Higher 25-hydroxyvitamin D Is Associated with Lower Relapse Risk in Multiple Sclerosis

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Objective: A protective association between higher vitamin D levels and the onset of multiple sclerosis (MS) has been demonstrated; however, its role in modulating MS clinical course has been little studied. We investigated whether higher levels of serum 25-hydroxyvitamin D (25-OH-D) were associated with a lower risk of relapses in people with MS.

Methods: We conducted a prospective cohort study of 145 participants with relapsing-remitting MS from 2002 to 2005. Serum 25-OH-D levels were measured biannually, and the hazard of relapse was assessed using survival analysis.

Results: There was an inverse linear relationship between 25-OH-D levels and the hazard of relapse over the subsequent 6 months, with hazard ratio (HR) 0.91 (95% confidence interval [CI]: 0.85–0.97) per 10nmol/l increase in 25-OH-D level ($p = 0.006$). When variation due to timing of blood collection was removed by estimating 25-OH-D at the start of each season, this association persisted, with HR 0.90 (95% CI, 0.83–0.98) per 10nmol/l increase ($p = 0.016$). Taking into account the biological half-life of 25-OH-D, we estimated 25-OH-D at monthly intervals, resulting in a slightly enhanced association, with HR 0.88 (95% CI, 0.82–0.95) per 10nmol/l increase ($p = 0.001$). Adjusting for potential confounders did not alter these findings.

Interpretation: In this prospective population-based cohort study, in a cohort largely on immunomodulatory therapy, higher 25-OH-D levels were associated with a reduced hazard of relapse. This occurred in a dose-dependent linear fashion, with each 10nmol/l increase in 25-OH-D resulting in up to a 12% reduction in risk of relapse. Clinically, raising 25-OH-D levels by 50nmol/l could halve the hazard of a relapse.

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Multiple sclerosis (MS) is a chronic central nervous system disorder characterized in the majority of cases by relapsing-remitting inflammatory demyelination on a background of slowly progressing neurodegeneration. MS has a highly variable inter- and intrapersonal clinical course, in both pattern and rate of deterioration.[1] This suggests multiple contributory factors, including genetic and lifestyle determinants. However, few factors have been identified that precipitate the onset of relapses in people with MS.

One of the most striking features of MS epidemiology is that increasing latitude correlates with increasing prevalence and incidence.[2,3] One explanation for this latitudinal gradient is the decrease in winter sunlight with increasing latitude.[4] A growing body of work[5–8] now indicates that sunlight and vitamin D may be involved in the etiology of MS.

Vitamin D is produced in the skin by ultraviolet radiation (UVR), is found in certain foods, and may be taken as a supplement.[9] The active form of vitamin D, 1,25-dihydroxyvitamin D (1,25-OH-D), is critical for bone metabolism, but also has important immunomodu-

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latory properties,[10] effecting a reduction in proinflammatory immune pathways.[11,12] The major circulating form, and that used to measure vitamin D, is 25-hydroxyvitamin D (25-OH-D).[13]

There is substantial epidemiological evidence, including prospective cohort studies, indicating that increased levels of sun exposure,[5] larger vitamin D dietary intake,[8] or higher levels of serum 25-OH-D[6] are associated with a lower risk of MS onset. However, the effect of vitamin D on clinical course is less clear. In population-level studies, correlations have been observed between the seasonal variation in levels of 25-OH-D and gadolinium-enhancing magnetic resonance imaging lesions[14] or monthly relapse rates.[15] Three other studies[7,16,17] found that 25-OH-D levels were significantly lower in patients during a relapse compared to patients with stable MS, but it is unknown whether relapses were the result or the cause of the observed low vitamin D levels. A retrospective cohort study found that higher 25-OH-D levels were associated with a lower relapse rate in the previous two years.[18]

Here we examine for the first time whether increasing levels of serum 25-OH-D are associated with a lower risk of relapse using a prospective cohort study design.

Patients and Methods

Study Design

The Southern Tasmanian Multiple Sclerosis Longitudinal Study was designed as a prospective longitudinal cohort study to evaluate the role of personal UVR exposure and 25-OH-D on the clinical course of MS. This study followed a cohort of 203 persons with clinically definite MS (using 2001 McDonald criteria[19]) living in Southern Tasmania, Australia from 2002 to 2005. An estimated 78% (203/259) of eligible cases in the region were included. The study retention rate was 90% (183/203), with 4% (8/203) withdrawing early and 6% (12/203) lost because they moved interstate or died.

A total of 146 participants had a relapsing-remitting[20] course of MS (RRMS). One person did not have any measures of 25-OH-D, leaving 145 persons included in this analysis.

