>• Main outcome measures accurate<br>*Internal Validity – Confounding*<br>• Participants stratified by sex and randomized by blocks of four to reduce sex differences in response to vitamin D supplementation<br>• Random allocation concealed from patients and staff<br>• Distribution of confounders balanced across groups; unnecessary to adjust<br>• Losses to follow-up reported but not accounted for<br>*Power*<br>• Sample size and power calculation disclosed | *Reporting*<br>• Main outcomes contained, but not explicitly stated in the methods section<br>• Some relevant baseline patient characteristics missing (e.g., BMI, smoking status)<br>• Characteristics of patients lost to follow-up not described<br>*External Validity*<br>• Source population and sampling method unclear<br>• No disclosure of differences between wiling and unwilling participants<br>• Generalizability of staff, places, and facilities where patients were treated unclear as only location (hospital) was disclosed<br>*Internal Validity – Bias*<br>• No adjustment of different lengths of follow-up due to discontinuation, dropouts, or change in treatment<br>*Internal Validity – Confounding*<br>• Unclear whether patients in each group were recruited from the same population or over the same time period<br>• Patients were aware that they were receiving vitamin D in both groups, may have been compelled to increase their own dose<br>• Intention to treat analysis not used<br>• Not all potential confounders considered at baseline |

**Table A5:** Strengths and Limitations of Randomized Controlled Trials using Downs and Black[40]

| Strengths | Limitations |
|---|---|
| Golan, 2013[36a] | |
| *Reporting*<br>• Primary and secondary objectives presented in introduction section<br>• Main outcomes to be measured clearly described in methods section<br>• Limited patient characteristics presented<br>• Interventions of interest clearly described<br>• Distribution of principal confounders clearly described<br>• Estimates of random variability provided for main outcomes<br>• Some adverse events reported<br>• Reasons for drop-outs at various follow-up time points presented<br>• Actual probability values reported for main outcomes<br>*External Validity*<br>• Subjects likely representative of entire population of patients with RRMS who attended the clinic at an MS center in Israel during a five-month period<br>*Internal Validity – Bias*<br>• Participants, outcomes assessors, and investigators were blinded to treatment allocation<br>• Statistical tests used to assess main outcomes were appropriate<br>• Main outcome measures accurate<br>*Internal Validity – Confounding*<br>• All study patients recruited from the same sources population over the same five month period of time<br>• "Assignment to groups was randomly set in advance according to recruitment order."[36]<br>• Random allocation concealed from patients and staff<br>• Proportion of patients lost to follow-up reported | *Reporting*<br>• Some relevant baseline characteristics (e.g., BMI, smoking status, skin color, vitamin D supplementation) not reported<br>• Not all relevant adverse events were reported on<br>• Characteristics of patients lost to follow-up not described<br>*External Validity*<br>• No disclosure of differences between wiling and unwilling participants<br>• Staff, places and facilities only representative of specific MS care facilities; patients may receive higher standard of care<br>*Internal Validity – Bias*<br>• No apparent adjustment for different lengths of follow-up<br>• Compliance assessed verbally during phone conversations and clinic visits, but drop in vitamin D levels at 12 months that was observed may indicate poor compliance<br>*Internal Validity – Confounding*<br>• Potential confounding was identified as an issue by authors, no adjustments made for seasonal variation despite a potential influence on the outcomes<br>• Unclear whether loss to follow-up affected the main findings as no adjustments were performed<br>*Power*<br>• Insufficient power to detect difference in the main outcomes based on the targets set by initial power analysis |
| ***Prevention***<br>*Vitamin D versus No Vitamin D* | |
| Derakhshandi, 2013[64] | |
| *Reporting*<br>• Hypothesis clearly stated in introduction<br>• Main outcomes clearly described in methods section<br>• Limited patient characteristics presented<br>• Intervention of interest clearly described<br>• Main findings of study clearly described<br>• Estimates of random variability provided for main outcomes<br>• Some relevant adverse events were reported on<br>• Reasons for loss to follow-up presented<br>• Actual probability values reported<br>*External Validity*<br>• Subjects representative of patients diagnosed with optic neuritis at ophthalmology referral centers during a 6 month period in Iran<br>*Internal Validity – Bias*<br>• Patients and outcome assessors were blinded to intervention<br>• No apparent data dredging<br>• Statistical tests used to assess main outcomes were | *Reporting*<br>• Some potentially important patient characteristics such as BMI, ethnicity and skin color, smoking status, EDSS, number of relapses were not discussed<br>• Very limited comparison of principal confounders between groups<br>• Not all relevant adverse events reported on<br>• Characteristics of patients lost to follow-up not described<br>*External Validity*<br>• No disclosure of differences between wiling and unwilling participants<br>• Staff, places, and facilities where patients are treated only representative of the care patients would receive at specialized ophthalmology centers; may not be generalizable to broader optic neuritis patients or patients at risk of MS<br>*Internal Validity – Bias*<br>• No adjustment for different lengths of follow-up |

**Table A5:** Strengths and Limitations of Randomized Controlled Trials using Downs and Black[40]

| Strengths | Limitations |
|---|---|
| appropriate<br>• Main outcome measures accurate<br>*Internal Validity – Confounding*<br>• Patients in both groups recruited from the same population over the same period of time<br>• Randomization of patients was conducted using permuted-block randomization in blocks of two | • Patients with poor compliance were excluded from analysis after 3 months<br>*Internal Validity – Confounding*<br>• Some patients were recruited during summer months and some during fall/winter months; effect on baseline vitamin D status unclear<br>• Randomization results concealed from patients and staff<br>• Inadequate adjustment for confounding<br>• Losses to follow-up reported but not accounted for<br>*Power*<br>• No power or sample size calculation disclosed |

