Vitamin D_3 supplementation in multiple sclerosis

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Vitamin D_3 supplementation in multiple sclerosis: Symptoms and biomarkers of depression

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ABSTRACT

Depressive symptoms are common in multiple sclerosis (MS), and both depression and MS have been associated with a poor vitamin D status. As cytokine-mediated inflammatory processes play a role in the pathogenesis of both disorders, we hypothesized that vitamin D_3 supplementation reduces depressive symptoms in MS via its immunomodulatory properties. In this randomized pilot study relapsing remitting (RR) MS patients received either vitamin D_3 supplementation ($n = 20$; 14.000 IU/day) or placebo ($n = 20$) during 48 weeks. Pre- and post-supplementation depression scores, measured using the Hospital Anxiety Depression Scale (HADS) depression subscale (HADS-D), showed a significant decrease within the vitamin D_3 group (median HADS-D 4.0 to 3.0, $p = 0.02$), a trend towards a decrease within the placebo group (median HADS-D 3.0 to 2.0, $p = 0.06$), but no significantly different reductions between groups ($p = 0.78$). Furthermore, no reductions in pro- and anti-inflammatory cytokine balances, secreted by stimulated leukocytes and $CD8^+$ T cells, were found in the vitamin D_3 compared to the placebo arm. Therefore, we found no evidence for a reduction of depressive symptoms or related biomarkers upon vitamin D_3 supplementation in RRMS patients in this exploratory study. Whether vitamin D_3 supplementation is of benefit in manifest depression in MS needs to be assessed by additional studies.

1. Introduction

Multiple sclerosis (MS) can have a major impact on quality of life. Local inflammation in the central nervous system (CNS) causes disturbed signaling of affected neurons, leading to a large variety of symptoms, such as motoric or sensory dysfunction or visual disturbances. Other MS symptoms cannot be explained by local inflammation alone, including fatigue and depression. With up to 90% of the patients with MS complaining of fatigue, it is the most reported symptom of MS. [1] Fatigue is a complex symptom with a multifocal etiology. Also, depressive disorder is frequent in MS, with lifetime prevalence rates going up to 50% [2], whereas in the general population a prevalence of up to 20% is reported [3]. Depressive disorder in MS needs to be considered as a major concern, since it negatively affects quality of life and may cause or perpetuate fatigue [2]. Also, it may lead to medication nonadherence [4], which could influence long-term disease outcomes.

The underlying mechanisms for depression (in MS) are not completely understood, and several (combinations of) theories have been proposed in order to explain the complex pathogenesis. These theories include the monoamine hypothesis [5], based on the deficiency of particularly serotonin and noradrenalin, the neurogenesis or neurodegeneration hypothesis [6], particularly involving atrophy of the hippocampus [7], the hypothalamic-pituitary-adrenal (HPA)-axis dysfunction theory [8], and the inflammatory theory [9]. The latter describes the importance of cytokines in the development of depression. Both in MS and in depressive disorder increased pro-inflammatory cytokine levels (i.e. the monocyte/macrophage derived cytokines tumor necrosis factor alpha (TNFα), interleukin (IL)-1 and IL-6) have been observed in the circulation and in the cerebrospinal fluid (CSF) [10,11,12]. These cytokines were positively associated with depression severity, [10] and were reduced upon antidepressant therapies [10,13]. Also, the proportion of $CD8^+$ (but not $CD4^+$) T cells producing TNFα and interferon-gamma (IFNγ) was shown to be increased in patients with MS with a depressive disorder compared to the ones without a depressive disorder [14]. Anti-inflammatory therapy such as anti-TNF therapy markedly improved mood in treatment-

Abbreviations: 25(OH)D, 15-hydroxyvitamin D; FSS, fatigue severity scale; HADS, hospital anxiety and depression scale; IFNγ, interferon-gamma; IL, interleukin; PBMC, peripheral blood mononuclear cells; RRMS, relapsing remitting multiple sclerosis; TNFα, tumor necrosis factor alpha.