The study methodology has been previously described.[15,21] Briefly, at each biannual review, participants were asked about their lifestyle, including physical activity, whether they smoked, used immunomodulatory therapy, took vitamin D supplements, or were pregnant. At each review, a serum sample was taken for measurement of 25-OH-D levels. Clinical disability was measured every winter by a single physician, including the Expanded Disability Status Scale (EDSS). Participants completed a weekly diary, recording the occurrence of acute infection, changes in their neurological symptoms or immunomodulatory therapy, and onset of pregnancy.

Ethics approval was obtained from the Southern Tasmania Human Research Ethics Committee; all participants provided informed consent.

Measurement of Relapses

In line with other studies, a relapse was defined as the acute or subacute appearance or reappearance of a neurological abnormality (lasting at least 24 hours), immediately preceded by a stable, improving, or slowly progressive neurological state for 30 days, in the absence of fever, known infection, concurrent steroid withdrawal, or externally derived increases in body temperature.[19] Using a real-time relapse notification system, participants telephoned the study center if they thought they were experiencing a relapse; 82 relapses were reported in this fashion. Additionally, at each biannual review, participants were queried about the occurrence of a relapse in the preceding 6 months; 63 relapses were recorded in this manner. To ensure each relapse was a true relapse, the study nurse or physician administered a relapse questionnaire detailing relapse symptoms, medical practitioner review, treatment, and co-occurrence of infection or fever. For quality control, the study physician also performed a physical examination for those reporting a relapse by telephone in year 1. Very few of these (2/35 relapses) were not true relapses, and these 2 would have been identified as such using the relapse questionnaire, validating its effectiveness. Throughout the study, each relapse was reviewed rigorously by the study physician and further by the study neurologist. After study completion, both study physician and neurologist scrutinized all relapse data, excluding events that did not fulfill the strict relapse definition. In total, 5 relapses were excluded in this manner; 6 additional relapses were excluded for being duplicates and 4 for occurring outside the study period. This left 122 validated relapses for use in the analysis.

Measurement of Personal Sun Exposure and Skin Type

At each biannual review, participants estimated how much time they spent in the sun during weekends and holidays. Additionally, participants quantified to the nearest quarter hour how much time they spent per week in the current and preceding season engaged in various activities outside. Outdoor times were summated to yield a value of the total time outside per week each season.

Polysulfone badges were used to measure personal UVR objectively.[22,23] In summer and winter, participants wore 1 badge on Saturday and 1 on Sunday, placed on the outer clothing in the chest region. The badges were analyzed in the UVR laboratory at the Australian Radiation Protection and Nuclear Safety Agency.

Skin melanin density was measured on the upper inner arm using a spectrophotometer as described elsewhere.[5]

Measurement of 25-OH-D

Serum 25-OH-D levels were measured with a commercially available radioimmunoassay (DiaSorin, Stillwater, MN), which has a detection range of 12.5 to 250nmol/l. Interbatch reproducibility was 4.6% at 32nmol/l and 6.4% at 125nmol/l. All samples were stored at −80°C and shielded from light.

Samples were taken at each biannual review, but all quantification of 25-OH-D levels were performed in sequential

batches in order of blood draw following the conclusion of the study. Consequently, neither participants nor study personnel were aware of the participants' 25-OH-D levels during the study.

Statistical Analysis

SEASONAL PATTERN OF 25-OH-D. The seasonal pattern of 25-OH-D was modeled using methods described previously.[24] Briefly, the sinusoidal regression model was:

$$y_t = \beta_0 + \beta_1 \sin\left(\frac{2\pi t}{365}\right) + \beta_2 \cos\left(\frac{2\pi t}{365}\right)$$

where y_t denotes measured serum 25-OH-D concentration, t denotes the day of the year the sample was collected, and $\beta_j (j = 0,1,2)$ are estimated regression coefficients. Excluded from the sinusoidal regression model were those measurements made following a significant period of travel to a location of differing ambient UVR (n = 60 measurements) and all measurements on subjects with levels of disability precluding their going outside (n = 30 measurements).

Two uses were made of the fitted model. It was used to define a summer season of highest mean 25-OH-D levels and a winter season of lowest mean 25-OH-D levels. Based on the fitted model, the highest and lowest 25-OH-D mean concentrations occurred on the 19th of February and the 19th of August. Therefore, summer season was defined as the period from November 20 until May 19, and winter season as the period from May 20 until November 19. 25-OH-D–determined season was used rather than calendar season because it was postulated to more accurately reflect the correlates of season most relevant to MS.

The model was also used to predict 25-OH-D concentrations for subjects at times of the year other than the measurement date. The predicted value at any point in time was the estimated mean 25-OH-D concentration of all subjects at that time plus the difference between the subject's last 25-OH-D measurement and the estimated mean 25-OH-D level of all subjects at the time of that measurement.

