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Original article

Neuroprotective role of high dose Vitamin D supplementation in multiple sclerosis: Sub-analysis of the EVIDIMS trial

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ABSTRACT

Background: Previous evidence suggests that vitamin D may help with neurodegeneration in multiple sclerosis (MS). Considering this, the study aims to investigate whether higher-dose vitamin D supplementation in individuals with MS can lead to reduced atrophy, as measured by optical coherence tomography (OCT) and MRI structural outcomes. This objective builds upon the established notion of thalamic and brainstem atrophy as reliable markers of disease progression and the potential association between adequate vitamin D levels and improved visual outcomes in MS patients.
Objective: To assess the impact of vitamin D as a neuroprotective intervention on visual and imaging biomarkers.
Method: Participants enrolled in the EVIDIMS trial underwent an assessment to investigate the impact of high and low-dose vitamin D supplementation at 12 and 18 months using a nonparametric multiple contrast test procedure (MCTP). The primary outcomes included four MRI-radiological measures: change in the mean upper cervical cord area (MUCCA) and hippocampal, thalamic and brainstem volumes, and the visual system outcomes included assessment of the peripapillary retinal nerve fiber layer (pRNFL) thickness and macular combined ganglion cell and inner plexiform layer (GCIPL), and inner nuclear layer (INL).
Results: MCTPs indicated that there were no statistically significant differences in the volumes of the thalamus, hippocampus, and brainstem, as well as in MUCCA, between the low and high-dose supplementation groups. Moreover, MTCPs only revealed a different thinning pattern in the INL at 18 months between the low versus high-dose treatment arm attributable to patients who suffered optic neuritis (NO), with no significant differences in neither pRNFL nor GCIPL.
Conclusion: Vitamin D supplementation did not affect MS imaging parameters. However, future studies should thoroughly evaluate patient histories of attacks and endogenous vitamin D levels before inclusion in dose-related investigations.

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1. Introduction

Numerous sources have shown an association between low levels of serum 25-hydroxyvitamin D [25(OH)D] and increased risk of developing multiple sclerosis (MS), as well as a potential protective role against the inflammatory activity of the disease once diagnosed (Ascherio et al., 2014; Munger et al., 2006; Stewart et al., 2012; Soilu-Hänninen et al., 2005). Vitamin D can increase the anti-inflammatory response at the molecular and cellular levels by elevating CD4+ and regulatory T cell levels and promoting neurotrophic factors (Colotta et al., 2017; Miclea et al., 2020). The controlled double-blinded phase II 'Efficacy of Vitamin D supplementation In Multiple Sclerosis' (EVIDIMS) clinical trial tested the effect of high dose (20,400 UI every other day) versus low dose (800 UI every other day) vitamin D in people with relapsing-remitting MS (pwMS) under stable disease-modifying treatment (Dörr et al., 2020). Although EVIDIMS did not reveal significant effects of vitamin D supplementation on several clinical and primary outcome magnetic resonance imaging (MRI) measures, secondary structural visual system and MRI outcomes of disease progression, such as thalamic or brainstem atrophy, were not evaluated. In the same line, mean upper cervical cord area (MUCCA) has been described as a potential biomarker for assessing spinal cord atrophy (Weeda et al., 2019) but has not yet been evaluated in the EVIDIMS cohort.

Both structural evaluation of the retina through optical coherence tomography (OCT) and assessment of the MUCCA have consistently demonstrated thinning and atrophy in pwMS, respectively (Abalo-Lojo et al., 2018; Ayoubi et al., 2022; Mina et al., 2021; Weeda et al., 2019). Similarly, thalamic and brainstem atrophy has proven to be an adequate measure of progression and disability in these people. In addition, animal models have demonstrated clustering of vitamin D receptors in deep grey matter structures - particularly the thalamus-, specific nuclei within the brainstem, and the spinal cord (Deluca et al., 2013) . Studies in larger populations have suggested that adequate vitamin D serum levels are associated with a decrease probability of developing macular degeneration (Graffe et al., 2014; Kim and Park, 2018) and are related with better visual outcomes in patients mainly classified as an isolated clinical syndrome (CIS) or pwMS (94.4 %) who suffered from optic neuritis (Burton et al., 2016) . Under this basis, we theorized that patients under vitamin D supplementation in a higher dose would have less atrophy measured by OCT and MRI structural outcomes.

