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Vitamin D and disease activity in multiple sclerosis before and during interferon-β treatment

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ABSTRACT

Objective: Studies based on deseasonalized vitamin D levels suggest that vitamin D may influence the disease activity in multiple sclerosis (MS), and high doses are suggested as add-on treatment to interferon-β (IFN-β). Seasonal fluctuation of vitamin D varies between individuals, thus the relationship to disease activity should preferentially be studied by repeated and simultaneous vitamin D and MRI measurements from each patient.

Methods: This was a cohort study comprising 88 patients with relapsing-remitting MS who were followed for 6 months with 7 MRI and 4 25-hydroxyvitamin D measurements before initiation of IFN-β, and for 18 months with 5 MRI and 5 25-hydroxyvitamin D measurements during IFN-β treatment.

Results: Prior to IFN-β treatment, each 10 nmol/L increase in 25-hydroxyvitamin D was associated with 12.7% ($p = 0.037$) reduced odds for new T1 gadolinium-enhancing lesions, 11.7% ($p = 0.044$) for new T2 lesions, and 14.1% ($p = 0.024$) for combined unique activity. Patients with the most pronounced fluctuation in 25-hydroxyvitamin D displayed larger proportion of MRI scans with new T1 gadolinium-enhancing lesions (51% vs 23%, $p = 0.004$), combined unique activity (60% vs 32%, $p = 0.003$), and a trend for new T2 lesions (49% vs 28%, $p = 0.052$) at the lowest compared to the highest 25-hydroxyvitamin D level. No association between 25-hydroxyvitamin D and disease activity was detected after initiation of IFN-β. HLA-DRB1*15 status did not affect the results.

Conclusion: In untreated patients with MS, increasing levels of 25-hydroxyvitamin D are inversely associated with radiologic disease activity irrespective of their HLA-DRB1*15 status. ***Neurology***® **2012;79:267–273**

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GLOSSARY

25(OH)D = 25-hydroxyvitamin D; **CI** = confidence interval; **CUA** = combined unique activity; **EDSS** = Expanded Disability Status Scale; **IFN-β** = interferon-β; **MS** = multiple sclerosis; **RRMS** = relapsing-remitting MS.

A poor vitamin D status is a potential risk factor for multiple sclerosis (MS),[1] and may possibly also affect the disease course. Seasonal variations in sunlight and vitamin D levels are correlated with relapse rate at the population level.[2] Lower levels of vitamin D have been recorded at relapse compared to remission in cross-sectional, retrospective, and prospective studies.[3–7] Prospective studies are few and restricted by a limited number of vitamin D measurements in each patient,[7–9] but have suggested that increasing deseasonalized vitamin D levels are associated with reduced relapse rate.[8,9] Pilot studies of vitamin D treatment have mainly focused on safety,[10–16] but have also indicated potentially beneficial immunologic,[12–14] clinical,[12,15] and radiologic[16] effects.

Editorial, page 208

See pages 254 and 261

Supplemental data at www.neurology.org

Supplemental Data

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Go to Neurology.org for full disclosures. Disclosures deemed relevant by the authors, if any, are provided at the end of this article.

The extent of seasonal fluctuation of vitamin D varies between individuals,[17] and the relationship with disease activity should therefore preferentially be studied with repeated assessments in each patient. Notably, vitamin D has been shown to regulate the expression of the MS-associated HLA-DRB1*15 allele,[18] HLA-DRB1 and smoking interact in the risk of MS,[19] further advocating that HLA determines the impact of environmental triggers. We performed a prospective 2-year study of 88 HLA-DR typed patients, before and during treatment with interferon-β-1a (IFN-β) (Rebif™, Serono International, Geneva, Switzerland), to examine the relationship between vitamin D and disease activity in relapsing-remitting MS (RRMS). The study comprised 12 MRI scans and 9 vitamin D measurements in each patient.