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resistant depressive disorder, [15] and pro-inflammatory cytokines are shown to induce sickness behaviour (i.a. fatigue, depression, loss of appetite) [10]. Furthermore, in each theory previously mentioned a role for pro-inflammatory cytokines has been suggested: they may cause serotonergic depletion [10], induce imbalance in the kynurenine pathway with neurotoxicity as a result, [6] and induce HPA-axis activity [16]. Although some studies report decreased anti-inflammatory IL-10 levels in depressed people [17], and antidepressant therapies have shown to increase IL-10 [13], it is not clear whether also a decrease in anti-inflammatory cytokines contributes to depression [18]. However, an imbalance in pro- and anti-inflammatory cytokines may play a central role in the development of depression (in MS) and well-balanced pro- and anti-inflammatory cytokines may prevent and/or ameliorate depression [13].

Insufficiency of vitamin D has been associated with MS risk and MS disease activity, [19,20] as well as with the presence and severity of depressive disorder (in MS) [21,22]. Vitamin D is a steroid hormone with immunomodulatory properties, causing a decrease in the production of pro-inflammatory cytokines and an increase in the production of anti-inflammatory cytokines [23]. Therefore, particularly in the context of the chronic inflammation in MS, vitamin D supplementation might prevent or ameliorate depressive disorder. However, data appear conflicting with respect to the role of vitamin D in depressive disorder in patients with MS [22,24], and with respect to the benefit of vitamin D supplementation in depression in general [25]. Therefore we used a randomized placebo-controlled trial (RCT) to explore the effect of high dose vitamin D_3 supplementation on depressive symptoms in MS. Fatigue was assessed as a potential confounder [2,14]. We also assessed the TNFα/IL-10 balance before and after supplementation, and the proportion of $CD8^+$ T cells producing pro- and anti-inflammatory cytokines, since the mode of action by which vitamin D could ameliorate depression may reflect a normalization of the pro-inflammatory/anti-inflammatory cytokine ratio.

2. Methods

2.1. Patient recruitment

This randomized pilot study describes some of the secondary outcomes of the Dutch sub-study SOLARIUM of the SOLAR trial, which are both described in more detail elsewhere [26]. In brief, all patients were recruited in four hospitals in the Netherlands. Patients were eligible when they were aged between 18 and 55, diagnosed with RRMS according to the original or 2005 revised McDonald criteria [27] confirmed by MRI, treated with interferon-β1α (Rebif®, Merck. Darmstadt, Germany), had their first clinical event in the previous 5 years, and had active disease in the previous 18 months, but not in the 30 days prior to inclusion. Among exclusion criteria were: use of oral or systemic glucocorticoids or ACTH within 30 days prior to inclusion, a history or presence of severe depression, a history of suicide attempt or current suicidal ideation, and current or past drug or alcohol abuse. Patients were randomized and allocated to one of the two treatment arms, being either placebo or vitamin D_3. Randomization for the SOLAR trial, however, was based on international patient inclusion, eventually resulting in different numbers of patients for each treatment arm in SOLARIUM. Patients allocated to the vitamin D_3 supplementation arm received cholecalciferol (Vigantol Oil, Merck Serono S.A.) dosed at 7000 IU/day in the first 4 weeks, followed by 14,000 IU/day up to week 48, being the adjusted endpoint of the SOLAR trial (https://clinicaltrials.gov/ct2/show/NCT01285401).

The SOLARIUM sub-study and the amendment for this pilot study, was approved by the Medical Ethical Committee METC Z (Heerlen, the Netherlands), and patients gave their written informed consent for participation in both SOLARIUM and SOLAR.

2.2. Serum 25(OH)D assessment

To determine the vitamin D status serum 25(OH)D levels (with an upper limit of 250 nmol/L) were measured using the Packard Cobra II Gamma Counter radioimmunoassay (GMI, Ramsey, Minnesota), performed by Covance (Princeton, New Jersey) as part of the SOLAR trial.

2.3. Questionnaires

Questionnaires were obtained in the same visit blood samples were drawn.

