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Original article

Low vitamin D, but not tobacco use or high BMI, is associated with long-term disability progression in multiple sclerosis

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ABSTRACT

Background: Low vitamin D levels, tobacco use and high body mass index (BMI) have been linked to adverse disease outcomes in multiple sclerosis (MS), but their influence on long-term disability progression remains unclear. Therefore, we explored whether these modifiable lifestyle factors were associated with 10-year clinical disability progression in patients with MS.

Methods: In this prospective study, a cohort of 88 patients with relapsing-remitting MS completed a randomized controlled study on ω-3 fatty acids between 2004 and 2008. During 24 months, serum 25-hydroxyvitamin D (25(OH)D), serum cotinine (nicotine metabolite), and BMI were repeatedly measured. In 2017, a follow-up study was conducted among 80 of the participants, including disability assessment by the Expanded Disability Status Scale (EDSS). Linear regression was used to explore associations between the lifestyle factors and the EDSS change over 10 years.

Results: Higher seasonally adjusted 25(OH)D levels were associated with lower 10-year EDSS progression (change in EDSS per 1 SD increase in 25(OH)D in a model adjusted for sex, age and baseline EDSS: -0.45 point, 95% CI: -0.75 to -0.16, p=0.003). Further adjustments for potential confounders related to lifestyle and disease status gave similar results. The association was mainly driven by low 25(OH)D levels during spring, as well as seasonally adjusted levels below 80 nmol/L. No clear association was found for BMI and cotinine.

Conclusion: Lower 25(OH)D levels, but apparently not tobacco use or higher BMI, were significantly associated with worse long-term disability progression in MS.

Abbreviations: DMT, disease-modifying treatment; 25(OH)D, 25-hydroxyvitamin D; RCT, randomized controlled trial; IFN-β, interferon beta 1a; aHSCT, autologous hematopoietic stem cell transplantation; CUA, combined unique activity.

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1. Introduction

Multiple sclerosis (MS) is a disabling chronic disease with several disease-modifying treatment (DMT) options, but so far, no curable treatment exists (Dobson and Giovannoni, 2019). Established risk factors related to lifestyle such as vitamin D deficiency, tobacco smoking, and obesity may also affect disease course (Waubant et al., 2019). Higher serum levels of 25-hydroxyvitamin D (25(OH)D) have been associated with less radiological inflammatory activity and lower relapse rate in observational studies (Smolders et al., 2019). However, two larger randomized controlled trials (RCTs) on high dose vitamin D supplementation failed to demonstrate a clear effect on relapse rate and disability progression in the intention-to-treat population (Hupperts et al., 2019, Camu et al., 2019). Further, several (Hernan et al., 2005, Healy et al., 2009, Manouchehrinia et al., 2013), but not all (Koch et al., 2007, Kvistad et al., 2016, Munger et al., 2015) studies, suggest that smoking increases the risk of a faster disease progression and earlier transition to secondary progressive MS (SPMS). For obesity, some studies indicate that higher body mass index (BMI) leads to more disease activity through weaker therapy response (Kvistad et al., 2015, Huppke et al., 2019), and may affect brain volume loss (Mowry et al., 2018), whereas other studies have failed to demonstrate any association between BMI and disease progression (Pilutti et al., 2012, Bove et al., 2016).

Only a few studies have examined associations between lifestyle factors and long-term disability progression in MS (Cortese et al., 2020, University of California, San Francisco MS-EPIC Team, 2016). To address this, we conducted a study to examine whether 25(OH)D levels, tobacco use, and BMI were associated with disability progression over 10 years, using prospective data from a well-defined Norwegian cohort of patients with MS.

2. Methods

2.1. Study population and design

2.1.1. The OFAMS baseline study

A total of 92 patients with relapsing-remitting MS (RRMS) aged 18-55 years were enrolled in an RCT on marine ω-3 fatty acids versus placebo (the OFAMS study) between 2004 and 2006, and then closely followed for 24 months. A detailed description of the study is reported elsewhere (Torkildsen et al., 2012). In the following text, we will refer to this study as "the baseline study". Frequent clinical examinations, blood samples and MRI scans of the brain were performed during the study period. No particular advice on lifestyle changes or vitamin D supplementation was given to the patients. Overall, the study demonstrated no significant effect of ω-3 fatty acids on disease activity (Torkildsen et al., 2012). However, in subsequent analyses, lower 25(OH)D levels were associated with more inflammatory MRI-activity *before* initiation of subcutaneous interferon beta 1a (IFN-β) at study month 6 (Loken-Amsrud et al., 2012), and higher BMI was associated with more disease activity *after* initiation of IFN-β (Kvistad et al., 2015).