METHODS

Study population and design. This study comprised all patients with RRMS who completed at least 12 months of a 24-month randomized, placebo-controlled trial of ω-3 fatty acids in MS (the OFAMS study).[20] The original OFAMS study comprised 92 patients with RRMS according to the McDonald criteria, age 18–58 years, Expanded Disability Status Scale (EDSS) score $\leq$5 at inclusion, and $\geq$1 relapse or 1 new T1-weighted gadolinium-enhancing lesion (T1 Gd+ lesion) or T2-weighted lesion (T2 lesion) on MRI in the year prior to inclusion. All patients were treatment naive, except 3 who had stopped using immunomodulatory drugs (1 IFN-β, 2 glatiramer acetate) >6 months before inclusion. During the whole study period, the participants received either ω-3 fatty acids (Triomar™, Pronova Biocare, Sandefjord, Norway) or placebo (corn oil). From month 6, all participants received subcutaneous injections of IFN-β 3 times weekly. None of the treatments contained vitamin D. The participants were not given any particular advice regarding vitamin D supplementation. The inclusion period extended from December 2004 until July 2006. The timing of clinical examinations, MRI, and serum sampling is shown in table 1. The patients were recruited at 13 Norwegian MS centers situated at latitudes 58°–63° north.

Clinical examination. EDSS and relapses were recorded by an experienced study neurologist. A relapse was defined as the appearance of new or worsening of old neurologic symptoms or signs, in the absence of fever, persisting for more than 48 hours and causing objective changes on neurologic examination.

Table 1 Timing of MRI, clinical scoring (EDSS), and 25(OH)D assessment

		Month											
	Baseline (month 0)	1	2	3	4	5	6	7	8	9	12	18	24
MRI examination	•	•	•	•	•	•	•	•	•	•	•		•
EDSS evaluation	•						•				•	•	•
Serum 25(OH)D	•	•		•			•	•		•	•	•	•

Abbreviations: 25(OH)D = 25-hydroxyvitamin D; EDSS = Expanded Disability Status Scale.

Measurement of 25-hydroxyvitamin D. Serum samples were stored at −80°C until analysis. The analyses were performed simultaneously for all samples from each patient. The laboratory technicians were not aware of the clinical or radiologic status of the patients. The 25-hydroxyvitamin D [25(OH)D_3 and 25(OH)D_2] was measured by radioimmunoassay (RIA kit, ImmunoDiagnostic Systems) at the Department of Medical Biochemistry, St. Olavs Hospital, Trondheim, Norway. The coefficient of variation was 5.4% and 6.3% at 29 and 112 nmol/L, respectively.

HLA-DRB1 typing. The HLA-DRB1 status was determined by DNA sequencing using SeCore Loc DRB1 SEQ kit (Invitrogen) at the Institute of Immunology, Oslo University Hospital, Rikshospitalet.

MRI. The MRIs were performed according to a standardized protocol comprising T2-weighted and T1-weighted Gd+ scans using a standard head coil with a 1.5 Tesla MRI unit. Blinded assessments of T1 Gd+ lesions, T2 lesions, and combined unique activity (CUA; the sum of T1 Gd+ lesions and new or enlarging T2 lesions) were conducted by 2 experienced neuroradiologists.

Statistics. A large proportion of the scans did not display new T1 Gd+ lesions, new T2 lesions, or CUA, and the MRI outcomes were therefore dichotomized as present or absent. Due to repeated measurements of 25(OH)D and MRI in each patient, the association between 25(OH)D levels and MRI outcomes was modeled by a logistic regression model for hierarchical data.[21] The SAS GLIMMIX procedure was used to fit the model with random intercepts for patients and fixed 25(OH)D effects. The association was further adjusted for gender, age, and HLA-DRB1 status. The SAS GLIMMIX procedure works well with unbalanced data; therefore, patients with one or more missing values were not excluded from the analysis. McNemar test for dependent proportions, z test for proportions, and Wilcoxon signed-rank test were used for the comparison of proportion of active MRI scans. t Test was used for the comparison of means. Regression to the mean of MRI outcomes was analyzed by linear regression. The statistical analyses were conducted using Statistical Package for the Social Sciences version 15.0 and SAS version 9.2. The figures were constructed using GraphPad Prism 5.0. Mean values (SEM) are given unless otherwise indicated. Findings with $p < 0.05$ were considered significant.