[a]Both primary and secondary analyses critiqued together
BMI = body mass index; EDSS = Expanded Disability Status Scale; MS = multiple sclerosis; RRMS = relapsing remitting multiple sclerosis

**Table A6:** Strengths and Limitations of Non-Randomized Studies using Downs and Black[40]

| Strengths | Limitations |
|---|---|
| Cortese, 2015[68] | |
| *Reporting*<br>• Study objectives clearly stated in introduction<br>• Main outcomes clearly described in methods section<br>• Patient characteristics clearly described<br>• Intervention of interest clearly described<br>• Distribution of potential confounders presented for cases and controls<br>• Main findings clearly described<br>• Estimates of random variability provided<br>• No losses to follow-up as the survey was cross-sectional; however, recall bias is a risk<br>• Probability values reported categorically<br>*External Validity*<br>• Context of vitamin D treatment is likely representative of intake of vitamin D as cod liver oil that occurs in the Norwegian community<br>*Internal Validity – Bias*<br>• Statistical tests used to assess main outcomes were appropriate<br>• Main outcome measures were accurate<br>*Internal Validity – Confounding*<br>• All participants were recruited from the same Norwegian MS-registry and Biobank during 2008 | *Reporting*<br>• No adverse events reported<br>*External Validity*<br>• More women were willing to participate than men; no other characteristics of willing versus unwilling participants were reported<br>• Patients only representative of MS patients in Norway with a disease duration shorter than 10 years<br>*Internal Validity – Bias*<br>• No blinding of study subjects or outcome assessors<br>• Doses of vitamin D were derived from self-reported intake of cod-liver oil and may not be exact<br>• No direct evidence of data-dredging; however, with absence of protocol and the variety of analyses presented, excessive analysis may have been conducted<br>• No measures of compliance with cod liver oil consumption over time were recorded<br>*Internal Validity – Confounding*<br>• No randomization of study subjects<br>• Adjustment for potential confounding factors was made using multiple linear regression<br>*Power*<br>• Authors commented that the study might be underpowered to observed "subtle" differences |
| Jelinek, 2015[69] | |
| *Reporting*<br>• Study aims and hypotheses presented in introduction<br>• Main outcomes to be measures clearly described in methods section<br>• Patient characteristics clearly described<br>• Intervention of interest clearly described<br>• Cross-sectional survey, no losses to follow-up<br>• Actual probability values reported<br>*External Validity* | *Reporting*<br>• Distribution of potential confounders in each group not described<br>• Main findings of the study underreported – regression coefficients, levels of significance not presented for all findings<br>• Adverse events not reported<br>*External Validity*<br>• Sampling included web-based advertising, may reduce generalizability to MS patients not engaged |

**Table A6:** Strengths and Limitations of Non-Randomized Studies using Downs and Black[40]

| Strengths | Limitations |
|---|---|
| • Use of intervention of interest likely generalizable to use of vitamin D supplements in the community<br>*Internal Validity – Bias*<br>• No apparent data dredging based on analyses presented in the study protocol<br>• Statistical tests used to assess the main outcomes were appropriate<br>• Main outcome measures accurate<br>*Internal Validity – Confounding*<br>• All patients recruited from the same broad population over the same 15 week period<br>• Adjustment for confounding performed for each main outcome using regression analysis<br>• No losses to follow-up due to study design<br>*Power*<br>• The sample size was large and all analyses were likely sufficiently powered | online<br>• Survey limited to English speakers<br>• Differences between willing and unwilling participants unclear<br>• Context of care may not extend to patients with more severe disease or those who are treated with vitamin D on the advice of a healthcare practitioner<br>*Internal Validity – Bias*<br>• No blinding of study participants or outcome assessors<br>• Temporality of vitamin D supplementation and development of MS could not be determined<br>• Compliance with intervention could not be assessed as it was self-reported<br>• Not all potential covariates were considered in the regression models<br>*Internal Validity – Confounding*<br>• No randomization of study subjects<br>*Power*<br>• No power or sample size calculation disclosed |
| Najafipoor, 2015[70] | |
| *Reporting*<br>• Study objective stated in introduction<br>• Main outcomes clearly described in introduction and methods section<br>• Intervention of interest clearly described<br>• Characteristics of patients included in study described<br>• Main study findings clearly described<br>• Estimates of random variability presented for main outcomes<br>• Actual probability values reported<br>*Internal Validity – Bias*<br>• No apparent data dredging<br>• Statistical test used to assess main outcomes were appropriate<br>• Main outcome measures accurate<br>*Internal Validity – Confounding*<br>• Patients in both groups recruited from the same population<br>• Recruitment was staggered over two periods (Autumn and winter, and spring and summer) to reduce potential bias of a narrow recruitment period | *Reporting*<br>• Distribution of potential confounders was not reported<br>• Very limited adverse event reporting<br>• Number of patients lost to follow-up unclear<br>*External Validity*<br>• Sampling method unclear, not certain whether participants are representative of all RRMS patients<br>• No disclosure of differences between willing and unwilling participants<br>• Patients were recruited from an MS clinic in Iran, therefore, context may not be generalizable to the treatment all MS patients receive, may be a higher standard of care<br>*Internal Validity – Bias*<br>• Blinding of patients and outcome assessors unclear<br>• Losses to follow-up not reported on, unclear whether adjustment required<br>• Compliance with intervention unclear<br>*Internal Validity – Confounding*<br>• Authors state that patients in the intervention group were 'randomly selected'; however, no method of randomization is described<br>• Insufficient and unclear adjustment for potential confounding factors<br>• No apparent losses to follow-up so they were not taken into account in analyses<br>*Power*<br>• No power or sample size calculation disclosed |