Three different models were used to account for exposure-disease temporality:

- In the *as-measured* model, each subject was assigned the measured value of 25-OH-D from the day of measurement until the day of the next measurement of 25-OH-D;
- In the *seasonal* model, the measured 25-OH-D concentration of each subject in summer was used to estimate that subject's 25-OH-D concentration on the preceding November 20, and the measured 25-OH-D concentration of each subject in winter was used to estimate that subject's 25-OH-D concentration on the preceding May 20. The 25-OH-D time-varying covariate for that subject was then assigned the summer season estimated value from November 20 until May 19, and the winter season estimated value from May 20 until November 19. This was done because 25-OH-D levels at Tasmania's latitude (approximately 40–44°S) show a strong seasonal variation, and the fieldwork for each review was spread over 3 months; by correcting to a common time point, this variation was removed;
- In the *monthly* model, the 25-OH-D of each subject was estimated at 30-day intervals. Given that the half-life of 25-OH-D in serum has been estimated to be between 20[25] and 90[26] days, this model was designed to provide a more accurate estimate of 25-OH-D over time.

Consider an individual with a measured 25-OH-D concentration of 72nmol/l when measured on January 10, when the average for all subjects was 70nmol/l, and a concentration of 50nmol/l on July 23, when the average was 40nmol/l. In the as-measured analysis, this person would be assigned a 25-OH-D level of 72nmol/l from January 10 until July 23. Also, consider a summer season stretching from November 20, when the cohort average value of 25-OH-D was 52nmol/l, until May 20 of the next year. In the seasonal analysis, this person would be assigned a 25-OH-D value that was 2 units above the cohort average value on November 20, from this date until May 20. In the monthly analysis, the fact that this person was 2nmol/l above the average on January 10 and 10nmol/l above the average on July 23 was used to estimate the concentrations for each month between January 10 and July 23.

We assessed serum 25-OH-D as a continuous term indicating linear risk. We found this was appropriate by comparing the fit of each model, with the log-likelihood ratio statistic suggesting that the linear form fitted the model best.

SEASONAL PATTERN OF UVR. The same regression function described above was used to estimate personal UVR exposure at the monthly level using polysulfone badge data. Excluded from this regression model were those measurements where the participants' reported time spent outdoors was significantly different from their norm (n = 312 measurements), or where participants wore the badge incorrectly (n = 17 measurements).

DATA ANALYSIS. The effect of 25-OH-D and other covariates, including use of immunomodulatory therapy during the study, smoking, and pregnancy, on time-to-relapse was calculated using Cox proportional hazards models for repeated events, using the time-gap model described by Prentice et al,[27] where multiple relapses by the same persons are treated as independent observations, and the time until a prior event does not influence the composition of the risk set for a subsequent event.

All covariates satisfied the proportional hazards assumption with the exception of the binary variable for sex. For this reason, univariate results are not reported for sex, and all multivariate models are stratified to allow the baseline hazards to differ by sex.

Multivariate models were adjusted for age. Survival proportions are depicted using Kaplan-Meier survival curves showing time to relapse, where multiple relapses by the same persons are treated as independent observations.

The significance of the difference in mean 25-OH-D between different subgroups was assessed using marginal models

TABLE 1: Characteristics of 145 Participants with RRMS in the MS Longitudinal Study Cohort

Characteristic	n/N (%)	Relapses[a]/ Person-Years	Relapse Rate[b]	Mean 25-OH-D, nmol/l
Total	145/145	122/330.2	0.37	55.0
Sex				
Female	109/145 (75.2)	100/248.1	0.40	54.8
Male	36/145 (24.8)	22/82.1	0.27	55.6
Age at study entry, yr				
21-38	34/145 (23.5)	29/70.9	0.41	62.5
39-44	36/145 (24.8)	32/85.6	0.37	51.7[c]
45-51	34/145 (23.5)	26/78.0	0.33	58.7
52-76	41/145 (28.3)	35/95.8	0.36	49.2[c]
Relapse during study?				
Yes	70/145 (48.3)	122/167.5	0.73	51.7
No	75/145 (51.7)	0/162.8	0.00	58.8[c]
Any immunomodulatory therapy during study?				
Yes	119/145 (82.1)	106/276.5	0.38	55.5
No	26/145 (17.9)	16/53.7	0.30	52.2
BMI[d,e]				
Normal	57/145 (39.3)	43/131.7	0.33	60.4
Overweight	57/145 (39.3)	52/124.6	0.42	54.5[c]
Obese	31/145 (21.4)	27/73.9	0.37	46.1[c]
Smoker during study?				
Yes	41/145 (28.3)	35/82.9	0.42	52.8
No	115/145 (79.3)	87/247.4	0.35	55.7