Depression was measured with a valid and reliable screening instrument for MS patients [28], namely the depression subscale of the Hospital Anxiety and Depression Scale (HADS) [29]. The HADS depression (HADS-D) score ranges from 0 to 21 with a higher score indicating more symptoms of depression. Honarmand and Feinstein showed that MS patients with a score of 8 or higher are likely depressed [28].

Fatigue was assessed by the Dutch version of the Fatigue Severity Scale (FSS) [30]. This 9-item scale measures the severity of fatigue and the impact of fatigue in daily functioning. All items are evaluated on a 7-point Likert scale (1 = I totally disagree, to 7 = I totally agree). A total score of 36 or higher indicates severe fatigue. The FSS is a valid and reliable scale for the assessment of fatigue in MS patients [31].

2.4. Cell isolation and cytokine secretion in culture supernatant

In order to investigate the effects of high dose vitamin D_3 supplementation on depression-associated cytokine secretion, TNFα and IL-10 concentrations were assessed in culture supernatants of lipopolysaccharide (LPS) stimulated peripheral blood mononuclear cells (PBMC). Hereto PBMC were isolated from peripheral venous blood samples as previously described [26]. Subsequently, these freshly isolated PBMC were cultured in a concentration of 10^5 cells per well (96-wells plate) in the presence of 1 μg/mL LPS (*E. Coli*, serotype 0111: B4; Difco Laboratories, Detroit, Michigan). After 72 h of culture, cells were centrifuged, and supernatants were collected and stored until further analysis. At the end of the study, TNFα and IL-10 concentrations in these supernatants were analyzed using an in-house developed and validated multiplex immunoassay (Laboratory of Translational Immunology, University Medical Center Utrecht, the Netherlands) based on Luminex technology (Luminex®, Luminex Corporation, Austin, Texas), and data were analyzed using Bio-Plex Manager software, version 6.1.1 (Biorad laboratories, Hercules, California).

2.5. Intracellular cytokine detection in $CD8^+$ T cells

After isolation of PBMC and 5 h of in vitro activation with 50 ng/mL phorbol myristate acetate (PMA; Sigma Aldrich, Zwijndrecht, the Netherlands) and 1 μg/mL ionomycin (Sigma Aldrich) in the presence of 1.25 μg/mL monensin (BD Biosciences, Breda, the Netherlands), cells were stained to identify $CD8^+$ T cells producing the pro-inflammatory cytokines TNFα and IFNγ. Hereto, they were stained for surface markers with anti-CD3-Horizon V450 and anti-CD8-APC-H7 (both BD Biosciences), and after fixation and permeabilisation (cytofix/cytoperm, BD Biosciences) the cells were stained intracellularly using anti-TNFα-PECy7 (eBioscience, Vienna, Austria) and anti-IFNγ-FITC (BD Biosciences). Cells were measured by flow cytometry (FACS Canto II flow cytometer, BD Biosciences) and analyzed with FACS Diva software (version 8.01, BD Biosciences).

2.6. Statistical analyses

All statistical analyses were performed using SPSS software (SPSS Inc., version 20, Chicago, Illinois). Data were checked for normality. Data are provided as means with standard deviations (SD) or medians with the 25th–75th percentile ranges (Q1–Q3). All outcome measures

Table 1
Baseline characteristics of the study population with questionnaires available at both baseline and week 48.

	Placebo (n = 20)	Vitamin D_3 (n = 20)
Sex (n) F/M	12/8	14/6
Age (years)		
Mean (±SD)	37.6 (±9.6)	38.5 (±7.8)
Disease duration (months)		
Median (Q1–Q3)	5.7 (3.9–11.7)	7.5 (4.4–11.7)
IFNβ treatment duration (months)		
Median (Q1–Q3)	3.8 (3.3–6.1)	4.4 (3.7–6.9)
EDSS score		
Median (Q1–Q3)	2.0 (1.5–2.3)	2.0 (1.5–2.5)
Relapses in previous 2 years (n)		
≤1	16	11
≥2	4	9
BMI (n)		
<25	10	9
≥25	10	11
25(OH)D (nmol/L)		
Median (Q1–Q3)	53 (43–63)	58 (38–82)
HADS-D		
Median (Q1–Q3)	3.0 (2.0–7.0)	4.0 (2.0–5.0)