MS = multiple sclerosis; RRMS = relapsing remitting MS

## APPENDIX 4: Main Study Findings and Author's Conclusions

**Table A7:** Summary of Findings of Included Systematic Reviews and Meta-Analyses

| Outcome | Vitamin D | Comparator | Effect estimate | Author's Conclusions |
|---|---|---|---|---|
| *Autier, 2014*[58a] | | | | |
| *Effectiveness Outcomes* | | | | |
| | **Vitamin D without concomitant therapy** | **Placebo or unspecified comparator** | N/A | |
| Relapse or disability | NR | NR | NR | None of the six intervention studies that reported on the effect of vitamin D supplementation on clinical endpoints in multiple sclerosis had results that were suggestive of a benefit |
| *James, 2013*[60] | | | | |
| *Effectiveness Outcomes* | | | | |
| | **High dose vitamin D** | **Placebo or low dose vitamin D** | **OR** | |
| Relapse, n = 5 studies | 30/129 | 29/125 | 0.98 (9,44 to 2.17), $I^2$ = 36% | There was no significant difference in the odds of relapse between high dose vitamin D and placebo or low-dose vitamin D-treated patients |
| ***Subgroup analyses*** | | | | |
| Relapse in patients treated for ≥ 52 weeks, n = 3 studies | 20/83 | 25/80 | 0.71 (0.35 to 1.44), $I^2$ = 2% | Subgroup analyses of trials focused on long-term follow-up (≥ 52 weeks), placebo only comparison, and use of vitamin D3 only were consistent with the overall findings of no significant difference in the odds of relapse between groups |
| Relapse in trials restricting to placebo comparison, n = 3 studies | 22/94 | 20/90 | 1.08 (0.53 to 2.19), $I^2$ = 0% | |
| Relapse in vitamin D3 only studies, n = 3 | 18/93 | 20/88 | 0.82 (0.33 to 2.02), $I^2$ = 32% | |
| *Safety Outcomes*[a] | | | | |
| Serious adverse events | No incidence of serious adverse events, nephrolithiasis, renal dysfunction or hypercalcemia were reported | | | |
| Nephrolithiasis | | | | |
| Renal dysfunction | | | | |
| Hypercalcemia | | | | |
| Constipation | Low rates of constipation were reported by two primary studies | | | |
| *Ganesh, 2013*[59a,b] | | | | |
| *Efficacy and Safety Outcomes* | | | | |
| Inflammation markers | Evidence from two double-blind placebo controlled trials suggests that:<br>• 6 months of supplementation with modest doses (1000 IU per day) of vitamin D significantly increases serum levels of TGF-β1 (an anti-inflammatory cytokine)<br>• Increasing doses of vitamin D over one year resulted in suppressed PBMC proliferative responses to neuron antigens, with no significant change in disease-associated PBMC responses.<br>The authors noted that changes in surrogate biomarkers may not translate to actual clinical benefit. | | | |
| Clinical outcomes | Twelve randomized controlled trials observed:<br>• Non-significant reductions in relapse event, reductions in T-cell proliferation, no safety concerns with escalating supplementation for one year[79]<br>• No significant difference in EDSS scores or MRI lesions after 6 months[46] | | | |

| Table A7: Summary of Findings of Included Systematic Reviews and Meta-Analyses | | | | |
|---|---|---|---|---|
| **Outcome** | **Vitamin D** | **Comparator** | **Effect estimate** | **Author's Conclusions** |
| | • No significant differences in clinical outcomes (though one study was not properly powered,[44] and the other used a relatively low dose of vitamin D)[47]<br>• One year of supplementation with 20 000 IU per day resulted in fewer new MRI lesions and a trend towards reduced disability accumulation and improved tandem walk time[48]<br>• Further unpublished ongoing studies likely to contribute further evidence regarding therapeutic vitamin D supplementation in MS[50]<br>• NNT to prevent a relapse ranged across comparative studies from 4.76 to 25[44,47,52,79]<br>• Authors noted that issues such as the wide range of doses, small sample sizes, and varying comparators and severity of disease impede the ability to make conclusions regarding the effectiveness of vitamin D supplementation in MS | | | |
| *Pozuelo-Moyano, 2013*[61a] | | | | |
| Overall findings | Four studies showed no effect of vitamin D on MS-related outcomes,[35,44,46,47] while one showed potential benefits.[43,48] The authors commented that there is very limited evidence speaking to the effectiveness of vitamin D supplementation in MS. Further, they raised concerns regarding variable dosing, type of vitamin D provided, outcome measures, and small sample sizes. Studies were likely underpowered to detect an effect; therefore, it is unclear whether well-designed large-scale RCTs would produce different outcomes than the studies reviewed. The authors anticipate that results from two ongoing studies (SOLAR[80] and EVIDIMS[81]) will contribute valuable evidence to this line of inquiry. | | | |
| Imaging studies | • No significant difference in the mean number of brain gadolinium-enhancing lesions with 300 000 IU per month injections[46], 6000 IU per day versus 1000 IU per day,[35]<br>• No significant difference in the total volume of T2 lesions with 6000 IU per day versus 1000 IU per day[35]<br>• Statistically significant reduction in the quantity of T1 enhancing lesions and T2 lesion volume with 20 000 IU per week for 1 year[48] — Effects more pronounced in subgroup of patients with at least one relapse during the year preceding or enhancing T1 lesions at baseline[43] | | | |
| Relapse rates | • No significant difference in relapse rates between groups with 300 000 IU per month injections[46]<br>• Significantly higher proportion of relapse in patients receiving high dose (6000 IU) versus low dose (1000 IU) vitamin D2, p = 0.04[35]<br>• No significant difference in relapse rate in patients who received low dose calcitriol for 12 months versus placebo[47] | | | |
| T cell function | • Lymphocyte proliferation significantly decreased in vitamin D-treated patients with 300 000 IU per month injections[46] | | | |
| EDSS | • Higher exit EDSS (p = 0.05) in high dose vitamin D2 (6000 IU) versus low dose (1000 IU) groups[35]<br>• No significant change in EDSS in patients who received low dose calcitriol for 12 months versus placebo[47] | | | |
| Disease activity | • Weekly 20 000 IU doses of vitamin D3 for 96 weeks did not result in changes in disease activity[44] | | | |
| Fatigue severity score | • Weekly 20 000 IU doses of vitamin D3 for 96 weeks did not result in changes in fatigue severity score[44] | | | |
| *Adverse Events* | | | | |
| Three studies reported on adverse events, which were deemed relatively mild in severity and comprised of gastrointestinal side effects such as diarrhea, constipation, dyspepsia, fever, fatigue and headache.[44,47,48] | | | | |