2.1.2. The OFAMS follow up study

In 2017, the OFAMS population was invited to a 10-year follow-up study to evaluate disease progression and current disability status. A trained neurologist at each participating centre performed a clinical examination of the patients. In addition, the patients answered a questionnaire regarding lifestyle habits, including sun exposure and tobacco use (smoking and/or snuff use) during the last 10 years.

2.2. Ethical approvals and Patient Consents

The OFAMS baseline study and the OFAMS follow-up study were approved by the Regional Committee for Medical and Health Research Ethics in Western Norway. All participants gave their written informed consent prior to the studies.

2.3. Assessment of lifestyle factors in OFAMS baseline study

2.3.1. Vitamin D measurement

Serum samples were collected at the baseline visit, and then at month 1, 3, 6, 7, 9, 12, 18, and 24. The samples were stored at -80°C until simultaneous analysis of all nine samples from each patient at the Department of Medical Biochemistry, St. Olav's University hospital, Trondheim, Norway (Loken-Amsrud et al., 2012). 25(OH)D levels in nmol/L were measured by radioimmunoassay (RIA kit; ImmunoDiagnostic Systems, Boldon, UK). The coefficient of variation was 5.4% at 29 nmol/l and 6.3% at 112 nmol/l.

2.3.2. Cotinine measurement

Cotinine levels, a sensitive and specific biomarker for nicotine intake (SRNT Subcommittee on Biochemical Verification, 2002), were measured simultaneously in serum samples collected at baseline visit, month 6, 12, 18, and 24 (Kvistad et al., 2016). The analysis was performed by liquid chromatography-tandem mass spectrometry (Bevital AS, Bergen, Norway). The within-day coefficient of variation was 2.0% to 6.6%, and the between-day coefficient of variation was 3.9%. The cut-off value for recent tobacco use was set to cotinine levels $>$ 85 nmol/L, with tobacco users defined as having $>$ 85 nmol/L in $\geq$ 60% of the samples.

2.3.3. Body mass index

The participants' height (in meters) and weight (in kg) were measured at screening, and then at baseline visit, month 1, 3, 6, 7, 9, 12, 18, and 24. From these values, BMI at each visit was calculated as kg/m^2.

2.4. Other relevant covariates

Current use of DMT at follow-up was categorized as "none", "less potent" (IFN-β, glatiramer acetate, teriflunomide, and dimethyl fumarate) and "potent" (fingolimod, natalizumab, autologous hematopoietic stem cell transplantation (aHSCT), and rituximab). For disease activity, we included two variables from the baseline study: the cumulative number of combined unique activity (CUA) lesions (Torkildsen et al., 2012) on subsequent MRI brain scans, and the annual relapse rate.

At the follow-up visit, the participants were asked about the frequency of outdoor activity in summer season (April-September) 10 years ago, 5 years ago and last year, categorizing this into "$<$ 1 time per week", "1-2 times per week", "3-4 times per week" and "approximately daily". From these data, we created a cumulative sun exposure variable.

2.5. Outcome measure

2.5.1. EDSS progression

The disability status was assessed by the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983) at baseline visit, month 6, 12, 18, and 24 during the baseline study and repeated once in the follow-up study 10 years later. The EDSS progression was defined as the EDSS change from the last score in the baseline study until the score at follow-up. For all patients but two the last EDSS score was at month 24; one patient had the last score at month 12 and the other one at month 18.

2.6. Missing values

92 patients were screened to participate in the baseline study, but four were lost to follow-up during the first six months of the study. In the follow-up study, 85 of the 91 patients still alive (93.4%) gave their consent to participate, including 81 of the 88 patients who completed at least 12 months of the baseline study. However, EDSS score at follow-up was missing for one of these 81 patients, leaving 80 patients eligible for the main analyses.