F = female; M = male; SD = standard deviation; Q1 – Q3 = 25th – 75th percentile; IFNβ = Interferon-beta 1α (Rebif®); EDSS = expanded disability status scale; BMI = body mass index.

were non-normally distributed and therefore group differences were analyzed using Mann-Whitney U tests, and differences within groups with Wilcoxon signed rank tests. Absolute differences for 25(OH)D levels and HADS-D, and relative differences using baseline/week 48 ratio's for immune parameters were used for comparisons between groups. Furthermore, to compare changes in HADS-D over time between groups, corrected for fatigue and cytokine balances, ANCOVA tests were performed with week 48 data as dependent variable, trial medication as fixed factor and baseline data and FSS or cytokine ratios as covariates. Spearman's rank correlation was used to assess the association between baseline HADS-D scores and 25(OH)D levels or cytokine measurements. p-Values <0.05 were considered statistically significant.

3. Results

3.1. Study population

In the SOLARIUM study 58 patients were included, of which 33 were allocated to the vitamin D_3 and 25 to the placebo arm. Of these randomized patients 5 were lost to follow-up. Patient characteristics of the total study population of n = 53 are described elsewhere [26]. The questionnaires were implemented in the study several months after the start of the study. Therefore, the HADS-D and FSS scores were only available for 20 patients in each treatment arm at both baseline (T0) and week 48 (T1). Patient characteristics of this population can be found in Table 1, and a flow diagram is shown in Fig. 1.

3.2. Serum 25(OH)D levels

Serum 25(OH)D levels within the placebo arm remained stable between T0 and T1 measurements (median vitamin D_3 (Q1–Q3) at baseline vs week 48: 53 (43–63) nmol/L and 61 (44–84) nmol/L resp.; p = 0.19), while within the vitamin D_3 supplementation arm 25(OH)D levels significantly increased (median vitamin D_3 (Q1–Q3) at T0 vs T1: 58 (38–82) and 226 (159–250) nmol/L respectively; p < 0.01), resulting in significantly higher 25(OH)D levels at T1 and a significantly larger T1–T0 difference in the vitamin D_3 supplemented arm compared to the placebo arm (both p < 0.01) (Fig. 2).

3.3. High dose vitamin D supplementation does not decrease depression and fatigue scores

Previously it was found that 25(OH)D levels correlated negatively with depression scores [22]. Here, this correlation was not observed at baseline (data not shown). When HADS-D scores at T0 and T1 were compared within groups a significant decrease was observed within the vitamin D_3 treated patients (p = 0.02, Fig. 2D), whereas a trend towards a decrease was found for the placebo group (p = 0.06; Table 2, Fig. 2E). However, the T1–T0 changes of the HADS-D scores did not significantly differ between groups (median vitamin D_3 (Q1–Q3) = −1.0