Missing and invalid data. Fourteen (2.7%) and 9 (2.0%) MRI scans were missing during study months 1–6 and 7–24, respectively. One (1.1%) 25(OH)D value was missing at baseline, and 3 (1.1%) and 8 (1.8%) during study months 1–6 and 7–24, respectively. At the account of the half-life of 25(OH)D in serum,[22,23] we defined the MRI-25(OH)D pairs which were within an interval of 31 days as valid. Thus, 11 MRI-25(OH)D pairs of which the interval exceeded 31 days were excluded. Due to invalid or missing values, 1 patient was excluded during study months 7–24 for the logistic regression analysis, while for the comparison of the proportion of active MRI scans between the highest [25(OH)D^{max}] and the lowest [25(OH)D^{min}] level of 25(OH)D, we excluded 1 patient during both study months 1–6 and 7–24, and another patient during study months 7–24. Two (2.3%) EDSS scores were missing at month 24. EDTA blood for HLA-DRB1 typing was missing from 4 patients. Missing values were not replaced.

Standard protocol approvals, registrations, and patient consents. The study was approved by the Regional Committee for Medical and Health Research Ethics in Western Norway

Table 2 Baseline and clinical characteristics of the 88 study participants

Characteristics	Values
Women:men, n	57:31
Caucasian, n (%)	88 (100)
HLA-DRB1*15 positive, n (%)	58 (66)[a]
Baseline 25(OH)D, nmol/L (SEM)	61.2 (2.1)
Baseline proportion T1 Gd+ lesions, % (SEM)	52 (5.4)
Median age at examination, y (range)	39 (19–58)
Median age at diagnosis, y (range)	38 (18–58)
Median duration from diagnosis, y (range)	1 (0–13)
Median duration from first symptom, y (range)	3 (0–23)
Median EDSS at inclusion (range)	2.0 (0.0–4.9)

Abbreviations: 25(OH)D = 25-hydroxyvitamin D; EDSS = Expanded Disability Status Scale.
[a] Not available in 4 patients.

regional health authority. All participants gave written informed consent.

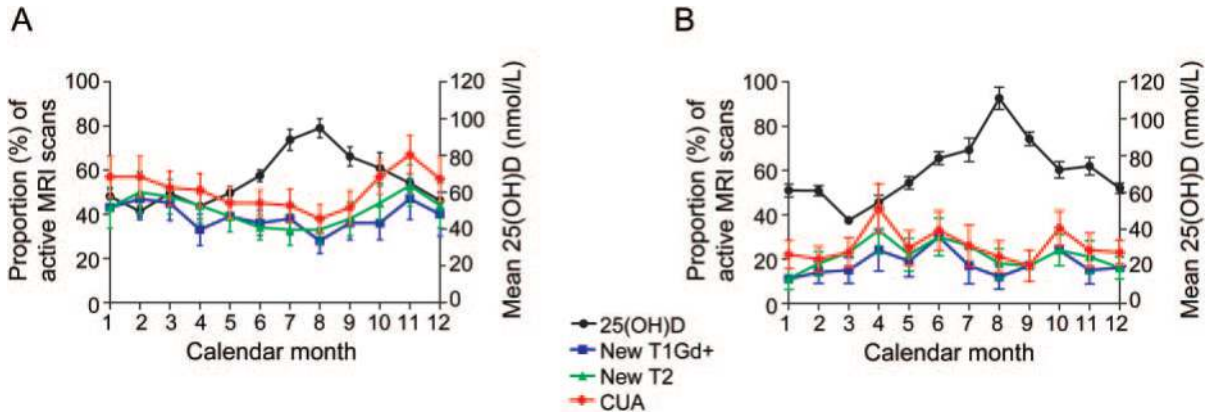
RESULTS

Study population. Demographic and baseline characteristics of the 88 participants are shown in table 2.

Vitamin D status. Including baseline, 780 values of 25(OH)D were available for analyses. The seasonal fluctuation of 25(OH)D levels is shown in figure e-1 on the *Neurology*® Web site at www.neurology.org. The mean (range) 25(OH)D level was 67.0 (26.3–121.5) nmol/L prior to and 70.8 (32.2–140.2) nmol/L during IFN-β treatment ($p = 0.20$, independent sample t test), and 69.5 (30.9–128.6) nmol/L for females and 68.7 (35.4–109.3) nmol/L for males during the whole study period. The mean (range) ratio between the highest and the lowest level of 25(OH)D among the patients was 2.25 (1.27–4.20). A total of 195 (25.0%) of the measured 25(OH)D values were <50 nmol/L (deficient), 314 (40.3%) were between 50.0 and 74.9 nmol/L (relatively insufficient), and 271 (34.7%) were ≥75 nmol/L (sufficient).[24] None of the participants had 25(OH)D levels <50 nmol/L at all recordings throughout the study, 35 (39.8%) had levels >50 nmol/L, and 4 (4.5%) had levels ≥75 nmol/L at all recordings.