2.7. Statistical analyses

For each lifestyle factor, we estimated the mean value per patient based on all available measurements during the baseline study. Since vitamin D levels vary with season in Norway, the 25(OH)D levels were seasonally adjusted by a sine function modelled within the baseline study, as previously described (Saltyte Benth et al., 2012).

We used linear regression models to estimate the association between the lifestyle factors and the EDSS progression from the last score in the OFAMS baseline study to the assessment in the follow-up study. All exposures were modelled as both categorical (quartiles) and continuous variables to maximize power and to explore possible nonlinear associations. In continuous analyses, we standardized the variables (mean = 0, standard deviation (SD) = 1) to estimate the change in EDSS per 1 SD increase in the exposure variable. To test for a linear trend across the quartiles, the median value of each quartile was included in the regression model as a continuous variable. All available measurements in the OFAMS baseline study were used to standardize and categorize variables. All models were adjusted for sex, age and baseline EDSS score (= last score in the baseline study). In multivariable models, we mutually adjusted for all three lifestyle factors, disease activity (CUA and annual relapse rate) in the baseline study, disease duration (from year of diagnosis until follow-up), and use of DMT at follow-up. We also adjusted for cumulative sun exposure in the follow-up period, but as this only had a minor influence on the effect estimates, we omitted this variable in the final models.

To illustrate the monthly fluctuations of 25(OH)D levels in our population, a Locally Estimated Scatterplot Smoothing (LOESS) curve was fitted to the available measures, with corresponding 95% confidence intervals (CI). To evaluate whether an association between 25(OH)D levels and EDSS progression varied by season, we computed a dichotomized variable of < median and ≥ median 25(OH)D levels per season based on each patient's mean 25(OH)D level for that season. The four seasons were summer (June-August), fall (September-November), winter (December-February), and spring (March-May). We then included the dichotomized seasonal variables (< median or ≥ median) as independent variables in linear regression analyses, with the change in EDSS score as the dependent variable, adjusted for sex, age and baseline EDSS score. Finally, to investigate whether there was a nonlinear relationship between seasonally adjusted 25(OH)D levels and disease progression, we plotted a LOESS-curve to the available data.

All the statistical analyses were performed in IBM SPSS Statistics, version 25.0 (SPSS Inc., Chicago, Ill., USA). The plots were made in R version 3.6.0 (The R Foundation) using the *ggplot2* package. P-values were considered significant at values <0.05. All tests were two-sided.

3. Results

3.1. Patient characteristics

The study population comprised 80 participants who completed more than 12 months in the baseline study and had an available EDSS score in the follow-up study. Table 1 gives the main baseline characteristics of this population. The mean EDSS score increased from 1.9 (SD: 0.84) at the baseline visit to 2.8 (SD: 1.6) at the follow-up visit, and seven (8.8%) of the patients converted to SPMS during the follow-up period. At follow-up, 72.5% received any kind of DMT, including seven patients still on IFN-β and two patients on past aHSCT treatment. Fewer used tobacco (40.0% vs. 61.3% in the baseline study), and 76.3% used vitamin D containing supplements in various doses and formulas. For most patients, BMI remained stable over the years, with mean BMI 25.6 kg/m^2 (SD: 4.2) and 25.7 kg/m^2 (SD: 4.6) during the baseline and follow-up study, respectively.

Table 1
Characteristics of the study population at OFAMS baseline visit or during the baseline study.

Variable	Values
Patients, N	80
Females, N (%)	52 (65)
Age, mean (SD)	38.3 (8.3)
Years from diagnosis, mean (SD)	1.9 (3.2)
EDSS score, mean (SD)	1.9 (0.84)
Seasonally adjusted 25(OH)D during baseline study, mean (SD)	74.1 (18.1)
Tobacco users during baseline study, N(%)[a]	49 (61.3)
BMI in kg/m^2 during baseline study, mean (SD)	25.6 (4.2)

SD: standard deviation; 25(OH)D: 25-hydroxyvitamin D nmol/L; BMI: body mass index.
[a] Tobacco users defined as serum cotinine levels > 85 nmol/L in ≥60% of five consecutive samples.