2. Methods

EVIDIMS (NCT01440062) is a multicenter, stratified, randomized, double-blind, controlled trial performed in seven German centers. Recruitment began in 2011; however, the trial enrollment was halted early, did not reach its originally planned sample size. Relapsing-remitting pwMS under stable treatment for at least three months with interferon-beta-1b with no Vitamin D supplementation before inclusion were recruited and randomized for supplementation with a high dose (20,400 UI every other day) and low dose (800 UI every other day) of vitamin D orally. Patients maintained their previous stable dose of interferon β−1b during the complete 18-month duration of the study. In addition, patients were stratified according to gender and Vitamin D serum levels at recruitment. The EVIDIMS exploratory cohort comprised 53 pwMS in the intention-to-treat protocol, with 28 allocated to the high-dose arm and 25 to the low-dose arm.

The primary outcomes analyzed included MRI-radiological outcomes: the mean upper cervical cord area (MUCCA) change and the thalamic, hippocampal and brainstem volumes. Visual system outcomes included measurements of the thickness of the macular retinal nerve fiber layer (RNFL), combined ganglion cell and inner plexiform layer (GCIPL), and inner nuclear layer (INL).

2.1. MRI acquisition and processing

The full MRI scanning protocol was described previously (Bäcker-Koduah et al., 2020), where in this study we used the retrospectively collected cerebral 3D magnetization prepared rapid gradient echo (MPRAGE) T1-weighted sequence (repetition time (TR): 1900 ms, echo time (TE): 3.03 ms, inversion time (TI): 900 ms, resolution: 1 × 1 × 1 mm (Stewart et al., 2012) isotropic) and fluid-attenuated inversion recovery (FLAIR) T2-weighted (TR: 6000 ms, TE: 388 ms, TI: 2100 ms, resolution: 1 × 1 × 1 mm (Stewart et al., 2012) isotropic) cerebral sequences for further longitudinal processing. Briefly, each patient's MPRAGE at baseline was N4-bias corrected (Tustison et al., 2010), cropped to standard space, linearly co-registered to MNI-152 space (Jenkinson et al., 2002), and brain extracted (Smith, 2002). Each corresponding FLAIR image was co-registered to the MNI-152 space and then co-registered linearly to the MPRAGE. For each patient follow-up session, the MPRAGE and FLAIR were linearly co-registered to MNI-152 space, then using transformation matrices from baseline, co-registered to the baseline MPRAGE (Cooper et al., 2021).

2.2. MRI analysis

Lesion segmentation was performed by two experienced (ten years of MS-related MRI research) MRI technicians on FLAIR images manually using ITK-SNAP (Yushkevich et al., 2006). For whole brain and deep grey matter subregional segmentation, longitudinally co-registered, lesion-filled MPRAGE (Battaglini et al., 2012) scans were used as inputs for FSL FIRST. For volume extraction of each summed region, we used FSLtools and converted volumes to milliliters. SIENAX (Smith et al., 2002, 2004) was applied to extract the VScaling normalization factor for deep grey matter volume normalization. A longitudinal correction based on the baseline SIENAX volume-correction factor was used to normalize thalamic, hippocampal, and brainstem volumes regarding head size or angle variations. For longitudinal MUCCA, MPRAGE cerebral scans were N4-bias corrected but not cropped into standard space nor co-registered to each follow-up session as the site of segmentation is at the C2/C3 intervertebral space level of the cord (Chien et al., 2018). The active surface method (JIM version 7.0, http://www.xinapse.com/home.php) was used by the same MRI technicians to segment MUCCA longitudinally, following standard practices and considerations for quality control of outputs (C. Chien et al., 2020).