MRI outcomes. The mean proportions of active MRI scans during IFN-β treatment were lower than before treatment (new T1 Gd+ lesions 17% [2.4%] vs 38% [3.6%], new T2 lesions 20% [2.5%] vs 41% [3.5%], CUA 25% [2.8%] vs 49% [4.0%]), $p <$ 0.001 for all comparisons (Wilcoxon signed rank test). The relations between MRI outcomes and 25(OH)D values are shown in figure 1. For analyses of the association between 25(OH)D levels and MRI outcomes using a logistic regression model for hierarchical data, 254 valid MRI 25(OH)D pairs (mean interval 2.8 [0.3] days) from 88 patients were available from study months 1–6, and 333 (mean interval 3.9 [0.3] days) from 87 patients from study months 7–24. There was an inverse association between 25(OH)D and MRI outcomes before but not after initiation of IFN-β treatment. Prior to IFN-β each 10 nmol/L increase in 25(OH)D was associated with reduced odds (95% confidence interval [CI]) at 12.7% (0.8–23.1) for occurrence of new T1 Gd+ lesions, 11.7% (0.3–21.8) for new T2 lesions, and 14.1% (2.0–24.7) for CUA (table 3). When adding DRB1*15 as an explanatory variable in the regression model, the level of significance was somewhat strengthened, whereas the odds ratios remained al-

Figure 1 Distribution of 25(OH)D and MRI outcomes clustered to calendar month



The mean 25(OH)D levels (black) (right y-axis) and proportion of active MRI scans (new T1 Gd+ lesions: blue, new T2 lesions: green, CUA: red) (left y-axis) during study months 1–6 (A) and study months 7–24 (B). Panel (A) is generated from 261 25(OH)D values and 514 MRI scans, and (B) is generated from 432 25(OH)D values and 431 MRI scans. Error bars represent SEM.

Table 3 Odds ratios for new MRI disease activity for each 10 nmol/L increase in 25(OH)D during study months 1–6 and 7–24, without and with HLA-DRB1*15 added as an explanatory factor

	Study months 1–6 (n = 88)[a]		Study months 7–24 (n = 87)[b]	
	Odds ratio (95% CI)	p Value[c]	Odds ratio (95% CI)	p Value[c]
New T1 Gd+ lesions	0.873 (0.769–0.992)	0.0368	1.055 (0.939–1.185)	0.3699
New T2 lesions	0.883 (0.782–0.997)	0.0442	1.028 (0.920–1.149)	0.6252
CUA	0.859 (0.753–0.980	0.0236	1.023 (0.921–1.136)	0.6701
	HLA-DRB1*15-status added as an explanatory factor			
	Study months 1–6 (n = 84)[d]		Study months 7–24 (n = 83)[e]	
	Odds ratio (95% CI)	p Value[c]	Odds ratio (95% CI)	p Value[c]
New T1 Gd+ lesions	0.826 (0.718–0.950)	0.0076	1.055 (0.937–1.188)	0.3745
New T2 lesions	0.852 (0.747–0.972)	0.0174	1.030 (0.920–1.153)	0.6098
CUA	0.818 (0.708–0.945)	0.0067	1.013 (0.910–1.128)	0.8080

Abbreviations: 25(OH)D = 25-hydroxyvitamin D; CI = confidence interval; CUA = combined unique activity.
[a] Based on 254 25(OH)D/MRI pairs.
[b] Based on 333 25(OH)D/MRI pairs.
[c] p Values calculated by a logistic regression model for hierarchical data.
[d] Based on 242 25(OH)D/MRI-pairs.
[e] Based on 320 25(OH)D/MRI-pairs.

most unchanged. Adjusting the analyses for gender and age did not significantly alter the results. No association was found between 25(OH)D levels and MRI outcomes the following 1 or 2 months (data not shown). No or only very weak associations were found between deseasonalized 25(OH)D levels and MRI outcomes (reduced odds [95% CI]) with each 10 nmol/L increase in 25(OH)D for the occurrence of new T1 Gd+ lesions 1.1% (−0.4 to 2.5), new T2 lesions 1.3% (−0.1 to 2.7), CUA 1.6% (0 to 3.1).