3.2. Vitamin D

Higher 25(OH)D levels were significantly associated with lower 10-year EDSS progression (Table 2). In the continuous model adjusted for sex, age and baseline EDSS score, 1 SD increase in seasonally adjusted average 25(OH)D levels was associated with 0.45 point (95% CI: 0.16-0.75, p=0.003) lower progression in EDSS scores at follow-up. Further adjustment for other covariates, including mean cotinine levels, mean BMI values and disease activity during the baseline study, did not influence the results. In the categorical analyses, there was a significant dose-response relationship between 25(OH)D and change in EDSS score with a p-trend of 0.024 in the simplest model (Table 2). The effect estimates and the p-trend remained similar when more covariates were added to the model.

Fig. 1 illustrates the seasonal fluctuation of repeated measures of 25(OH)D throughout the baseline study, with the highest levels seen in August and the lowest levels seen in March. In the model that included dichotomized 25(OH)D variables for all four seasons, only higher (≥ median) 25(OH)D levels during the spring, when the levels were lowest, were significantly associated with 10-year EDSS progression (Fig. 2).

When exploring the possible nonlinear relationship between 25(OH)D and disease progression with a LOESS-curve (Fig. 3), an increase in seasonally adjusted 25(OH)D levels from around 50-60 nmol/L to 80 nmol/L was associated with approximately one point decrease in EDSS progression, whereas little additional benefit was seen for higher 25(OH)D levels.

3.3. Cotinine levels

Tobacco use based on cotinine levels showed no significant association with EDSS progression, neither in the simple model adjusted for sex, age, and baseline EDSS score, nor in the models adjusted for additional variables (Table 2). Although five of seven patients (71%) who converted to SPMS were classified as tobacco users during the baseline study, this finding was not significant (p= 0.70) according to Fisher's exact two-sided test for small samples.

3.4. BMI

For BMI, there was a tendency towards a beneficial effect for the patients with BMI values in the highest quartile, but no significant dose-response curve was present (Table 2). We found a similar non-significant trend in the continuous model.

4. Discussion

In this prospective study, we found a significant and consistent association between higher 25(OH)D levels and lower 10-year disability progression independent of potential confounders related to lifestyle

Table 2
The association between mean values of lifestyle factors during the baseline study and the 10-year EDSS progression from last EDSS score in the baseline study.

Lifestyle factors	Quartile 1	Quartile 2 Change in EDSS(95%CI)	Quartile 3 Change in EDSS(95%CI)	Quartile 4 Change in EDSS(95%CI)	p-trend	Per 1 SD increase[a] Change in EDSS (95%CI)	p-value
25(OH)D[b]							
Patients, N	20	18	21	21			
Median (range), nmol/L	54.9 (36.4- 60.1)	66.9 (60.3- 70.7)	77.5 (71.1- 83.8)	97.6 (84.0- 118.4)			
Model 1[c]	Reference	0.13 (-0.67- 0.93)	-0.61 (-1.41- 0.19)	-0.78 (-1.59- 0.03)	0.024	-0.45 (-0.75- -0.16)	0.003
Model 2[d]	Reference	0.29 (-0.53- 1.11)	-0.59 (-1.38- 0.21)	-0.76 (-1.56- 0.05)	0.022	-0.46 (-0.75- -0.17)	0.002
Model 3[e]	Reference	-0.06 (-0.93- 0.82)	-0.86 (-1.72- 0.00)	-0.99 (-1.83- -0.15)	0.010	-0.49 (-0.79- -0.20)	0.002
Cotinine[b]							
Patients, N	20	19	19	22			
Median (range), nmol/L	0.4 (0.0- 1.2)	123.8 (1.2- 400.8)	738.9 (407.7- 946.6)	1140.6 (980.3- 2443.6)			
Model 1[c]	Reference	0.55 (-0.25- 1.35)	0.30 (-0.51- 1.10)	-0.17 (-0.98- 0.64)	0.353	-0.09 (-0.38- 0.20)	0.557
Model 2[d]	Reference	0.48 (-0.28- 1.24)	0.16 (-0.62- 0.94)	-0.11 (-0.88- 0.66)	0.393	-0.07 (-0.34- 0.20)	0.618
Model 3[e]	Reference	0.34 (-0.45- 1.12)	-0.07 (-0.88- 0.75)	-0.19 (-0.98- 0.60)	0.296	-0.09 (-0.37- 0.20)	0.538
BMI[b]							
Patients, N	22	20	19	19			
Median (range), kg/m^2	21.5 (17.7- 22.9)	23.8 (22.9- 25.2)	26.3 (25.3- 28.2)	31.1 (28.8- 38.3)			
Model 1[c]	Reference	0.04 (-0.75- 0.82)	-0.10 (-0.90- 0.69)	-0.51 (-1.31- 0.28)	0.157	-0.20 (-0.48- 0.08)	0.160
Model 2[d]	Reference	0.11 (-0.64- 0.86)	-0.13 (-0.89- 0.63)	-0.43 (-1.19- 0.33)	0.182	-0.20 (-0.47- 0.06)	0.134
Model 3[e]	Reference	0.02 (-0.74- 0.78)	-0.15 (-0.91- 0.62)	-0.40 (-1.16- 0.36)	0.247	-0.18 (-0.44- 0.09)	0.189