2.3. OCT protocol

All OCT measurements were carried out with a Spectralis SD-OCT and Heidelberg Eye Explorer (HEYEX) version 5.7.5.0 (Heidelberg Engineering, Heidelberg, Germany) by three individual operators, with automatic real-time (ART) function for image averaging and an activated eye tracker in a dimly lit room. Macular 3D volumes were assessed by a custom scan comprising 61 vertical B-scans (each with 768 A-Scans, with ART of 13 frames) with a scanning angle of 30°x25° focusing on the fovea using a cylinder of 6 mm. Peripapillary ring scans were derived from the 3.5 mm diameter circle scan from the ONH-RC protocol, centered on the Bruch's membrane opening center, using the device-internal segmentation (HRA version 6.9a). All scans were quality controlled according to the OSCAR-IB criteria (Schippling et al., 2015; Tewarie et al., 2012), and reporting adheres to APOSTEL 2.0 recommendations (Aytulun et al., 2021). Scans not passing the quality control ($N = 5$) were excluded from the analysis. The macular scans were exported from the device and stored in HEYEX Vol file format (*.vol files), and then intraretinal segmentation was performed using the SAMIRIX segmentation pipeline (Motamedi et al., 2019). We acquired the mean thickness (μm) of the peripapillary retinal nerve fiber layer from the ring scan and macular volumes (mm^3) of the combined ganglion cell and inner plexiform layers (GCIPL) and inner nuclear layer (INL).

2.4. Statistical analysis

First, data was analyzed descriptively using appropriate summary statistics such as the median and inter-quartile range (IQR) for metric variables and absolute and relative frequencies for categorical variables. Baseline characteristics were compared using the T-test (for age and 25 (OH)D serum baseline and control levels) or the Wilcoxon test (EDSS, all tested volumes, and OCT parameters). To evaluate differences in proportion between the treatment arms, the χ^2 test was performed. To assess the changes to baseline after twelve and 18 months within and between both groups, we applied a nonparametric multiple contrast test procedure (MCTP). Since for some variables (PRNFL, GCIPL, and INL thickness), both eyes were investigated, a clustered data structure is present. Therefore, we used the methodology by Rubarth et al. (Rubarth et al., 2022) to account for the clustering and missing values. By using this procedure, all available information is used instead of only using complete observations. The hypotheses were formulated regarding the relative (treatment) effect (Konietschke et al., 2010), which is the probability that a randomly sampled observation from one group is larger than a randomly sampled observation from the other groups. Groups or time points are compared by calculating the differences in these relative effects. Thus, under the null hypothesis, it is postulated that the difference between the two relative effects equals zero. According to our hypotheses, we evaluated the effect of vitamin D supplementation at twelve and 18 months on differences to baseline in each group and compared the change between each dose arm at the same time points. The MCTP controls the type-I error for each family of hypotheses regarding one outcome parameter separately, and due to the exploratory characteristic of the study, we did not apply additional techniques to adjust the type-I error for all outcome parameters. Hence, all p-values are interpreted in a non-confirmatory manner. All p-values ≤ 0.05 were considered significant. All statistics were performed with R Statistical Software version 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

EVIDIMS included 53 pwMS with 37 (69.8 %) females. The mean age at randomization was 42.7 (±10.1) years. Baseline characteristics were equally distributed between the high and low-dose arms (Table 1). Generally, all patients that entered the study were classified as vitamin D deficient, but there were no significant differences between the treatment groups at baseline. At 18 months, a significant difference in Vitamin D serum levels was observed between the high vs. low-dose arm (mean increase of 45.9 ± 5.4 ng/ml and 5.9 ± 2.3 ng/ml, respectively). Upon enrollment, 22 pwMS with a history of ON were identified. Among them, 8 (33.3 %) were allocated to the low-dose arm, while 14 (50.0 %) were in the high-dose arm, with no statistically significant difference observed ($p = 0.352$). Throughout the follow-up period, none of the patients experienced any relapses.

3.1. Longitudinal analysis of thalamus, hippocampus, spinal cord, and brainstem changes

Baseline imaging did not reveal differences between pwMS allocated to the low or high-dose group in the volume of the brainstem, the hippocampus, or the MUCCA (Table 1). A slightly reduced volume in the thalamus was found in pwMS allocated to the low-dose group compared with the high-dose group (median 19.28 [17.73, 20.47] vs 20.35 [18.85, 21.61], $p = 0.036$). Overall, the MCTPs revealed no statistically significant difference in the change in volume of the thalamus, hippocampus, brainstem, or the MUCCA for both the low and the high-dose supplementation arms (Fig. 1A-D). When evaluating the effect of supplementation between each arm, no significant differences were observed (Table 2).

Table 1
Baseline characteristics of patients included in the analysis.