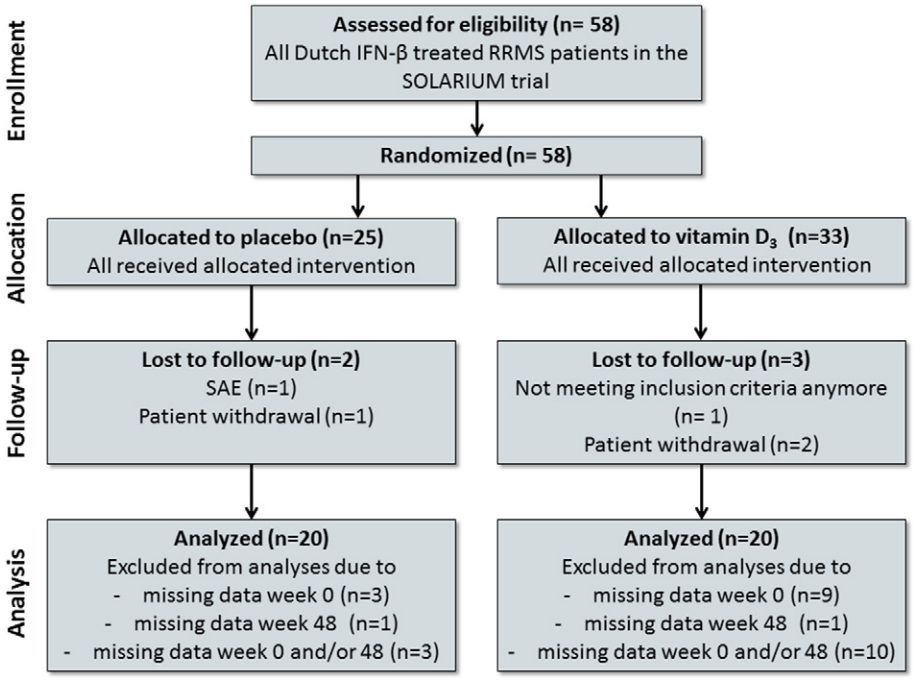

Fig. 1. Flow diagram of the study process. IFN-β = interferon-beta 1α (Rebif®); RRMS = relapsing remitting multiple sclerosis; SAE = serious adverse event; T0 = baseline; T1 = week 48.