[a]Results presented narratively
[b]Results on treatment with vitamin D analogues, or studies without comparators not presented
EDSS = Expanded Disability Status Scale; IU = international units; PBMC = peripheral blood mononuclear cell; MS = multiple sclerosis; MRI = magnetic resonance imaging; OR = odds ratio; RCT = randomized controlled trial

**Table A8:** Summary of Findings of Included Randomized Controlled Trials

| Outcome | Vitamin D | Comparator | Effect estimate | | Author's Conclusions |
|---|---|---|---|---|---|
| ***Treatment*** *Vitamin D versus No Vitamin D* | | | | | |
| *Aivo, 2015*[62] | | | | | |
| *Adverse Events* | | | | | |
| | **D3 20 000 IU for 12 months** | **Identical placebo tablets** | **N/A** | | |
| Hypercalcemia | 0(0) | NR | NR | | No incidence of hypercalcemia in the treatment group |
| Respiratory tract infections | NR | NR | NS | | The rate of respiratory tract infections and other mild infections did not differ significantly between groups |
| Other mild infections | NR | NR | NS | | |
| *Ashtari, 2015*[63] | | | | | |
| | **D3 50 000 IU every 5 days for 12 weeks** | **Placebo** | **p-value** | | |
| Serum 25(OH)D, ng/mL (median, IQR) at 3 months | 28.27 ± 29.03 to 84.67 ± 42.87 | 39.6 ± 20.97 to 28.66 ± 25.34 | <0.001 | | Significantly higher concentrations at 3 months were observed in the intervention group |
| Serum IL-10, pg/mL (median, IQR) at 3 months | 12.58 ± 11.97 to 13.76 ± 18.96 | 10.97 ± 9.97 to 11.31 ± 19.63 | 0.158 | | No differences between IL-10 levels were observed between groups at 3 months<br><br>Multiple linear regression analysis suggested a positive association between vitamin D consumption and the log of IL-10 measures (β = 0.737, p = 0.015, and $R^2$ = 0.91) when controlling for age, sex, and EDSS scores |
| *Adverse Events* | | | | | |
| Calcium, mg/dL (mean, SD) at 3 months | 9.41 ± 0.47 to 9.53 ± 0.60 | 9.40 ± 0.4 to 9.34 ± 0.46 | 0.302 | | No significant differences in serum calcium between groups at baseline or after 3 months;<br><br>Calcium levels did not cross the upper limits of normal |
| *Etemadifar, 2015*[65] | | | | | |
| *Effectiveness Outcomes*[a] | | | | | |
| | **D3 50000 IU weekly from 12 to 16 weeks gestation until delivery** | **Routine care** | **p-value** | | |
| Serum 25(OH)D level at baseline, ng/mL | 15.3 ± 2.9 | 18.3 ± 1.9 | **<0.05** | | Vitamin D supplemented patients had significantly lower 25(OH)D levels at baseline and significantly higher levels at 6 months;<br><br>"*Six months after delivery, average increase in serum 25(OH)D levels between vitamin D3 and routine care groups was 19.1 (0.3 to 29.9)*"[65] |
| Serum 25(OH)D level at 6 months, ng/mL | 33.7 ± 15.2 | 14.6 ± 1.3 | **<0.01** | | |
| EDSS at baseline | 1.2 ± 0.3 | 1.3 ± 0.4 | NS | **MD = -** | EDSS was significantly lower in |