SD: standard deviation; CI: confidence interval; 25(OH)D: 25- hydroxyvitamin D; BMI: body mass index.
[a] 1 SD for seasonally adjusted 25(OH)D =18.7 nmol/L, 1 SD for mean cotinine= 523.8 nmol/L, 1 SD for mean BMI= 4.2 kg/m^2
[b] Mean values for the baseline period based on N consecutive samples, where N= 9 for seasonally adjusted 25(OH)D, N=5 for cotinine and N=10 for BMI.
[c] Model 1: Adjusted for sex, age and EDSS score at last visit in the baseline study.
[d] Model 2: Model 1 + mutually adjusted for 25(OH)D, cotinine and BMI as standardized continuous variables.
[e] Model 3: Model 2 + further adjusted for disease duration from year of diagnosis until follow-up (2017), use of disease-modifying treatment at follow-up (none, less potent, potent), brain MRI activity (cumulative Combined Unique Activity) and relapse rate during the baseline study.

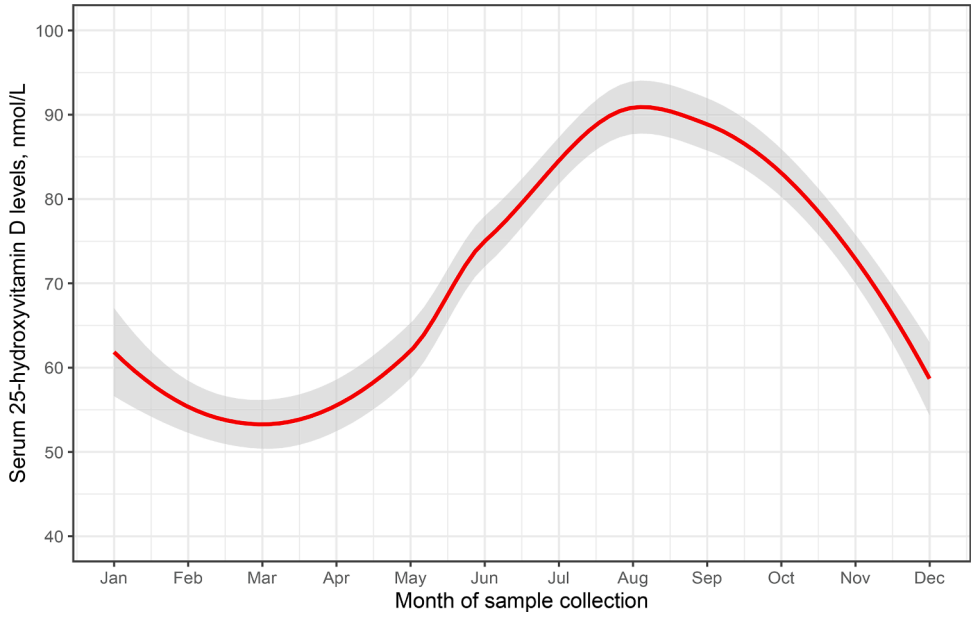

Fig. 1. The seasonal fluctuation of 25-hydroxyvitamin D levels based on sample analyses in the baseline study shown by a fitted LOESS curve with 95% confidence intervals.

and disease status. The association was mainly driven by levels during spring when 25(OH)D reached its seasonal nadir. Further, a ceiling effect in the association appeared around 80 nmol/L, as there were only minor changes in disease progression for 25(OH)D increases above this level. Tobacco use and BMI were not significantly associated with long-term disability in our study.