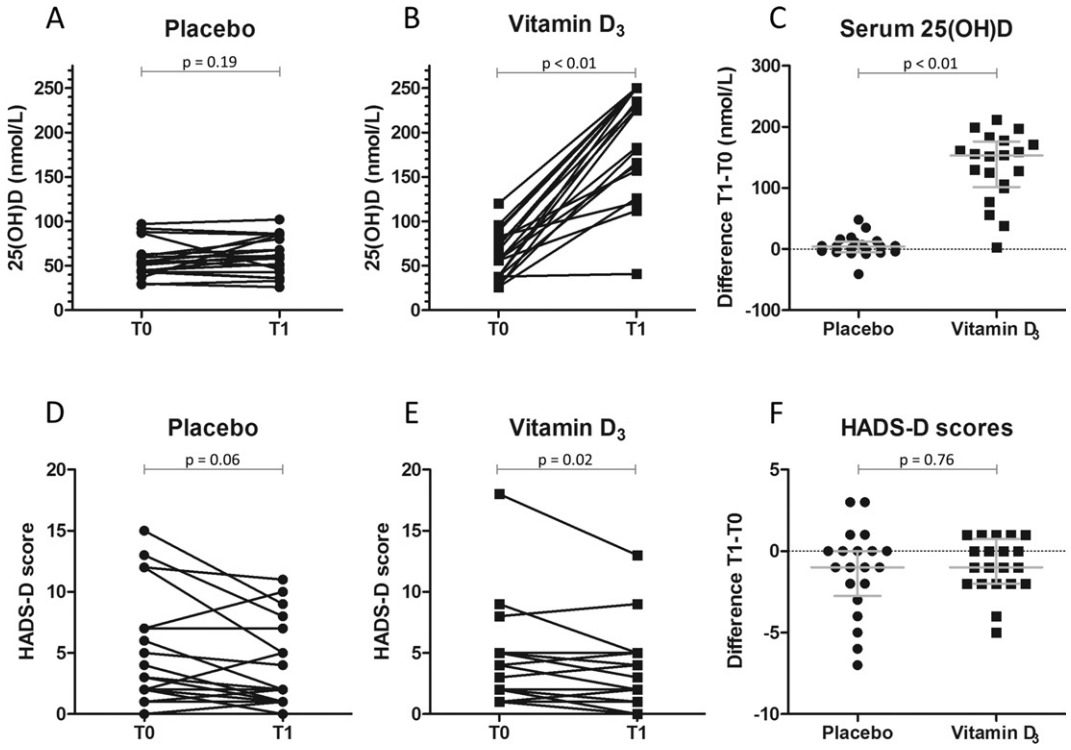

Fig. 2. Comparisons of serum 25(OH)D levels and HADS-D scores within and between groups. A) Within group comparisons of serum 25(OH)D levels at T0 (baseline) and T1 (week 48) in the placebo arm (n = 20). B) Within group comparisons of serum 25(OH)D levels at T0 and T1 in the vitamin D_3 supplementation arm (n = 20). C) Between group comparisons of the serum 25(OH)D level differences between T1 and T0. D) Within group comparisons of HADS-D scores at T0 and T1 in the placebo arm (n = 20). E) Within group comparisons of HADS-D scores at T0 and T1 in the vitamin D_3 supplementation arm (n = 20). F) Between group comparisons of the HADS-D score differences between T1 and T0. HADS-D = depression subscale of the Hospital anxiety and Depression Scale.

(−2.0 – +0.8); placebo = −1.0 (−2.8–0.0); p = 0.78; Fig. 2F). Also, in an ANCOVA model no additive effect of treatment on HADS-D scores could be found, neither when corrected for HADS-D at T0 (F (1,37) = 0.003, p = 0.96, r = 0.009), nor when corrected for HADS-D at T0 and FSS at T1 (F(1,36) = 0.008, p = 0.93. r = 0.015). Therefore, we cannot consider this reduction in HADS-D score as treatment dependent. In neither of the groups FSS scores changed significantly over time (Table 2).

3.4. High dose vitamin D supplementation does not decrease pro-inflammatory/anti-inflammatory cytokine ratios of PBMC

Since we did not find a significant change in depression score, we explored an effect of vitamin D supplementation on earlier identified biomarkers of depressive symptoms in MS. The production of TNFα and IL-10 by PBMC was assessed, as a reflection of the balance between pro- and anti-inflammatory cytokines relevant for depression. Results are shown in Table 3 and Fig. S1. At T0, neither TNFα and IL-10 levels nor the TNFα/IL-10 ratio correlated with HADS-D scores (Fig. S2A—C). Within the placebo arm a trend towards an increase in IL-10 (p = 0.06), with a significant reduction in the TNFα/IL-10 ratio (p = 0.01) was found after 48 weeks, but no cytokine changes were found in the vitamin D_3 supplemented arm. The change in the TNFα/IL-10 ratio after 48 weeks of treatment was significantly different between the groups (p = 0.02).

3.5. High dose vitamin D supplementation does not decrease pro-inflammatory/anti-inflammatory cytokine ratios of $CD8^+$ T cells

$CD8^+$ T cells producing pro-inflammatory cytokines were shown to be relatively increased in patients with MS with comorbid depressive disorder [14]. Therefore, we assessed the proportions of $CD8^+$ T cells producing TNFα and IFNγ (Table 4 and Fig. S3). Next to these pro-inflammatory cytokines we also assessed the $CD8^+$ T cell production of the anti-inflammatory cytokine IL-10. At T0, neither the proportions of cytokine-producing cells nor the ratios correlated with HADS-D scores (Fig. S2D—H). Also, in neither of the groups the proportions of $CD8^+$ T cells producing TNFα, IFNγ or IL-10 changed significantly after 48 weeks of treatment. With respect to the ratios of pro- and anti-inflammatory $CD8^+$ T cells, there was a significant reduction for the IFNγ/IL-10 ratio only within the vitamin D_3 group (p = 0.04). This reduction, however, was again not significantly different from the non-significant reduction in the placebo group (p = 0.94).

Table 2
Between and within group comparisons for HADS-D scores.

	Placebo (n = 20)			Vitamin D_3 (n = 20)			
	T0 M (Q1 – Q3)	T1 M (Q1 – Q3)	P-value	T0 M (Q1 – Q3)	T1 M (Q1 – Q3)	P-value	P-value[a]
HADS-D	3.0 (2.0–7.0)	2.0 (1.0–6.5)	0.06	4.0 (2.0–5.0)	3.0 (2.0–5.0)	0.02	0.78
FSS	4.6 (3.5–5.2)	4.3 (3.4–5.1)	0.56	4.9 (3.3–6.0)	4.6 (2.8–6.0)	0.39	0.95

HADS-D = depression subscale of the Hospital anxiety and Depression Scale; FSS = fatigue severity scale; T0 = baseline; T1 = week 48; Q1–Q3 = 25th – 75th percentile.
[a] Between group comparisons of the T1–T0 differences.