	Overall	High dose	Low dose	p
n	53	28	25	
Age (years, mean (SD))	42.70 (10.10)	41.35 (11.02)	44.21 (8.93)	0.308
Female (N (%))	37(69.8)	20(71.0)	17(68.0)	1.000
Baseline serum Vitamin D levels (ng/ml, mean (SD))	18.21 (9.38)	18.80 (10.12)	17.55 (8.62)	0.632
18 months increase in Vit D serum (unit, mean (SD))	28.61 (4.17)	45.9 (5.4)	5.9 (2.3)	**<0.001**
EDSS (median [IQR])	2.00 [1.50, 3.00]	2.00 [1.50, 2.50]	2.50 [1.50, 3.50]	0.180
History of ON (N(%))	22 (42.3)	14 (50.0)	8 (33.3)	0.352
Thalamic volume (mL, median [IQR])	19.95 [18.51, 21.10]	20.35 [18.85, 21.61]	19.28 [17.73, 20.47]	**0.036**
Brainstem volume (mL, median [IQR])	29.75 [27.80, 31.11]	29.97 [27.89, 31.82]	29.36 [27.80, 30.49]	0.336
Hippocampal volume (mL, median [IQR])	9.87 [8.91, 10.61]	9.84 [9.36, 10.57]	10.04 [8.60, 10.61]	0.373
MUCCA (mm^2, median [IQR])	73.90 [67.12, 78.13]	73.97 [68.82, 79.78]	73.65 [66.75, 77.10]	0.68
pRNFL (µm, median [IQR])	88.00 [78.25, 97.00]	88.50 [82.25, 97.75]	88.00 [78.00, 96.00]	0.707
GCIPL (mm^3, median [IQR])	65.08 [60.13, 69.76]	64.72 [61.54, 69.67]	66.49 [58.53, 70.38]	0.65
INL (mm^3, median [IQR])	35.54 [33.60, 37.49]	35.37 [33.60, 37.14]	36.08 [32.89, 37.49]	0.987

Abbreviations: EDSS = expanded disability status scale, ON = optic neuritis, MUCCA = mean upper cervical cord area, RNFL = retinal nerve fiber layer, GCIPL = combined ganglion cell and inner plexiform layers, INL = inner nuclear layer.

3.2. Effect on OCT parameters

Baseline OCT characteristics were equally distributed in both treatment arms. MTCPs did not reveal any statistical effect on vitamin D supplementation in either of the dose groups at twelve nor 18 months of follow-up in any of the retinal layers evaluated (Fig. 1E-G). Similarly, no longitudinal significant difference was found when evaluating low vs. high-dose treatment arms at either twelve or 18 months (Table 2) except for the INL, where thinning appeared to be more significant when comparing the low-dose vs. high-dose treatment arms at 18 months. When assessing these distinctions using a mixed model that incorporated the history of ON as a fixed factor in the MCTP, no statistically significant differences were observed in the pRNFL, GCIPL, or INL in any of the treatment arms **(Supplementary Tables 1–3).**

4. Discussion

This study investigated the association between different vitamin D supplementation regimens and changes in visual, brain and spinal cord degeneration parameters in stable patients with relapsing-remitting MS (RRMS). Our results indicate that high-dose vitamin D supplementation did not confer a benefit on MRI parameters (MUCCA, thalamic, hippocampal, and brainstem volume) or OCT-derived retinal layer thicknesses (pRNFL, GCIPL, and INL).

In line with prior vitamin D supplementation trials in MS (Camu et al., 2019; Cassard et al., 2023; Hupperts et al., 2019), we did not observe a protective effect against thalamic, hippocampal, or brainstem atrophy. Although vitamin D receptors are widely distributed in the central nervous system, they are primarily found in the prefrontal