Our findings on vitamin D are consistent with previous findings on a likely role of vitamin D on disease course in MS. While several studies have shown a significant relationship between vitamin D levels and inflammatory activity in MS over a few years, few have demonstrated any significant association between vitamin D levels and disease progression (Smolders et al., 2019). This may be due to shorter follow-up time, as use of DMTs delay disability progression and the time to secondary progressive MS (Claflin et al., 2018, Brown et al., 2019). A recent study found poorer long-term (11 years) cognitive performance in the Paced Auditory Serial Addition Test in patients with lower 25(OH)D levels at baseline (Cortese et al., 2020), which in part supports our results. Thus, a longer observational period may be necessary to detect a potential effect of vitamin D levels on physical and cognitive disability scores.

In our data, a ceiling effect appeared in the association between 25 (OH)D and disability progression as there was almost no additional

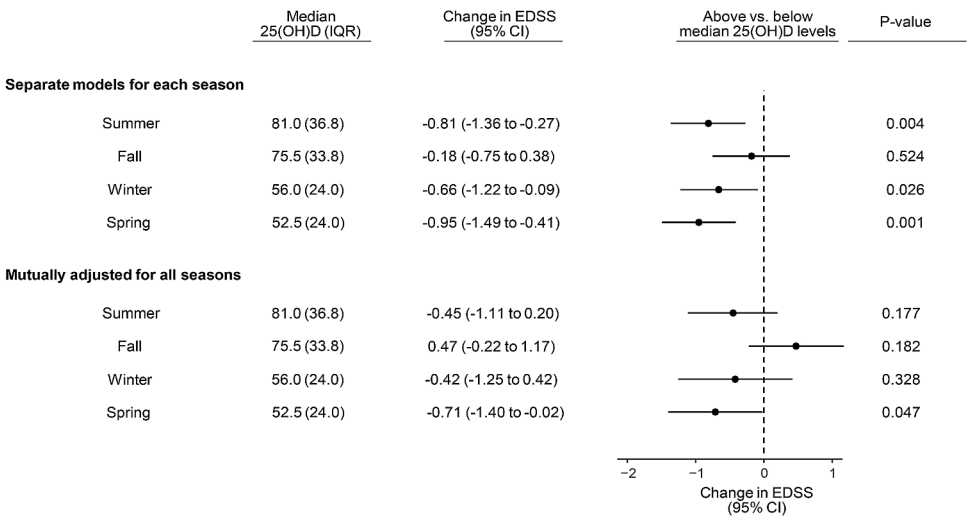

Fig. 2. The association between dichotomized seasonal 25-hydroxyvitamin D levels and long-term EDSS progression.
The seasonal 25-hydroxyvitamin D levels are dichotomized into "< median" and "≥ median" values and further adjusted for sex, age and EDSS score at last visit in the baseline study. Change in EDSS is the difference between the EDSS score at follow-up and the last EDSS score in the baseline study. The plots on the right side illustrate the estimates. 25(OH)D: 25-hydroxyvitamin D; IQR: interquartile range; CI: confidence interval.