	Mean (SD; range)
Age at study entry, yr	44.8 (10.8; 21, 76)
MS duration from diagnosis, yr	6.8 (7.2; 0, 43)
MS duration from 1st symptoms, yr	11.1 (9.1; 0, 58)
EDSS at study entry	2.8 (1.6; 0, 8.5)

[a]Total number.
[b]Relapses per person-year.
[c]Significantly different ($p < 0.05$) from initial category using random-effects generalized estimating equations.
[d]Underweight = BMI < 17.5, Normal = BMI 17.5-25.0, Overweight = BMI 25-29.9, Obese = BMI ≥ 30.
[e]No participants were underweight by BMI, and thus this category was not included in the table.
RRMS = relapsing-remitting multiple sclerosis; MS = multiple sclerosis; 25-OH-D = 25-hydroxyvitamin D; BMI = body mass index; SD = standard deviation; EDSS = Expanded Disability Status Scale.

estimated by generalized estimating equations. For the analysis of the determinants of 25-OH-D, estimated monthly 25-OH-D levels were applied. The results were similar to those found when biannually measured 25-OH-D levels were used.

All analyses were performed using STATA/SE for Windows (version 10.1; StataCorp LP, College Station, TX).

Results

Participant Characteristics

The cohort of 145 participants with RRMS was followed for an average of 2.3 (standard deviation, 0.6) years. A total of 122 confirmed relapses occurred in 70 partici-

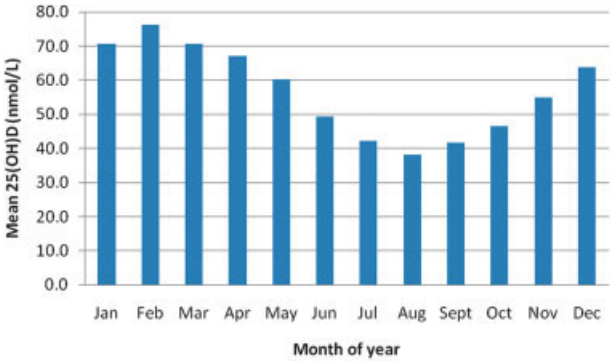

FIGURE 1: Annual variation in modeled 25-hydroxyvitamin D (25-OH-D) in nanomoles per liter by month of year. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

pants (mean, 0.37 relapses per person/yr). The mean 25-OH-D was 41.2nmol/l in winter and 74.8nmol/l in summer. During the study, 77/145 (53.1%) took a vitamin D supplement; of these, only 23/77 (29.9%) took >400IU/day. Other features of the cohort are shown in Table 1.

Determinants of Serum 25-OH-D Levels

Figure 1 shows the sinusoidal pattern of modeled 25-OH-D in this cohort.

As expected, summer season and measures of personal sun exposure were important determinants of 25-OH-D levels. Modeling personal UVR exposure at the monthly level increased the magnitude of the association between higher UVR exposure and subsequent 25-OH-D.

Neither female sex (coefficient, −2.4; 95% confidence interval [CI] −9.5 to 4.6) nor smoking status (coefficient, 0.3; 95% CI, −2.7 to 3.3) were significant determinants of 25-OH-D. Vitamin D supplement dosage was not a significant determinant of 25-OH-D (p = 0.46). Physical activity was a strong predictor of 25-OH-D (coefficient, 1.4; 95% CI, 0.8–2.0), even after adjusting for time spent outdoors (coefficient, 1.5; 95% CI, 0.5–2.5). Melanin density was positively associated with 25-OH-D (coefficient, 4.7; 95% CI, 1.7–7.8). Age, body mass index, and EDSS score were negatively associated with 25-OH-D, and these effects persisted after adjustment for time spent outside (Table 2).

From these results, the following findings should be interpreted as relating to predominantly UVR-derived 25-OH-D stores, in keeping with previous work.[24]

Univariate Analysis of Associations with the Hazard of Relapse

We examined the association between specific participant characteristics and the hazard ratio (HR) of having a relapse in the subsequent 6 months (Table 3). There was no association between the EDSS score at study entry (HR, 1.02; 95% CI, 0.89–1.17), MS duration from first symptom (HR, 0.98; 95% CI, 0.96–1.00), or pregnancy (HR, 0.88; 95% CI, 0.27–2.87) and the hazard of relapse.