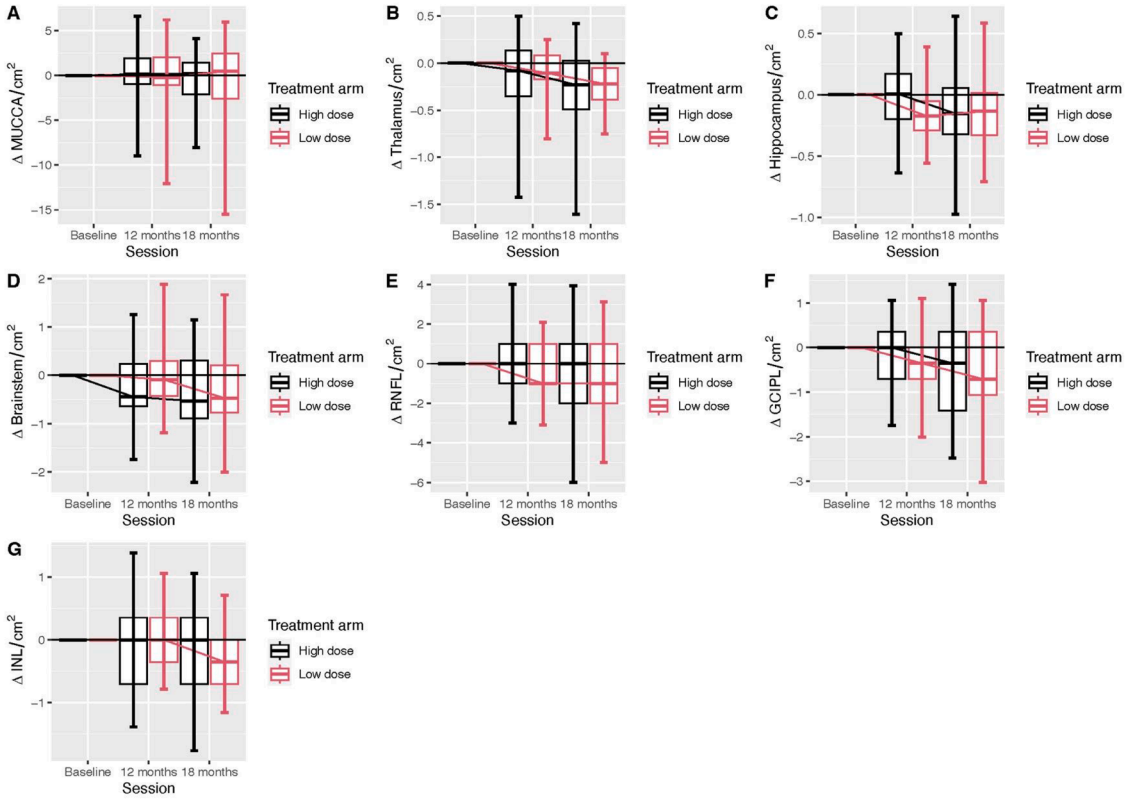

Fig. 1. Plot of longitudinal change in OCT and MRI.
Legend: The median difference between baseline and cutoff points at 12- and 18-months post-randomization are represented. Abbreviations: MUCCA = mean upper cervical cord area, RNFL = retinal nerve fiber layer, GCIPL = combined ganglion cell and inner plexiform layers, INL = inner nuclear layer.

Table 2
Longitudinal OCT and MRI parameters according to the treatment arm.

	Change from baseline to month 12							
	High dose			Low dose			High vs Low dose	
	median [IQR]	difference of rel. eff.	p	median [IQR]	difference of rel. eff.	p-val.	rel. eff.	p
MUCCA	−0.14 (−1.93,0.94)	−0.02	0.93	0.23(−2.03, 1.09)	−0.01	1.00	0.03	0.92
Thalamus	−0.08 (−0.34, 0.13)	−0.00	1.00	−0.11 (−0.17, 0.08)	0.01	0.93	0.035	0.90
Brainstem	−0.45 (−0.64, 0.24)	−0.01	1.00	−0.09(−0.43, 0.30)	0.04	0.67	0.10	0.44
Hippocampal	0.01 (−0.20, 0.17)	−0.01	1.00	−0.17 (−0.29, −0.05)	−0.01	0.97	−0.09	0.47
RNFL	0.00 (−1.00, 4.00)	−0.02	0.90	−1.00 (−1.00, 1.00)	−0.07	0.25	−0.05	0.76
GCIPL	0.00 (−0.71, 0.35)	0.03	0.91	−0.35 (−0.70, 0.00)	−0.04	0.76	−0.09	0.37
INL	0.00 (−0.71, 0.35)	0.01	1.00	0.00 (−0.35, 0.35)	0.07	0.68	−0.02	0.96
	Change from baseline to month 18							
	High dose			Low dose			High vs Low dose	
	median [IQR]	difference of rel. eff.	p	median [IQR]	difference of rel. eff.	p	difference of rel. eff.	p
MUCCA	−0.23 (−1.42, 2.10)	−0.02	0.96	−0.48 (−2.47, 2.57)	−0.01	1.0	−0.16	0.11
Thalamus	−0.23 (−0.49, 0.03)	0.00	1.00	−0.22 (−0.39, −0.05)	0.01	1.00	0.−0.11	0.29
Brainstem	−0.53 (−0.89, 0.31)	0.00	1.00	−0.47 (−0.77, 0.21)	0.01	1.00	−0.11	0.32
Hippocampal	−0.15 (−0.32, 0.05)	−0.02	0.94	−0.13 (−0.33, 0.01)	−0.00	1.00	−0.13	0.23
RNFL	0.00 (−2.00, 4.00)	−0.04	0.67	−1.00 (−2.00, 4.00)	−0.05	0.63	−0.11	0.21
GCIPL	−0.35 (−1.41, 0.35)	−0.00	1.00	−0.70 (−1.06, 0.35)	−0.01	1.00	−0.15	0.08
INL	0.00 (−0.71, 0.35)	0.02	0.96	−0.35 (−0.71, 0.00)	0.08	0.66	−0.16	0.03