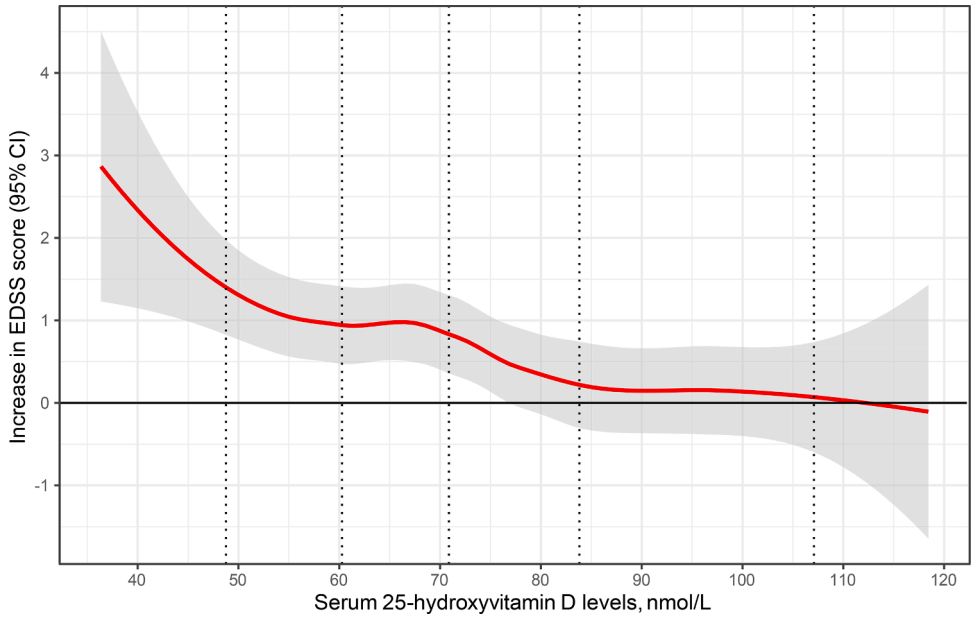

Fig. 3. Seasonally adjusted 25-hydroxyvitamin D levels and the increase in EDSS score fitted by a LOESS curve.
The increase in EDSS score is defined as the follow-up EDSS score subtracted by the last EDSS score in the baseline study.
The vertical lines correspond to the fifth, 25th, 50th, 75th, and 95th percentile of serum 25-hydroxyvitamin D levels.

benefit for levels above 80 nmol/L. This finding is in line with a previous observational study among 156 RRMS patients on IFN-β or glatiramer acetate who were supplemented with vitamin D3. During follow-up, the relapse incidence rate significantly decreased until 25(OH)D levels reached 110-120 nmol/L - above this, the relapse rate stabilized (Pierrot-Deseilligny et al., 2012). Overall, this may suggest that the optimal 25(OH)D level for MS patients could lay within a high normal range of 80-120 nmol/L.

In our study population, 25(OH)D levels during spring had the strongest association with long-term disability. This may be explained by the "vitamin D winter" (Engelsen et al., 2005) period at latitudes above 50° when UVB radiation, the main natural source of vitamin D (Prietl et al., 2013), is too weak to induce any meaningful cutaneous synthesis of pre-vitamin D (Engelsen et al., 2005). This lack of synthesis cannot be fully compensated by a 15-25 days half-life of 25(OH)D in non-supplemented individuals (Martinaityte et al., 2017), making early spring extra prone for insufficient levels. Other studies have similarly found higher relapse rate during (early) spring (Miclea et al., 2017, Spelman et al., 2014). Vitamin D supplementation can compensate for the seasonal UVB-related variations in 25(OH)D (Miclea et al., 2017), and may also increase the half-life through storage in adipose tissue (Martinaityte et al., 2017), thus likely avoiding the lowest levels during the winter months at high latitudes.

The association between vitamin D and MS can be explained through plausible biological mechanisms. Both antigen-presenting cells of the innate immune system and T- and B-lymphocytes of the adaptive immune system express vitamin D receptors and are able to synthesize the active vitamin D compound calcitriol (Prietl et al., 2013, Hart et al., 2011). Through various mechanisms, calcitriol modulates the immune system into a more tolerogenic and anti-inflammatory state, thus likely

preventing and down-scaling autoimmune actions (Prietl et al., 2013). On the other hand, UVB radiation itself has likely immunomodulatory effects independent of the vitamin D pathway (Hart et al., 2011). However, when adjusting for cumulative sun exposure in our models, only a minor influence on the estimates was seen, suggesting that our results likely represent effects of vitamin D rather than UVB radiation.

In contrast to other cohorts (Healy et al., 2009, Manouchehrinia et al., 2013), we found no significant association between tobacco use and EDSS progression. Our results may have been affected by generally low disease progression in the population and beneficial effect of smoking cessation (Ramanujam et al., 2015) during follow-up (21.3% fewer tobacco users at follow-up visit). Since we used a nicotine metabolite to classify tobacco use in the baseline study, the results could potentially have been influenced by snuff use, which also contains nicotine and has been associated with a decreased risk for MS (Hedstrom et al., 2009). However, only three tobacco users in the baseline study reported a history of solely snuff use at follow-up, making it unlikely that our results can be explained by many snuff-users relative to smokers.