Association between Serum 25-OH-D Levels and Hazard of Relapse

We examined whether 25-OH-D levels, measured as a 10-unit continuous variable every 6 months, were associated with the hazard of relapses. We found that increasing levels of 25-OH-D were associated with a lower age- and sex-adjusted hazard (adjusted HR [AHR]) of relapse (AHR, 0.91; 95% CI, 0.85–0.97; p = 0.006), with each 10nmol/l increase in 25-OH-D reducing the hazard by 9% (95% CI, 3–15%). When the seasonal analysis was used to standardize all measures to the start of each season, the association persisted (AHR, 0.90; 95% CI, 0.83–0.98; p = 0.016), with each 10nmol/l increase in 25-OH-D reducing the hazard by 10% (95% CI, 2–17). The monthly analysis slightly enhanced the association (AHR, 0.88; 95% CI, 0.82–0.95; p = 0.001), with each 10nmol/l increase in 25-OH-D reducing the hazard of relapse 12% (95% CI, 5–18) (Table 4).

The association between 25-OH-D and hazard of relapse was linear for all 3 models, with no evidence of a threshold effect (see Figure 2 for monthly model). Figure 3 shows the survival curve for the monthly model, demonstrating that those with higher 25-OH-D levels experienced fewer relapses and a longer time until relapse occurrence.

Further Analyses

The association between monthly 25-OH-D and relapse (AHR, 0.88; 95% CI, 0.82–0.95) was not significantly altered by further adjustment for immunomodulatory therapy (AHR, 0.88; 95% CI, 0.82–0.95), smoking (AHR, 0.88; 95% CI, 0.82–0.95), higher physical activity (AHR, 0.88; 95% CI, 0.82–0.95), melanin density (AHR, 0.88; 95% CI, 0.82–0.95), pregnancy (AHR, 0.88; 95% CI, 0.81–0.95), number of acute infections (AHR, 0.90; 95% CI, 0.83–0.97), EDSS score at study entry (AHR, 0.88; 95% CI, 0.82–0.95), or MS disease duration from first symptom (AHR, 0.88; 95% CI, 0.85–0.95).

The beneficial effect of higher 25-OH-D levels was evident in both winter (AHR, 0.86; 95% CI, 0.75–0.97) and summer (AHR, 0.91; 95% CI, 0.82–1.02) (p = 0.45) and did not differ by the amount of personal UVR exposure, being present in both those below (AHR, 0.88; 95% CI, 0.80–0.96) and those above (AHR, 0.93; 95% CI, 0.80–1.08) the mean cohort UVR (p = 0.27). Therewas no difference between patients taking immuno-

TABLE 2: Determinants of Serum 25-OH-D

Determinant	Coefficient (95% CI)	*p*	Adjusted Coefficient[a] (95% CI)	*p*
Season[b]				
Winter	1.0[c]		1.0[c]	
Summer	17.2 (16.5, 18.0)	<0.001	18.0 (16.5, 19.4)	<0.001
Time in sun (h/day)				
<½	1.0[c]			
½–<1	1.2 (−0.7, 3.0)	0.221		
1–<2	3.3 (1.4, 5.2)	0.001		
2–<3	5.1 (3.0, 7.2)	<0.001		
3+	6.4 (4.1, 8.7)	<0.001		
Trend		<0.001		
Time spent outside, h/wk				
<4	1.0[c]			
4–<7	8.1 (5.4, 10.8)	<0.001		
7–<13	11.8 (8.9, 14.7)	<0.001		
13+	18.5 (15.3, 21.6)	<0.001		
Trend		<0.001		
Polysulfone-measured UVR (SEDs)				
<0.14	1.0[c]			
0.14-0.39	0.6 (−1.0, 2.1)	0.463		
0.40-0.79	4.5 (3.0, 6.1)	<0.001		
0.80+	10.6 (9.1, 12.2)	<0.001		
Trend		<0.001		
Monthly modeled UVR (SEDs)				
<0.14	1.0[c]			
0.14-0.39	−1.6 (−3.1, −0.1)	0.040		
0.40-0.79	6.6 (5.1, 8.2)	<0.001		
0.80+	15.3 (13.8, 16.7)	<0.001		
Trend		<0.001		
Skin melanin density, %				
<1.00	1.0[c]		1.0[c]	
1.00-1.99	6.2 (−2.2, 14.6)	0.146	4.8 (−3.2, 12.8)	0.238
2.00-2.99	14.1 (6.0, 22.2)	0.001	12.4 (4.7, 20.1)	0.002
3.00+	10.7 (0.5, 20.8)	0.039	9.4 (−0.3, 19.1)	0.057
Trend		0.003		0.004
Vitamin D supplementation in each 6-month period				
None	1.0[c]		1.0[c]	
<400IU/day	−0.4 (−1.9, 1.0)	0.608	0.5 (−2.1, 3.2)	0.701
400-720IU/day	−0.9 (−3.7, 1.8)	0.500	0.3 (−4.5, 5.1)	0.914
Trend		0.464		0.777