Abbreviations: MUCCA = mean upper cervical cord area, RNFL = retinal nerve fiber layer, GCIPL = combined ganglion cell and inner plexiform layers, INL= inner nuclear layer.
Note: A nonparametric multiple contrast test procedure was executed at 12 months (top) and 18 months (bottom) following randomization. Noteworthy is the absence of a statistically significant alteration within either the high or low-dose group. However, upon comparing the relative effects between both treatment arms, a significant distinction in the atrophy of the inner nuclear layer (INL) was exclusively identified in the low-dose arm at the 18-month mark.

cortex, cingulate gyrus, CA1 and CA2 regions of the hippocampus, hypothalamus, and substantia nigra (Eyles et al., 2005). Importantly, these regions were not the focus of our study. This raises the possibility that the absence of significant findings may be due to a mismatch between the brain regions most influenced by vitamin D and those selected for volumetric analysis. Additionally, a meta-analysis has suggested a possible association between vitamin D depletion and loss of volume in vertex brain regions (Annweiler et al., 2014) further underscoring the

importance of evaluating a broader range of neuroanatomical structures in future studies. Moreover, the lack of a significant effect could be attributed to the relatively short study duration, as substantial atrophy differences often require longer follow-up periods to become evident. Additionally, our cohort consisted primarily of stable RRMS patients with nonactive disease, reducing the likelihood of observing rapid neurodegenerative changes over 18 months. Interestingly, our results did not show a decline in MUCCA in either supplementation group. This contrasts with findings from a previous longitudinal study in MS, which reported consistent spinal cord atrophy in patients who later transitioned to secondary progressive MS. (Bischof et al., 2022) The stability observed in our study is likely due to differences in cohort characteristics, particularly the inclusion of early-stage, stable RRMS patients with minimal disease activity. Other studies have shown that supplementation with high doses of vitamin D in isolated clinical syndrome found decreased radiological activity. Highlighting the possible benefit of this supplementation in patients with recent onset (Thouvenot et al., 2025).

Regarding retinal neurodegeneration, we observed a significant degree of INL thinning in the low-dose vitamin D group compared to the high-dose group at 18 months. INL thinning has been associated with both neuroinflammation and late-stage neurodegeneration in MS.40,41 While it is tempting to attribute this observation to a neuroprotective effect of high-dose vitamin D, our analysis suggests that the difference is more likely related to the distribution of prior ON between groups and the subsequent resolution of inflammation. Indeed, the low-dose group had a lower incidence of prior ON, which may have contributed to a distinct INL thinning pattern, characterized by an initial increase at 12 months—potentially reflecting transient inflammation—followed by a greater degree of atrophy at 18 months (Balk et al., 2019; Cellerino et al., 2019) . Notably, vitamin D receptors are present in several layers of the retina, particularly in amacrine cells, horizontal cells, and the bipolar layer within the INL, as well as in the inner and outer plexiform layers, with smaller amounts in the ganglion cells of the GCIPL (Verstappen et al., 1986; Schreiner et al., 1985). This distribution suggests a potential role for vitamin D in retinal structure and function, particularly in conditions involving inflammation, such as optic neuritis. However, the absence of significant changes in pRNFL and GCIPL in our study indicates that any potential effects of vitamin D supplementation were insufficient to counteract the structural changes associated with MS-related retinal atrophy. These findings align with prior research showing that while low vitamin D levels are linked to worse visual outcomes in optic neuritis, vitamin D supplementation has not demonstrated clear neuroprotective benefits in OCT measures in progressive MS or in recent ON trials (Abbatemarco et al., 2021; Burton et al., 2016; Salari et al., 2015).