| Table A8: Summary of Findings of Included Randomized Controlled Trials | | | | | |
|---|---|---|---|---|---|
| **Outcome** | **Vitamin D** | **Comparator** | **Effect estimate** | | **Author's Conclusions** |
| | | | | **0.6 (-1.2 to -0.1),** p = NR | vitamin D supplemented patients at 6 months versus routine care |
| EDSS 6 months after treatment | 1.1 ± 0.2 | 1.7 ± 0.6 | <0.05 | | |
| Relapse events at baseline | 1.3 ± 0.5 | 1.1 ± 0.4 | NS | **MD = -0.4 (-0.9 to 0.2); p <0.060** | The mean number of relapses within 6 months was significantly decreased in both study groups (data not shown); the average number of relapses within 6 months was reduced to a greater extent in the vitamin D group versus routine care despite no differences in the baseline or end line rate of relapse events between groups |
| Relapse events 6 months after treatment | 0.0 0.0 | 0.4 ± 0.5 | NS | | |
| *Adverse Events* | | | | | |
| Urinary dysfunction, frequency (%) | 0 (0) | 0 (0) | NR | | Vitamin D supplementation well tolerated; no adverse events reported |
| Symptomatic nephrolithiasis, frequency (%) | 0 (0) | 0 (0) | NR | | |
| Disturbances of cardiac rhythm, frequency, (%) | 0 (0) | 0 (0) | NR | | |
| *Røsjø, 2015*[66] | | | | | |
| *Effectiveness Outcomes* | | | | | |
| | **D3 20 000 IU per week for 2 years** | **Placebo capsules** | **Group difference, p-value** | | |
| Serum 25(OH)D levels (change from baseline), n mol/L | 67.6 (37.7) | 6.2 (15.7) | **Group difference = 60, p = <0.001** | | 25(OH)D levels increased significantly in patients receiving vitamin D3 supplementation versus placebo over 96 weeks |
| ***Mean change of inflammation markers from baseline to end line, change (SD)*** | | | | | |
| ALCAM (ng/mL) | 4 (31) | 9 (22) | 0.130 | | No differences in change from baseline values for markers of inflammation between groups |
| CCL21 (pg/mL) | 66 (129) | 30 (115) | 0.284 | | |
| CXCL16 (pg/mL) | 42 (224) | 47 (242) | 0.763 | | |
| IL-1Ra (pg/mL) | 90 (478) | -10 (115) | 0.175 | | |
| MMP-9 (ng/mL) | 170 (464) | 84 (529) | 0.719 | | |
| OPG (pg/mL) | 43 (307) | -33 (228) | 0.390 | | |
| OPN (ng/mL) | -1.4 (3.1) | -2.0 (2.5) | 0.252 | | |
| PTX3 (pg/mL) | -39 (566) | -95 (458) | 0.638 | | |
| sFRP3 (pg/mL) | 13 (3775) | 129 (736) | 0.995 | | |
| sTND-r1 (pg/mL) | 107 (164) | 83 (139) | 0.589 | | |
| TGF-β1 (ng/mL) | 0.0 (2.9) | 0.1 (2.2) | 0.717 | | |
| *Toghianifar, 2015*[67] | | | | | |
| *Effectiveness Outcomes* | | | | | |
| | **D3 50 000 IU every 5 days for 12 weeks** | **Placebo** | **p-value** | | |
| Baseline serum 25(OH)D, ng/m | 28.27 ± 29.03 | 39.6 ± 20.97 | 0.412 | | After 12 weeks, vitamin D treated individuals had significantly higher vitamin D status than controls |
| 12 week 25(OH)D, ng/m, baseline | 84.67 ± 42.87 | 28.66 ± 25.34 | **<0.001** | | |
| Baseline IL-17, pg/mL | 56.75 ± 28.72 | 30.31 ± 78.85 | 0.338 | | No difference in baseline or end line IL-17 levels, or the proportion of |

| Table A8: Summary of Findings of Included Randomized Controlled Trials | | | | |
|---|---|---|---|---|
| **Outcome** | **Vitamin D** | **Comparator** | **Effect estimate** | **Author's Conclusions** |
| 12 week IL-17, pg/mL | 58.93 ± 67.93 | 46.13 ± 94.70 | 0.960 | patients with increased IL-17 levels after 12 weeks between vitamin D treated individuals and controls;<br><br>In multiple linear regression analysis, vitamin D consumption showed a significant positive correlation with the log of IL-17 measures in the intervention group (β = 1.719; p = 0.002, $R^2$ = 0.91) when adjusted by EDSS measures; thus, a large amount of the variation in IL-17 measures may be attributed to vitamin D supplementation |
| Proportion with increase in IL-17 levels | 60% | 67% | 0.567 | |
| Baseline calcium, mg/dL | 9.41 ± 0.47 | 9.40 ± 0.4 | 0.980 | No difference in baseline or end line calcium levels between vitamin D treated individuals and controls |
| 12 week calcium, mg/dL | 9.53 ± 0.60 | 9.34 ± 0.46 | 0.302 | |
| ***High versus Low Dose Vitamin D*** | | | | |
| *Sotirchos, 2016*[37] | | | | |
| *Effectiveness Outcomes* | | | | |
| | **D3 10400 IU every day for 6 months** | **D3 800 IU every day for 6 months** | **p-value** | |
| Serum 25(OH)D (ng/mL), mean change from baseline[b] | 34.9 ng/mL (25.0 to 44.7) | 6.9 ng/mL (1.0 to 13.7) | <0.0001 | 25(OH)D levels were not significantly different between baseline and mid-study visits, or mid and end-study visits, but were significantly different between baseline and end-study visits in the low-dose group<br><br>25(OH)D levels were significantly higher than baseline at mid and end-study visits, but not between mid and end-study visits in the high dose group.<br><br>Baseline 25(OH)D levels were not significantly different between low-dose and high-dose groups, but were significantly higher in the high-dose group at mid and end-study visits |
| Relapse, n | 1 | 1 | NR | One relapse occurred in each treatment group over the course of the study |
| ***Immune cell subtype changes,*** *Comparison of change during study between groups, mean difference, % (95% CI)* | | | | |
| IL-17$^+$CD4$^+$ T cells, proportion | -3.70 (-0.80 to -6.58) | -0,44 (-1.89 to 1.01) | **2.68 (0.13 to 5.23)** | Greater negative change in IL-17$^+$CD4$^+$ T cells observed in the high-dose group compared to low-dose group |
| IFN- γ $^+$CD4$^+$ T cells | -7.50 (017.16 to 2.16) | -2.27 (-9.87 to 5.33) | 5.32 (-2.89 to 13.53) | Numerically greater negative change in high-dose group compared to low-dose group, not statistically significant |
| IFN- γ $^+$ IL-17$^+$ CD4$^+$ T cells | -1.70 (-4.03 to 0.65) | -0.15 (-0.99 to 0.69) | 1.21 (-0.66 to 3.07) | |
| Effector memory | -9.87 (-18.05 | -0.80 (-11.37 to | 8.24 (-2.19 to | |