TABLE 2: Continued

Determinant	Coefficient (95% CI)	*p*	Adjusted Coefficient[a] (95% CI)	*p*
Physical activity (METs/day)				
0-5.9	1.0[c]		1.0[c]	
6.0-23/9	2.0 (0.5, 3.5)	0.011	2.2 (−0.5, 4.9)	0.106
24.0-47.9	2.8 (1.1, 4.4)	<0.001	3.7 (0.7, 7.8)	0.014
48+	4.5 (2.7, 6.3)	<0.001	4.7 (1.5, 7.8)	0.004
Trend		<0.001		0.003
Age at study entry, yr				
21-39	1.0[c]		1.0[c]	
40-45	−9.9 (−17.9, −2.0)	0.014	−8.7 (−16.3, −1.1)	0.024
46-50	−1.9 (−10.8, 6.9)	0.667	−2.8 (−11.2, 5.6)	0.514
50-76	−11.2 (−18.5, −3.8)	0.003	−11.9 (−18.9, −4.8)	0.001
Trend		0.020		0.005
BMI[d,e]				
Normal	1.0[c]		1.0[c]	
Overweight	−6.9 (−13.4, −0.4)	0.038	−6.6 (−12.9, −0.4)	0.036
Obese	−14.5 (−22.2, −6.8)	<0.001	−13.2 (−20.6, −5.8)	<0.001
Trend		<0.001		<0.001
EDSS score at study entry				
0–<1.0	1.0[c]		1.0[c]	
1.0–<2.0	−2.6 (−13.4, 8.3)	0.645	−4.2 (−14.7, 6.2)	0.425
2.0–<5.0	−1.5 (−12.2, 9.2)	0.787	−2.2 (−12.4, 8.1)	0.681
5.0+	−15.9 (−27.9, 3.9)	0.010	−14.2 (−25.7, 2.7)	0.016
Trend		0.013		0.038
Pregnancy				
No	1.0[c]		1.0[c]	
Yes	0.3 (−3.8, 4.4)	0.896	1.3 (−5.4, 8.1)	0.697

[a]Adjusted for time spent outside per season.
[b]Summer season was defined as the period from November 20 until May 19 (with February 19 as its midpoint), and the winter season was defined as the period from May 20 until November 19 (with August 19 as its midpoint).
[c]Reference.
[d]Underweight = BMI < 17.5, Normal = BMI 17.5-25.0, Overweight = BMI 25-29.9, Obese = BMI ≥ 30.
[e]No participants were underweight by BMI, and thus this category was not included in the table.
25-OH-D = 25-hydroxyvitamin D; CI = confidence interval; UVR = ultraviolet radiation; SED = standard erythema dose; MET=metabolic unit; BMI = body mass index; EDSS = Expanded Disability Status Scale.

TABLE 3: Univariate Associations of Selected Factors and Relapse Rate/Hazard, As-Measured Analysis

Factor	No. Relapses/ Person-Years	Relapse Rate[a]	HR (95% CI)
25-OH-D			
<40nmol/l	59/124.8	0.50	1.00[b]
≥40nmol/l	62/195.8	0.30	0.60 (0.43-0.84)
25-OH-D by season[c]			
Winter	66/172.2	0.38	1.00[b]
Summer	56/158.1	0.35	0.92 (0.64-1.32)
Vitamin D supplementation in each 6-month period			
None	94/242.4	0.39	1.00[b]
<400IU/day	22/63.5	0.35	0.91 (0.55-1.49)
400+ IU/day	6/22.0	0.27	0.76 (0.33-1.74)
Trend			$p = 0.47$
Number of acute infections in each 6-month period			
0	41/107.6	0.38	1.00[b]
1	31/100.8	0.31	0.80 (0.52-1.25)
2+	30/83.7	0.36	0.90 (0.55-1.46)
Trend			$p = 0.63$
Immunomodulatory therapy in each 6-month period[d]			
No	27/74.7	0.36	1.00[b]
Yes	95/255.5	0.37	1.03 (0.65-1.63)
Sex[e]			
Male	22/82.1	0.27	
Female	100/248.1	0.40	
Age at study entry, yr			
21-38	29/70.8	0.41	1.00[b]
39-44	32/85.6	0.37	0.95 (0.52-1.74)
45-51	26/78.0	0.33	0.84 (0.46-1.55)
52-76	35/95.8	0.37	0.91 (0.52-1.57)
Trend			$p = 0.67$
Smoker during study			
No	87/247.4	0.35	1.00[b]
Yes	35/82.9	0.42	1.21 (0.76, 1.95)

[a]Relapses/person-years.
[b]Reference.
[c]Summer season was defined as the period from November 20 until May 19 (with February 19 as its midpoint), and the winter season was defined as the period from May 20 until November 19 (with August 19 as its midpoint).
[d]Of those on immunomodulatory therapy, 82.6% were using a beta-interferon product.
[e]Sex could not be evaluated for its HR due to its violation of the proportional hazards assumption.
HR= hazard ratio; CI = confidence interval; 25-OH-D = 25-hydroxyvitamin D.