For BMI, we observed a non-significant trend towards less EDSS progression with higher BMI. Studies on BMI and long-term outcomes in MS may be difficult to interpret, as MS itself or changes in diet and activity may affect BMI (Habek et al., 2010), making findings prone to reverse causation. Patients with MS tend to have lower mean BMI (Nortvedt et al., 2005, Dardiotis et al., 2019), and gain less weight with age as compared to the general population (Bove et al., 2016, Wesnes et al., 2015), which could suggest that maintaining a higher BMI over the years reflects a more benign MS with less chronic disease burden affecting weight. This is consistent with other observations in our study, as use of potent DMT at follow-up was more prevalent in the lowest BMI quartile (54.5%) than in the highest quartile (31.6%).

Our study has several strengths. First, it benefits from a prospective design, a well-defined cohort, and a long follow-up time. Second, the lifestyle variables are based on objective and repeated measures over 24 months, making the results less prone to extreme values in single observations. Third, we could adjust for several potential confounders and explore the importance of seasonality in the relationship between 25 (OH)D levels and disability progression.

There are also some limitations to our study. The relatively small sample size may have limited the statistical power to detect associations in our study (i.e., increasing the likelihood of a type II error). In addition, the low level of disease progression in the study group could have influenced our findings, and factors that were not associated with progression in our study (e.g., smoking and BMI) may be more relevant for patients with a more aggressive disease course. Since the baseline study and the follow-up study was separated by a long period, we did not have detailed information on lifestyle habits between the two studies. It is therefore possible that lifestyle changes, such as increasing use of vitamin D supplements, may have attenuated the associations. At follow-up, only one EDSS score per patient was available, which could have been influenced by the patients' mood and level of fatigue at the time of assessment. However, such day-to-day changes may act in both directions, and are therefore less likely to affect the results. Our study population was originally recruited for a randomized clinical trial based on specific inclusion and exclusion criteria (Torkildsen et al., 2012), and may not be fully representative of the general MS population. Still, our findings on vitamin D are consistent with previous prospective studies and are biologically plausible. Lastly, we cannot exclude the possibility that our findings may be affected by residual or unmeasured confounding that we could not account for.

5. Conclusions

In summary, we found a significant association between higher vitamin D levels and lower long-term disability progression in patients with MS, suggesting that vitamin D may have a favourable effect on long-term outcomes in MS. This association seems to be driven by seasonal low levels during late winter/early spring at latitudes above 50°. No clear association was found between tobacco use or BMI and long-term disability scores, indicating that these factors may have less relevance for long-term prognosis.

CRediT authorship contribution statement

Kristin Wesnes: Conceptualization, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Visualization, Project administration, Funding acquisition. **Kjell-Morten Myhr:** Investigation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Trond Riise:** Writing - review & editing, Supervision, Methodology. **Silje Stokke Kvistad:** Investigation, Resources, Writing - review & editing. **Øivind Torkildsen:** Resources, Data curation, Writing - review & editing. **Stig Wergeland:** Data curation, Writing - review & editing. **Trygve Holmøy:** Investigation, Resources, Writing - review & editing. **Rune Midgard:** Investigation, Resources, Writing - review & editing. **Alla Bru:** Investigation, Resources, Writing - review & editing. **Astrid Edland:** Investigation, Resources, Writing - review & editing. **Randi Eikeland:** Investigation, Resources, Writing - review & editing. **Sonia Gosal:** Investigation, Resources, Writing - review & editing. **Hanne F. Harbo:** Investigation, Resources, Writing - review & editing. **Grethe Kleveland:** Investigation, Resources, Writing - review & editing. **Yvonne S. Sørenes:** Investigation, Resources, Writing - review & editing. **Nina Øksendal:** Investigation, Resources, Writing - review & editing. **Kjetil Bjørnevik:** Conceptualization, Formal analysis, Methodology, Validation, Formal analysis, Data curation, Writing - review & editing, Visualization, Supervision.

Declaration of Competing Interests

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