Table 3
Between and within group comparisons for cytokine secretion.

	Placebo (n = 16)			Vitamin D_3 (n = 20)			
	T0 M (Q1 – Q3)	T1 M (Q1 – Q3)	P-value	T0 M (Q1 – Q3)	T1 M (Q1 – Q3)	P-value	P-value[a]
TNFα (pg/mL)	1035 (331–1701)	765 (302–1535)	0.92	879 (393–1513)	932 (648–1662)	0.35	0.37
IL-10 (pg/mL)	1306 (919–1754)	1762 (1117–2496)	0.06	1100 (673–2125)	1543 (1021–2104)	0.71	0.54
TNFα/IL-10 ratio	0.81 (0.41–1.20)	0.37 (0.24–0.81)	0.01	0.56 (0.31–0.88)	0.69 (0.46–1.29)	0.23	0.02

TNFα = tumor necrosis factor alpha; T0 = baseline; T1 = week 48; IL-10 = interleukin-10; Q1–Q3 = 25th – 75th percentile.
[a] Between group comparisons of the relative changes using T1/T0 ratios.

4. Discussion

Little is known about the possible role of vitamin D on depressive symptoms in MS. Here we explored the effects of high dose vitamin D_3 supplementation on depressive symptoms in RRMS using a randomized controlled design. Although a significant decrease in HADS-D scores was observed within the vitamin D_3 supplementation arm, this reduction was not significantly different from the decrease seen in the placebo group. This observation emphasizes the need for blinded and controlled studies on vitamin D and MS-related outcomes. Furthermore, we did not observe the expected shift in pro- and anti-inflammatory cytokine levels in the supernatant of LPS-stimulated PBMC after vitamin D supplementation, nor did we observe a reduction in the proportions $CD8^+$ T cells producing TNFα and IFNγ or a more reduced pro-inflammatory/anti-inflammatory cytokine ratio. Therefore, our data do not support a general reduction of depressive symptoms or related inflammatory biomarkers in MS after supplementation of high doses vitamin D_3.

In non-MS cohorts, low serum 25(OH)D levels were shown to be associated with more depressive symptoms [32,33]. Patients with a current depression had higher odds of having a low vitamin D status compared to healthy controls, and the vitamin D status of these patients was inversely correlated with depression severity [21]. Although most studies have shown this relation between vitamin D and depression, some others did not find these associations [34,35], and causality is uncertain. In depressive disorder several RCTs with vitamin D_3 have been conducted, again with conflicting results. Even meta-analyses, assessing largely the same studies, are not unanimous, showing either beneficial effects of vitamin D_3 supplementation on depression or no effect at all [36,37,25]. However in some RCTs, as well as in the current study, patients had low depression scores and relatively normal 25(OH)D levels at baseline [37,25]. Therefore, it may be that effects of vitamin D_3 supplementation can only be measured in actual depressive disorder or in case of vitamin D insufficiency.

In MS patients also associations between vitamin D status and depression have been observed in cohorts not selected for manifest depression. However, they were not maintained after adjustment for confounders [22]. By contrast, sun exposure was found to be associated with depression in MS, independent of 25(OH)D levels [24]. Therefore, previous studies showing associations between vitamin D and depression may have captured associations as an epiphenomenon. This may also explain why supplementation of high daily dosages of vitamin D, which substantially increase serum 25(OH)D levels, does not have a clear effect on depression scores in our study. Alternatively, we cannot exclude that our groups were too small and lacked the power to detect an effect of vitamin D_3 supplementation. This might be related to the study design based on only two evaluations in a timeframe of 48 weeks, and influence of confounding factors. Furthermore, other studies need to show whether our results are also applicable to other cohorts of MS patients, especially those with longstanding disease or with major depression.