TABLE 4: Association between Serum 25-OH-D Levels and the Hazard of a Relapse, Using the 3 Models

Analysis Method	25-OH-D-10, Crude HR (95% CI)	25-OH-D-10, Adjusted[a] HR (95% CI)
As-measured	0.92 (0.86-0.98), $p = 0.013$	0.91 (0.85-0.97), $p = 0.006$
Seasonal	0.91 (0.84-0.99), $p = 0.028$	0.90 (0.83-0.98), $p = 0.016$
Monthly	0.89 (0.82-0.96), $p = 0.001$	0.88 (0.82-0.95), $p = 0.001$

HR is expressed in 10nmol/l increments; 10-unit increment continuous 25-OH-D HRs reflect the change in the hazard of relapse for each 10-unit increase in serum 25-OH-D.
[a]Analyses are adjusted for age at study entry and sex.
25-OH-D = 25-hydroxyvitamin D; HR = hazard ratio; CI = confidence interval.

modulatory therapy and those who were not ($p = 0.11$). Importantly, even when patients with 25-OH-D insufficiency[28] (<40nmol/l) were excluded, an inverse association remained (AHR, 0.91; 95% CI, 0.81–1.01).

To examine the possibility of reverse causality, we stratified our analysis between those with an EDSS at study entry of ≤4.5 versus >4.5. We found no significant difference ($p = 0.56$) in the relationship between 25-OH-D and the hazard of relapse between those with lower disability (AHR, 0.89; 95% CI, 0.82–0.96) and those with higher disability (AHR, 0.80; 95% CI, 0.63–1.03).

Discussion

This is the first prospective study to demonstrate an association between increasing levels of 25-OH-D and a subsequent reduced hazard of relapse in people with RRMS. This relationship was dose-dependent and linear, with no evidence of a threshold effect. Importantly, this effect persisted when measurements were corrected for sampling date variations (in the seasonal analysis) and became slightly stronger when 25-OH-D estimates were used that were closer in time to a relapse (in the monthly analysis).

These associations persisted on adjustment for a diverse range of potential confounders, including immunomodulatory therapy, disease course, and behavioral and environmental factors. The association was evident among those with low personal UVR exposure, suggesting that the results can be generalized to locations with lower ambient summer UVR than Tasmania. Also, the association was still observed after excluding patients who were 25-OH-D insufficient, indicating that the association was not driven solely by 25-OH-D levels in the insufficiency range. There was no difference in the relationship be-

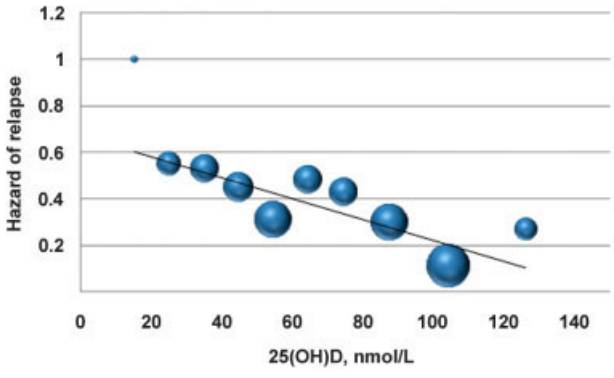

FIGURE 2: Hazard ratios for category of 25-hydroxyvitamin D (25-OH-D) in 10nmol/l increments, where level of 25-OH-D is determined using the monthly model. Analysis is adjusted for age at study entry and stratified to allow the baseline hazards to differ by sex. Size of points is proportional to the inverse of the variance (larger bubbles represent greater precision). The plots and findings were very similar for the as-measured and seasonal models (not shown). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