| Table A8: Summary of Findings of Included Randomized Controlled Trials | | | | |
|---|---|---|---|---|
| **Outcome** | **Vitamin D** | **Comparator** | **Effect estimate** | **Author's Conclusions** |
| $CD4^+$ T cells | to -1.69) | 9.76) | 18.67) | |
| Central memory $CD4^+$ T cells | 10.89 (2.09 to 19.67) | 1.45 (-8.29 to 11.18) | -8.08 (-19.23 to 3.07) | Numerically greater positive change in high-dose group compared to low-dose group, not statistically significant |
| Naive $CD4^+$ T cells | 3.26 (0.10 to 6.41) | 1.82 (-1.31 to 4.95) | -1.99 (-6.0 to 2.02) | |
| $CD161^+$ $CD4^+$ T cells | -0.81 (-1.54 to -0.08) | -0.41 (-1.28 to 0.46) | 0.48 (-0.57 to 1.52) | Numerically greater negative change in high-dose group compared to low-dose group, not statistically significant |
| $CD85j^+CD8^+$ T cells | -3.19 (-5.91 to -0.48) | -0.54 (-2.57 to 1.49) | 2.64 (-0.18 to 5.46) | |
| 51 cytokines | NR | NR | NR | No differences in the levels of 51 cytokines measured at baseline and end line in either group |
| *Golan, 2013*[36] | | | | |
| *Effectiveness Outcomes, Mean (SD) at baseline and 1 year unless otherwise specified* | | | | |
| | **D3 75 000 IU every 3 weeks plus 800 IU daily (4730 IU/day total) for1 year** | **D3 800 IU/day** | **p-value** | |
| Serum 25(OH)D[36c] | NR | NR | $p < 0.001$ | Significantly higher 25(OH)D levels achieved by the high-dose group versus low dose group throughout follow-up at 3 months and 1 year |
| Flu-like symptoms[36d] | NR | NR | NS | No significant change in flu-like symptoms was observed in either group, and there were no significant differences between study groups, insufficient power to detect a difference between groups |
| *Adverse Events* | | | | |
| Hypercalcemia[36] | 0 (0) | 0(0) | NR | Treatment with high and low dose vitamin D was well tolerated with no reports of treatment-attributable adverse events, or withdrawals due to adverse events |
| Possible vitamin D related adverse events[36] | 0 (0) | 0 (0) | NR | |
| WDAE[36] | 0 (0) | 0 (0) | NR | |
| ***PREVENTION*** | | | | |
| *Derakhshandi, 2013*[64] | | | | |
| *Efficacy Outcomes* | | | | |
| | **D3 50 000 IU weekly for 12 months** | **Placebo** | **p-value** unless otherwise specified | |
| Serum 25(OH)D levels | | | | |
| Incidence of second demyelinating attack, frequency (%) | 0 (0%) | 5 (45%) | Absolute risk reduction = 45.5%, NNT = 2 **0.007** | Significantly fewer cases of second demyelinating attack occurred in the treatment versus placebo group; |
| ***Magnetic Resonance Imaging Outcomes at 1-year follow-up, mean (SD)*** | | | | |
| Black holes In final MRI | 0.15 ± 0.55 | 1.64 ± 2.11 | **0.001** | Incidence of black holes, cortical, juxtacortical, corpus callosal, new gadolinium-enhanced and new T2 lesions were significantly lower in the vitamin D group versus placebo, |
| New gadolinium enhancing lesions | 0 ± 0 | 1.36 ± 2.24 | **0.002** | |
| Cortical plaques in | 1.08 ± 3 | 3.36 ± 4.29 | **0.001** | |

| **Table A8:** Summary of Findings of Included Randomized Controlled Trials | | | | |
|---|---|---|---|---|
| **Outcome** | **Vitamin D** | **Comparator** | **Effect estimate** | **Author's Conclusions** |
| final MRI | | | | whereas there was no difference in the incidence of periventricular or brain stem plaques and the difference in cerebellum plaques could not be computed |
| Periventricular plaques in final MRI | 4.62 ± 4.29 | 5.91 ± 6.12 | 0.16 | |
| Juxtacortical plaques in final MRI | 0.69 ± 1.10 | 2.82 ± 3.94 | **0.001** | |
| Corpus callosum plaques in final MRI | 0.15 ± 0.55 | 1.27 ± 1.90 | **0.005** | |
| Brain stem plaques in final MRI | 0.15 ± 0.55 | 0.27 ± 0.46 | 0.84 | |
| Cerebellum plaques in final MRI | 0 ± 0.0 | 0.18 ± 0.40 | Not computable | |
| New plaques in T2 | 0.64 ± 0.65 | 2.63 ± 3.31 | **0.001** | |
| *Adverse Events* | | | | |
| Hypercalcemia | 0 (0) | 0 (0) | NR | No incidence of hypercalcemia or vitamin D toxicity reported in either group |
| Vitamin D toxicity | 0 (0) | 0 (0) | NR | |