This study has several limitations that should be acknowledged. First, this sub-analysis is restricted to the cohort enrolled in the EVIDIMS trial, which was terminated prematurely. As a result, the sample size is small, limiting the statistical power and generalizability of the findings. Additionally, the inclusion criteria were not specifically designed to assess the effects of vitamin D supplementation on MRI and OCT biomarkers. Individual variability in imaging measures—particularly within a small sample—may have further influenced the observed outcomes. Another important limitation is the relatively short follow-up duration of 18 months. This time frame may be insufficient to detect meaningful changes in atrophy progression, especially in patients with clinically stable disease and no recorded relapses. Prior longitudinal studies in RRMS suggest that structural changes in the brain and spinal cord often require longer observation periods to become evident. Future studies with extended follow-up will be critical to determine whether vitamin D supplementation exerts any long-term neuroprotective effects. The absence of a placebo group also constrains the interpretability of our results, as we cannot assess the absolute effect of vitamin D supplementation on disease progression. Although our data show no significant difference in atrophy progression between high- and low-dose groups, previous studies have suggested that higher-dose regimens may be more efficacious (Thouvenot et al., 2025). Therefore, future trials exploring higher-dose vitamin D supplementation, ideally in comparison with placebo, are warranted.

It is also important to consider the therapeutic context in which this study was conducted. All participants were treated with interferon-beta-1b, a disease-modifying therapy that is now less commonly used due to the emergence of newer, high-efficacy agents. This limits the external validity of our findings for patients receiving contemporary therapies. Nevertheless, insights from studies involving less efficacious treatments remain valuable, particularly in settings where access to advanced therapeutic options is limited. Further research should explore whether vitamin D supplementation interacts differently with newer MS therapies.

An additional avenue for future investigation involves early or atypical disease presentations, such as isolated clinical syndrome or radiologically isolated syndrome. In these scenarios, vitamin D supplementation—alone or in combination with lower-efficacy treatments—may hold greater potential and deserves dedicated evaluation. Finally, technical aspects may have impacted the precision of our measurements. The transformation of longitudinal MRI scans to baseline introduces measurement variability, a recognized limitation in volumetric analysis. Similarly, the lack of co-registration in longitudinal spinal cord imaging may have introduced partial volume effects, affecting MUCCA quantification (Chien et al., 2020b; Reuter et al., 2012). A more refined approach to assess neurodegeneration would involve calculating annualized regional atrophy rates rather than relying on absolute volume differences. Notably, the slightly lower baseline thalamic volume in the low-dose group raises the possibility that group differences in annualized atrophy may have been obscured. Future analyses employing this methodology could enhance our understanding of the potential neuroprotective role of vitamin D.

In conclusion, our findings suggest that different doses of vitamin D supplementation do not significantly affect atrophy rates in the brain, spinal cord, or retina in stable RRMS patients. However, due to limitations such as short follow-up duration, small sample size, and lack of a placebo group, further research is needed to determine whether vitamin D plays a role in neuroprotection, especially in the context of modern MS therapies, early stage or active disease. Future studies should also investigate the relationship between vitamin D levels and acute relapses, examining whether vitamin D status at the time of relapse influences inflammatory activity and subsequent atrophy progression.

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CRediT authorship contribution statement

Enrique Gomez-Figueroa: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Carlos Moreno-Bernardino:** Writing – review & editing. **Priscilla Bäcker-Koduah:** Investigation, Data curation. **Jan Dörr:** Investigation, Formal analysis, Conceptualization. **Kerstin Rubarth:** Supervision, Methodology, Formal analysis. **Frank Konietschke:** Investigation, Formal analysis, Data curation. **Judith Bellmann-Strobl:** Resources, Investigation, Formal analysis, Conceptualization. **Klemens Ruprecht:** Resources, Investigation, Data curation. **Frederike Cosima Oertel:** Writing – review & editing, Supervision, Methodology, Investigation. **Friedemann Paul:** Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation. **Claudia Chien:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Conceptualization. **Hanna Zimmermann:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

"The authors report no conflicts of interest related to the design, conduct, or reporting of this research".

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.msard.2025.106567.

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