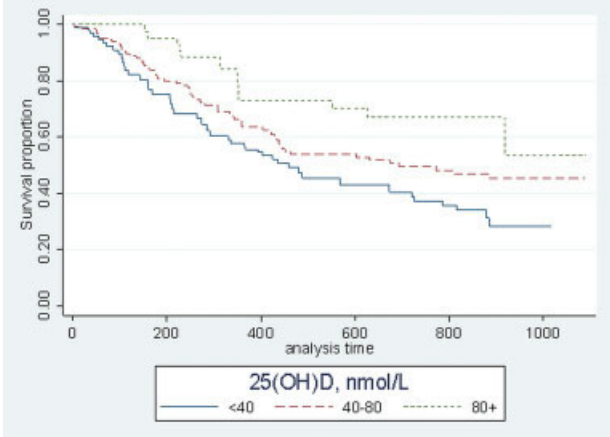

FIGURE 3: Kaplan-Meier survival plots by category of 25-hydroxyvitamin D (25-OH-D) where level of 25-OH-D is determined by the monthly model. The plots show the proportion of subjects relapse-free each day since study entry. Multiple relapses by the same persons are treated as independent observations. The plots and findings were very similar for the as-measured and seasonal models (not shown). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

tween 25-OH-D and relapse between those with higher (EDSS $>$ 4.5) and lower (EDSS $\leq$ 4.5) disability. This suggests that the association was not being driven by those with more active disease being less mobile and thus, having less UVR exposure and less 25-OH-D, and argues against reverse causality as a cause of the observed relationship.

A key strength of this work is the prospective nature of our study, using repeated measures of 25-OH-D and real-time relapse notification. The size of the cohort (145 participants), the duration of follow-up (mean, 2.3 years), and the comprehensive nature of the factors investigated allowed a thorough investigation of the relationship between 25-OH-D and relapse, taking into account potential confounders such as immunomodulatory therapy, smoking, and seasonal covariates.

An additional strength was the use of survival analysis rather than rates; this allowed the inclusion of all information on the times of events and the change in exposure status over time, increasing temporal precision. Furthermore, using Cox proportional hazards models for repeated events enabled inclusion of all relapses, rather than merely time to first relapse.[29]

The study method was limited by the biannual collection of 25-OH-D. The ideal experiment, where the level of 25-OH-D at the time of relapse is known, is well approximated by the monthly analysis here. The modeling methods used carry assumptions about the fluctuation of 25-OH-D levels over time and assume a sinusoidal variation over time for all participants. Justification of this approach is the observation that serum 25-OH-D has been found to vary with an approximately sinusoidal pattern[30,31] due to variation in ambient UVR and seasonal behavior. As interindividual variation in behavior does not always allow extrapolation of this assumption to every individual, we also took into account important factors likely to affect our models, excluding those with significant disability and trips to areas of differing ambient UVR, both of which have the potential to affect the levels of 25-OH-D.

We observed up to a 12% decrease in relapse risk for each 10nmol/l increase in serum 25-OH-D, in line with contemporaneous work by Mowry et al,[32] who found an inverse association between higher 25-OH-D levels and risk of relapse in pediatric-onset MS, with each 25nmol/l increase in serum 25-OH-D reducing the subsequent relapse rate by 34%. Our data imply that increasing serum 25-OH-D levels by 50nmol/l could more than halve the risk of relapse, a reduction at least on a par with most immunomodulatory therapies.[33] Importantly, these reductions were seen in a cohort that was largely using immunomodulatory therapy (82%), suggesting that 25-OH-D has additive beneficial effects. A 50nmol/l increase in serum 25-OH-D could be realized with supplementation of 2,000IU/day,[34,35] well below the tolerable upper limit of 10,000IU/day.[28] Compared to immunomodulatory therapy, vitamin D-based therapies are cheaper and have much less potential for side effects. Given that the mean 25-OH-D level in winter in this cohort was 41.2nmol/l and that beneficial effects were observed up to 120nmol/l, a significant reduction in relapse risk could be realized by treatment with sufficient doses of vitamin D.

There is biological plausibility for vitamin D as a protective factor against relapses. 1,25-OH-D shifts the immune response away from a proinflammatory profile and enhances anti-inflammatory pathways in multiple settings[11,12,36]; also, 25-OH-D reduces the proliferation of Th17 cells.[7] Calcitriol-fed mice have fewer clinical, histopathological, and immunological signs of experimental autoimmune encephalitis, an animal model of MS.[37–40]

This prospective cohort study demonstrates that for each 10nmol/l increase in serum 25-OH-D, there was up to a 12% reduction in the hazard of relapse. These findings provide strong support for randomized clinical trials of vitamin D-based therapies in treating relapse in RRMS.

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Authorship

B.T., I.v.d.M., A.-L.P., P.G., and T.D. were involved in the conception, planning, and acquisition of funding for the study. B.T., I.v.d.M., F.P., A.-L.P., and T.D. were involved in the acquisition of data for the study. P.G. was involved in the acquisition of polysulfone data for the study. S.S., B.T., L.B., H.T., and I.v.d.M. were involved in the conception and implementation of the analyses used in this articl. S.S., B.T., L.B., and I.v.d.M. were involved in the drafting of the manuscript. All authors were involved in the critical revision of the manuscript and approved it for submission.

B.T. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Potential Conflicts of Interest

Nothing to report.

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