[a]Change from baseline values not reported
[b]Deseasonalized and calculated as the predicted level on January 1st
[c]Data presented in a figure (exact values not presented), see Figure 2[36] for details
[d]Data presented in a figure (exact values not presented), see Figure 3[36] for details
ALCAM = Activated leukocyte cell adhesion molecule; CCL21 = Chemokine (C-C motif) ligand 21; CI = confidence interval; CXCL16 = Chemokine (C-X-C motif) ligand 16; EDSS = Expanded Disability Status Scale; IFN = interferon; IL = interleukin; IL-1Ra = Interleukin-1 receptor antagonist; MD = mean difference; MMP-9 = Matrix metalloproteinase 9; N/A = not applicable; NR = not reported; NS = not significant; OPG = osteoprotegerin; OPN = osteopontin; PTX3 = pentraxin 3; SD = standard deviation; sFRP3 = secreted frizzled-related protein 3; sTND-r1 = soluble tumor necrosis factor receptor 1; TGF-β1 = transforming growth factor β 1

| **Table A9:** Summary of Findings of Included Non-Randomized Studies | | | | | |
|---|---|---|---|---|---|
| **Outcome** | | **Vitamin D** | **Comparator** | **Effect estimate** | **Author's Conclusions** |
| *Cortese, 2015*[68] | | | | | |
| *Efficacy Outcomes* | | | | | |
| | | **Cod liver oil** | **No cod liver oil** | **OR**, unless otherwise specified | |
| Association between cod liver oil supplementation at different ages and MS risk | | NR | NR | NR | There was a significant association between cod liver oil supplementation (a source of vitamin D) during adolescence and reduced MS risk in unadjusted models as well as models adjusted for sun exposure, infectious mononucleosis, smoking, body size, oily fish consumption and education, but not during childhood or adulthood |
| Association between average daily intake | **Cod liver oil dose per month; equivalent** | Cases, n (%) | Controls, n (%) | OR (adjusted for age and sex), 95% | Cod liver oil consumption in the range of equivalent vitamin D doses of 0 to 200 IU, 201 to 400 IU, and 601 |

| Table A9: Summary of Findings of Included Non-Randomized Studies | | | | | |
|---|---|---|---|---|---|
| **Outcome** | | **Vitamin D** | **Comparator** | **Effect estimate** | **Author's Conclusions** |
| of vitamin D and MS risk | **vitamin D dose per day** | | | CI | to 800 IU was associated with a reduced odds of developing MS, while doses in the range of 401-600 and >800 trended towards a reduced odds of MS but this association was not statistically significant. While the authors claim a dose-response relationship, and a general pattern is observed, the outcome does not provide compelling evidence for this. |
| | No vitamin D | 525 (66.0) | 784 (56.1) | 1.00 | |
| | 1 to 15 teaspoons; ≤200 IU | 79 (9.9) | 160 (11.5) | 0.74 (0.55 to 0.99) | |
| | 16 to 30 teaspoons, 201 to 400 IU | 55 (6.9) | 125 (9.0) | 0.68 (0.48 to 0.95) | |
| | 31 to 45 teaspoons, 401 to 600 IU | 14 (1.8) | 38 (2.7) | 0.58 (0.31 to 1.08) | |
| | 46 to 60 teaspoons, 601 to 800 IU | 32 (4.0) | 104 (7.4) | 0.46 (0.31 to 0.70) | |
| | > 60 teaspoons, >800 IU | 90 (11.3) | 186 (13.3) | 0.77 (0.58 to 1.02) | |
| *Jelinek, 2015*[69] | | | | | |
| *Effectiveness Outcomes* | | | | | |
| | | **Vitamin D supplementation** | **No vitamin D supplementation** | **p-value,** unless otherwise specified | |
| Quality of life (MSQOL-54) | | NR | NR | NR | In a regression model that included deliberate sun exposure, latitude, and vitamin D supplementation and evaluated the effect on HRQOL, as well as a further adjusted model including gender, age, disability, physical activity and fish consumption, vitamin D supplementation was associated with improved quality of life, though the association was when additional covariates were added |
| <u>Level of Disability</u><br><br>No supplementation<br>Mild<br>Moderate<br>High<br>1-2000 IU | | | <br><br>207 (52.4)<br>140 (35.4)<br>48 (12.2) | NR | Patients taking 2000 to 5000 IU per day were more likely to be in the mild disability group and less likely to be in the high disability group; those taking 1 to 2000 IU per day |

| **Table A9:** Summary of Findings of Included Non-Randomized Studies | | | | |
|---|---|---|---|---|
| **Outcome** | **Vitamin D** | **Comparator** | **Effect estimate** | **Author's Conclusions** |
| Mild<br>Moderate<br>High<br>2001 to 5000 IU<br>Mild<br>Moderate<br>High<br>>5000 IU<br>Mild<br>Moderate<br>High | 293 (48.0)<br>243 (39.8)<br>74 (12.1)<br><br>439 (59.9)<br>236 (32.2)<br>58 (7.9)<br><br>257 (57.5)<br>138 (30.9)<br>52 (11.6 | | | were less likely to be in the mild disability group and more likely to be in the moderate disability group ($p > 0.001$);<br><br>Based on logistic regression modelling, vitamin D supplementation was not associated with the odds of being in a moderate disability or high disability category compared to the no or mild disability categories when controlled for a variety of confounders including latitude |
| <u>Relapse Rate</u><br><br>1-2000 IU<br>2001 to 5000 IU<br>>5000 IU | <br><br>0.63<br>0.61<br>0.62 | <br><br>0.92 | <br><br><0.001<br><0.003<br><0.001 | Significantly lower annualized relapse rate in patients receiving all ranges of vitamin D doses versus those taking no supplements, but there were no significant differences between varying ranges of vitamin D supplements;<br><br>Ordinal regression models did not find an association of vitamin D supplementation with relapse rate when controlled for latitude, gender, age, physical activity, fish consumption, and deliberate sun exposure |
| *Najafipoor, 2015*[70] | | | | |
| | **D3 50 000 IU per week for 6 months plus interferon-β** | **No vitamin D plus interferon-β** | **N/A** | |
| 25(OH)D levels mean (SD) | 66.7 (53.5) to 113.4 (28.3) | 61.7 (42.8) to 61.46 (23.1) | NR | Vitamin D status improved numerically in supplemented but not non-supplemented group |
| VCA IgG, mean (SD) | 212.14 (168.5) to 274.11 (193) | 182.07 (126.5) to 453.61 (221) | NR | All MS patients were seropositive for anti-VCA IgG and anti-EBNA1 IgG at the onset of disease;<br><br>Rise in EBV antibodies were significantly lower in vitamin D group than control group; significant |
| EBNA1 IgG, mean (SD) | 235.59 (238.9) to 250.07 (193.9) | 159.92 (26.4) to 339.53 (209.6) | NR | |