As more patients were included during the winter than during the summer (table e-1), it was important to rule out confounding by a regression to the mean effect.[25] MRI activity during study months 1–6 is shown in table e-2, and did not show any significant or pronounced regression to the mean effect. Thus, the mean proportion of MRI scans with new T1 Gd+ lesions at study months 1–2 and 5–6 were identical (40%), and only slightly reduced for new T2 lesions (45% to 40%) and CUA (54% to 48%).

To further address whether intraindividual fluctuations in 25(OH)D levels were associated with MRI outcomes, we compared the occurrence of new T1 Gd+ lesions, new T2 lesions, and CUA at $25(OH)D^{max}$ and $25(OH)D^{min}$ in each patient. For this analysis, 87 patients with ≥2 valid MRI-25(OH)D pairs (mean interval 3.1 [0.3] days) were available from study months 1 to 6, and 86 patients (mean interval 3.8 [0.5] days) from study months 7 to 24. Because a putative association between 25(OH)D levels and MRI outcomes is likely to depend on the extent of seasonal fluctuation of 25(OH)D, we divided the patients in 2 groups according to their median difference between $25(OH)D^{max}$ and $25(OH)D^{min}$. Prior to IFN-β treatment, the group with most pronounced fluctuation in 25(OH)D displayed larger proportions of MRI scans showing new T1 Gd+ lesions and CUA, and also a trend for new T2 lesions at $25(OH)D^{min}$ compared to $25(OH)D^{max}$ (table 4). This difference was mainly driven by reduced disease activity at $25(OH)D^{max}$. Thus, the patients with most pronounced 25(OH)D fluctuation had a smaller proportion of new T1 Gd+ lesions ($p = 0.001$), new T2 lesions ($p = 0.016$), and CUA ($p = 0.006$) at $25(OH)D^{max}$ than those with low fluctuation, but not at $25(OH)D^{min}$ (new T1 Gd+ lesions $p = 0.11$, new T2 lesions $p = 0.20$, CUA $p = 0.43$ [z test for proportions]). Corresponding to the findings for MRI outcomes, the difference in 25(OH)D fluctuation between the groups was mainly driven by higher $25(OH)D^{max}$ in those with most pronounced fluctuation, whereas $25(OH)D^{min}$ was quite similar (table 4). There were no differences in MRI outcomes at $25(OH)D^{max}$ and $25(OH)D^{min}$ after initiation of IFN-β treatment in either of the groups, although the extent of 25(OH)D fluctuations was maintained (table 4).

Clinical disease activity. A total of 42 relapses were recorded in 23 patients, of which 14 occurred during study months 1 to 6. The mean (range) 25(OH)D level during the entire study period was 73.3 (48.9–113.0) nmol/L in the patients with relapse and 67.7 (30.9–128.6) nmol/L in those without ($p = 0.22$, independent sample t test). The median (range) EDSS score were 2.0 (0.0–4.0) at baseline and 2.0 (0.0–6.5) at month 24. The mean 25(OH)D during the entire study period in the 26 patients who progressed ≥1 EDSS point was 68.5 (30.9–113.0) nmol/L, compared to 70.2 (35.4–128.6) nmol/L in the 60 stable patients ($p = 0.69$, independent sample t test). Analyzing study months 1–6 and 7–24 separately, neither the occurrence of relapse nor EDSS progression were associated with 25(OH)D levels.

DISCUSSION The main findings of this study are 1) increasing levels of 25(OH)D are associated with a significant and consistent reduction of active MRI scans in untreated patients with MS, irrespective of their DRB1*15 status, 2) no association between the 25(OH)D levels and MRI activity was observed after initiation of IFN-β, and 3) the association between 25(OH)D levels and MRI outcomes was mainly confined to patients with pronounced seasonal 25(OH)D fluctuation, and was driven by their high 25(OH)D levels and low radiologic disease activity during the summer.