Reductions of depressive symptoms may be the result of a normalized immune/cytokine profile [13,10]. Vitamin D has been shown to affect several immune parameters, including serum cytokine levels and several cell subsets of the adaptive and innate immune system [19,23]. Here, we measured pro- and anti-inflammatory cytokine secretion by PBMC and $CD8^+$ T cells, which have been shown to be associated with depression and comorbid depression in MS [14]. These biomarkers for depression were also not influenced by vitamin D_3 supplementation. Also, cytokine balances did not contribute to the HADS-D scores in ANCOVA models (data not shown). It would have been interesting to also study the effects of vitamin D supplements on other parameters, such as cytokine levels in CSF or inflammatory activity on MRI, which possibly better reflect the local effects of vitamin D in the CNS.

In this study patients of both groups showed a reduction in depressive scores after 48 weeks. As there are several theories to explain depression, several mechanisms besides normalization of cytokine profiles may account for this reduction. Our study population had a short median disease duration at baseline, below one year. The reduction in depressive symptoms in both groups, therefore, may reflect a depressive reaction after diagnosis which is normalized over time because of better acceptance and coping strategies [38]. Also, the reductions in both arms may be explained by nonspecific attention from participation in the trial [39]. Furthermore, Gold et al. suggest that different mechanisms, i.e. HPA-axis dysregulation and inflammation, mediate different aspects of sickness behaviour [14]. They observed that the proportions of pro-inflammatory $CD8^+$ T cells were more related to fatigue, whereas the cortisol slope as HPA-axis parameter was associated with depressive symptoms [14]. Indeed, we previously found no association between serum 25(OH)D levels and fatigue in RRMS [22], and also in the present study no changes were found for fatigue and immunological parameters after vitamin D_3 supplementation. We did not measure HPA-axis

Table 4
Between and within group comparisons for the cytokine producing $CD8^+$ T cells.

	Placebo (n = 20)			Vitamin D_3 (n = 20)			
	T0 M (Q1 – Q3)	T1 M (Q1 – Q3)	p-Value	T0 M (Q1 – Q3)	T1 M (Q1 – Q3)	p-Value	p-Value[a]
% $TNFα^+CD8^+$ T cells	21.5 (14.5–38.2)	19.7 (11.1–36.9)	0.22	27.1 (18.7–31.5)	22.5 (16.2–35.3)	0.91	0.21
% $IFNγ^+CD8^+$ T cells	18.9 (15.9–26.4)	16.1 (9.2–28.7)	0.58	28.1 (18.2–40.5)	22.8 (16.1–36.4)	0.37	0.82
% $IL10^+CD8^+$ T cells	0.7 (0.3–1.0)	0.9 (0.4–1.2)	0.49	0.7 (0.4–1.1)	0.8 (0.7–1.3)	0.14	0.46
TNFα/IL-10 ratio	42.8 (19.3–79.8)	32.5 (17.5–49.5)	0.28	34.7 (21.5–104.3)	28.0 (16.4–45.3)	0.16	0.94
IFNγ/IL-10 ratio	36.7 (17.7–63.9)	30.7 (10.7–40.6)	0.24	45.1 (25.2–78.8)	27.6 (18.2–42.9)	0.04	0.94

TNFα = tumor necrosis factor alpha; T0 = baseline; T1 = week 48; IFNγ = interferon-gamma; Q1–Q3 = 25th – 75th percentile.
[a] Between group comparisons of the relative changes using T1/T0 ratios.

parameters, therefore it might be that there is an improvement of the HPA-axis regulation.

Altogether, we found no evidence that vitamin D_3 supplementation reduces depressive symptoms or related biomarkers in early RRMS in our exploratory study. Whether vitamin D_3 supplementation is of benefit for manifest depression and/or preferentially in case of a poor vitamin D status in MS needs to be assessed by additional randomized controlled studies.

Conflicts of interest

LR, AM, YB, and JD report no disclosures. JS received lecture and/or consultancy fees from Biogen, Merck, Genzyme and Novartis. RH received honoraria for lectures and advisory boards, Institutional and Research Grants from Merck, Biogen, Sanofi-Genzyme, Novartis and TEVA.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jns.2017.04.017.

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