| **Table A9:** Summary of Findings of Included Non-Randomized Studies | | | | |
|---|---|---|---|---|
| **Outcome** | **Vitamin D** | **Comparator** | **Effect estimate** | **Author's Conclusions** |
| | | | | difference between supplemented and control groups after 6 months in levels of both antibodies (see Figure 1)[70] |
| Decline in anti-VCA titers | 6 (15%) | 0 (0%) | NR | The proportion of patients with a decline in both antibody titers was 15% in the supplemented and 0% in the control group |
| Decline in anti-EBNA1 titers | 6 (15%) | 0 (0%) | NR | |
| *Adverse Events* | | | | |
| Vitamin D intoxication defined as 25(OH)D > 250 nmol/L | 0 (0) | 0 (0) | NR | No evidence of vitamin D intoxication in intervention or control groups |

25(OH)D = 25 hydroxyvitamin D; EBNA1 = Epstein Barr virus nuclear antigen 1; EBV = Epstein Barr virus; HRQOL = health related quality of life; IgG = immunoglobulin G; IU = international units; MS = multiple sclerosis; MSQOL-54 = Multiple Sclerosis Quality of Life-54; N/A = not applicable; NR = not reported; NS = not significant; OR = odds ratio; VCA = Epstein Barr virus viral-capsid antigen

## APPENDIX 5: Additional References of Potential Interest

### *Ongoing Systematic Reviews*

1. Hempel S, Estrada E, Chen A, Miake-Lye I, Beroes J, Shanman R, et al. Modifiable risk factors in the progression of multiple sclerosis. 2015 [cited 2016 Feb 25]. In: PROSPERO: International prospective register of systematic reviews [Internet]. York (UK): University of York, Centre for Reviews and Dissemination. Available from: http://www.crd.york.ac.uk/prospero/display_record.asp?ID=CRD42015016461.

### *Ongoing Trials or Trials with Pending Publications*

#### *Treatment*

2. Bhargava P, Cassard S, Steele SU, Azevedo C, Pelletier D, Sugar EA, et al. The vitamin D to ameliorate multiple sclerosis (VIDAMS) trial: study design for a multicenter, randomized, double-blind controlled trial of vitamin D in multiple sclerosis. Contemp Clin Trials. 2014 Nov;39(2):288-93.

3. Dorr J, Ohlraun S, Skarabis H, Paul F. Efficacy of vitamin D supplementation in multiple sclerosis (EVIDIMS Trial): study protocol for a randomized controlled trial. Trials [Internet]. 2012 [cited 2016 Mar 3];13:15. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3298796

4. AlQuaiz AM. Role of vitamin D in reducing the relapse rate in patients with multiple sclerosis. 2012 Dec 17 [cited 2016 Mar 10] . In: ClinicalTrials.gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/show/NCT01753375 Identifier: NCT01753375.

5. Abedini M. Vitamine D in multiple sclerosis (MSVit). 2013 Jan 11 [cited 2016 Mar 10] . In: ClinicalTrials.gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available from: https://www.clinicaltrials.gov/ct2/show/NCT01768039 Identifier: NCT01768039.

6. Merck KGaA. A Multicentre study of the efficacy and safety of supplementary treatment with cholecalciferol in patients with relapsing multiple sclerosis treated with subcutaneous interferon Beta-1a 44 µg 3 times weekly (CHOLINE). 2010 Sep 8 [cited 2015 Mar 7; last updated 2015 Sep 8; ]. In: ClinicalTrials.gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available from: https://www.clinicaltrials.gov/ct2/show/NCT01198132 Identifier: NCT01198132.

#### *Prevention*

7. Thouvennot E. Efficacy of cholecalciferol (Vitamin D3) for delaying the diagnosis of MS after a clinically isolated syndrome (D-Lay-MS). 2013 Mar 20 [cited 2015 Mar 7; last updated 2016 Jan 18]. In: ClinicalTrials.gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available from: https://www.clinicaltrials.gov/ct2/show/NCT01817166?term=vitamin+d+multiple+sclerosis&rank=13 Identifier: NCT01817166.

8. O'Connell K, Kelly S, Kinsella K, Jordan S, Kenny O, Murphy D, et al. Dose-related effects of vitamin D on immune responses in patients with clinically isolated syndrome and healthy control participants: study protocol for an exploratory randomized double- blind placebo-controlled trial. Trials [Internet]. 2013 [cited 2016 Feb 16];14:272. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3844318