Table 4 Proportion of active MRI scans and 25(OH)D concentration at the highest [25(OH)D^{max}] and the lowest [25(OH)D^{min}] 25(OH)D value, according to the median difference between 25(OH)D^{max} and 25(OH)D^{min} during study months 1–6 and 7–24

	Study months 1–6[a]: Difference between 25(OH)D^{max} and 25(OH)D^{min}				Study months 7–24[b]: Difference between 25(OH)D^{max} and 25(OH)D^{min}			
	<28.0 nmol/L (n = 40)		≥28.0 nmol/L (n = 47)		<26.0 nmol/L (n = 43)		≥26.0 nmol/L (n = 43)	
	% (SEM)	*p* Value	% (SEM)	*p* Value	% (SEM)	*p* Value	% (SEM)	*p* Value
New T1 Gd+ lesions								
25(OH)D^{min}	38 (8)	0.21[c]	51 (7)	0.004[c]	19 (6)	1.0[c]	16 (6)	0.80[c]
25(OH)D^{max}	53 (8)		23 (6)		21 (6)		21 (6)	
New T2 lesions								
25(OH)D^{min}	40 (8)	0.48[c]	49 (7)	0.052[c]	26 (7)	0.77[c]	21 (6)	0.80[c]
25(OH)D^{max}	50 (8)		28 (7)		21 (6)		26 (7)	
CUA								
25(OH)D^{min}	50 (8)	0.61[c]	60 (7)	0.003[c]	26 (7)	1.0[c]	26 (7)	0.48[c]
25(OH)D^{max}	58 (8)		32 (7)		23 (7)		35 (7)	
Mean 25(OH)D (SEM), nmol/L								
25(OH)D^{min}	52.3 (2.4)		57.3 (2.6)	0.17[d]	52.3 (3.1)		53.2 (2.3)	0.82[d]
25(OH)D^{max}	69.6 (2.7)		101.7 (3.9)	<0.001[e]	68.3 (3.5)		103.8 (4.4)	<0.001[e]

Abbreviations: 25(OH)D = 25-hydroxyvitamin D; CUA = combined unique activity.
[a] Based on 174 pairs of MRI-25(OH)D.
[b] Based on 172 pairs of MRI-25(OH)D.
[c] *p* Values for the difference between the proportion of active MRI scans between 25(OH)D^{max} and 25(OH)D^{min} (McNemar test for dependent proportions).
[d] *p* Values for the difference of the 25(OH)D levels at 25(OH)D^{min} (independent sample *t* test).
[e] *p* Values for the difference of the 25(OH)D levels at 25(OH)D^{max} (independent sample *t* test).

The association between 25(OH)D levels and MRI outcomes, including that DRB1*15 status did not alter the results, concurs with previous findings for relapse rate.[8,9] We did not find any association between the occurrence of relapses and the mean level of 25(OH)D, possibly due to the limited number of relapses. The only previous prospective study that included MRI parameters did not find any significant correlation between the burden of disease or new or enlarging T2 lesions and 25(OH)D.[7] That study did, however, only comprise 23 patients undergoing a limited number of MRI scans.

Based on the rapid kinetics of immunologic vitamin D effects in vitro and in vivo,[26,27] we hypothesized that any association with MRI outcomes would appear within 1 month. This concurs with a previous study of relapse rate and estimated 25(OH)D levels,[2] but not with a previous observation of a relationship between 25(OH)D and MRI activity lagged by 2 months.[28] Notably, 25(OH)D levels and disease activity in these studies[2,28] were measured in different populations, and the results are therefore not directly comparable to those reported here.

Previous studies have used one or few deseasonalized 25(OH)D measurements from each patient to study the relationship with disease activity.[8,9] The intraindividual variation in 25(OH)D is largely driven by seasonal fluctuation.[24] As our study mainly addressed intraindividual fluctuations, and because intraindividual fluctuation is equalized by deseasonalization, 25(OH)D levels were not deseasonalized in the main analysis. For this reason, it was not surprising that no or only very weak associations remained after deseasonalization.

The present results extend previous findings[7–9] by showing a consistent association between seasonal fluctuation of 25(OH)D and MRI outcomes in individual nontreated patients. The difference in disease activity between 25(OH)D^{max} and 25(OH)D^{min} suggests that a beneficial effect of 25(OH)D might be most pronounced when the serum level of 25(OH)D exceeds 100 nmol/L. This corroborates an earlier study showing reduced MS risk in people with 25(OH)D levels in the same magnitude.[29]

The main strength of the present study is the prospective design with repeated and paired MRI scans and vitamin D measurements before and during IFN-β treatment in clinically well-characterized and DR typed patients. Comparing the MRI outcomes at the highest vs the lowest levels of 25(OH)D limited the possibility of confounding, as the comparison was made within each individual patient. The study

also has several limitations, however. First, the observation time prior to immunomodulatory treatment was relatively short. Moreover, the number of patients was rather small, and the statistical power of the subgroup analyses consequently limited. Notably, the skewed inclusion of patients could potentially affect our results through a regression to the mean effect of MRI outcomes.[25] The regression to the mean effect was, however, slight and not significant. Any effect of skewed inclusion is therefore modest.

A synergistic effect between vitamin D and IFN-β has been suggested in experimental allergic encephalomyelitis,[30] and high-dose vitamin D_3 is currently tested as add-on treatment to IFN-β in MS.[31] We did not find any association between 25(OH)D levels and MRI outcomes after initiation of IFN-β. This may seem surprising, as there is no evidence suggesting that the immunomodulatory effects of vitamin D are counteracted by IFN-β or vice versa. A reasonable explanation is that IFN-β reduced radiologic disease activity,[32] leaving relatively little left to be reduced. Other explanations, however, cannot be excluded. Interestingly, synthesis of interleukin 10, which may mediate anti-inflammatory effects of vitamin D,[13] showed seasonal variation with high summer levels only in patients treated with IFN-β.[33] Moreover, an association between seasonal variation in MS relapses and air pollution has been shown at a population level.[34] Relapses were associated with air pollution and airway infections only in IFN-β users, and not in untreated patients,[35] suggesting that environmental factors may affect treated and nontreated patients differently.

Importantly, the lack of association between physiologic variation in 25(OH)D levels and radiologic disease activity in IFN-β-treated patients does not exclude that supraphysiologic levels obtained through pharmacologic intervention may be effective, as suggested by immunologic[12,13] and radiologic[16] effects in pilot studies. The evidence in favor of vitamin D supplementation in MS is, however, limited. Thus, a recent study comprising 23 patients with MS followed for 6 months suggested a negative effect of high-dose vitamin D_2 on disease activity.[36]

The immunomodulatory effects of vitamin D make a causative relation between vitamin D levels and MRI outcomes biologically plausible.[26] Although our results are compatible with a causal relationship, other factors with similar seasonal fluctuation as vitamin D may have confounded our results. Thus, solar ultraviolet radiation has been shown to induce regulatory T cells through mechanisms independent of vitamin D.[37] Moreover, several infections that are more common during the winter than during the summer are also associated with an increased relapse rate.[2,38] Infections were not recorded in our study. A randomized controlled trial sufficiently powered to capture moderate, but still potentially important effects, is needed to prove a causal relationship between vitamin D levels and disease activity in MS.

AUTHOR CONTRIBUTIONS

K.I. Løken-Amsrud contributed by analysis and interpretation of the data and drafting of the manuscript. T. Holmøy contributed by design and conceptualization of the study, interpretation of the data, and drafting and revising the manuscript. S.J. Bakke contributed by designing the study and revising the manuscript. A.G. Beiske contributed by designing the study and revising the manuscript. K.S. Bjerve contributed by designing the study, analyzing 25-hydroxyvitamin D, and revising the manuscript. B.T. Bjørnarå contributed by designing the study and revising the manuscript. H. Hovdal contributed by designing the study and revising the manuscript. F. Lilleås contributed by designing the study and revising the manuscript. R. Midgard contributed by designing the study and revising the manuscript. T. Pedersen contributed by designing the study and revising the manuscript. J. Šaltytė Benth contributed by analyzing and interpreting of the data and revising the manuscript. L. Sandvik contributed by analyzing and interpreting of the data and revising the manuscript. Ø. Torkildsen contributed by revising the manuscript. S. Wergeland contributed by revising the manuscript. K.M. Myhr contributed by designing the study, interpreting the data, and revising the manuscript.

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DISCLOSURE

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Vitamin D and disease activity in multiple sclerosis before and during interferon-β treatment

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