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Vitamin D levels in people with multiple sclerosis and community controls in Tasmania, Australia

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■ **Abstract** *Background* Adequate 25(OH)D levels are required to prevent adverse effects on bone health. Population-based data on factors associated with 25(OH)D levels of people with MS have been lacking. *Objectives* To examine the prevalence and determinants of vitamin D insufficiency in a population-based sample of MS cases and controls, and to compare 25(OH)D status between MS cases and controls, taking into account case disability. *Methods* We conducted a population based case-control study in Tasmania, Australia (latitude 41–43°S) on 136 prevalent cases with MS confirmed by magnetic resonance imaging and 272 community controls, matched on sex and year of birth. Measurements included serum 25(OH)D, sun exposure, skin type, dietary vitamin D intake and disability including EDSS.

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Results A high prevalence of vitamin D insufficiency was found in MS cases and controls. Among MS cases, increasing disability was strongly associated with lower levels of 25(OH)D and with reduced sun exposure. Cases with higher disability (EDSS > 3) were more likely to have vitamin D insufficiency than controls (OR = 3.07 (1.37, 6.90) for 25(OH)D ≤ 40 nmol/l), but cases with low disability were not (OR = 0.87 (0.41, 1.86)).

Conclusion The strong associations between disability, sun exposure and vitamin D status indicate that reduced exposure to the sun, related to higher disability, may contribute to the high prevalence of vitamin D insufficiency found in this population-based MS case sample. Active detection of vitamin D insufficiency among people with MS and intervention to restore vitamin D status to adequate levels should be considered as part of the clinical management of MS.

■ **Key words** vitamin D · multiple sclerosis · deficiency · insufficiency · sun exposure · disability · healthy

Introduction

Most (90–100%) vitamin D is produced endogenously from the action of solar radiation (particularly UVB) on precursors within sun-exposed skin [18]. Only a few foods, such as oily fish, contain significant amounts of vitamin D. Vitamin D plays an important role in maintaining bone health by preserving calcium and phosphorous homeostasis. In addition, vitamin D appears to play roles in immunomodulation [6], cell differentiation [40] and neural development [14]. In particular, the active form of vitamin D (1,25(OH)₂D₃) can down-regulate the T helper type 1 (Th₁) cell activity through vitamin D receptors on activated T lymphocytes [24]. Multiple sclerosis (MS) is a disease where Th₁ over-activity appears to play an important role. Serum 25-hydroxyvitamin D (25(OH)D) is often used as a marker of vitamin D status [43], and reflects recent UVR exposure and vitamin D intake. Vitamin D deficiency is often defined as having levels below 25 nmol/l, vitamin D insufficiency as having levels between 25 and 40 or 50 nmol/l, and optimal levels as having levels above 50 nmol/l [31,44]. Recent evidence suggests that the optimal serum 25(OH)D levels may be even higher, above 80 nmol/l [20].

Low vitamin D levels can lead to osteoporosis and osteomalacia [19] through a reduction in calcium absorption, an elevation in the blood concentration of parathyroid hormone, and an increased rate of bone resorption [15]. Serum 25(OH)D levels of at least 50 nmol/L are required for calcium homeostasis without secondary parathyroidism [19]. Low vitamin D levels, over time, may result in osteoporotic bone fractures [15]. Fractures might also increase through a higher risk of falls, because vitamin D influences neuromuscular coordination [2]. People with MS seem to be at risk. In one study, people with MS had more fractures during adulthood (22%) compared with controls (2%) and lost bone mass three to six times more rapidly during two years of follow-up [9]. Apart from the substantial health care expenses, the consequences of fractures are severe, with high rates of permanent disability, particularly among elderly [26]. Prevention seems important and two meta-analyses of randomized controlled trials show that oral vitamin D supplementation indeed reduces the risk of fractures and falls [3, 4].

There are currently no population-based data on the occurrence of vitamin D insufficiency and deficiency among people with MS, although vitamin D deficiency has been shown to be common among female MS patients admitted to a tertiary care hospital [29]. A small Finnish study comparing MS patients with early phase MS and hospital controls showed that MS patients had lower 25(OH)D levels

than controls in summer, but similar levels in winter [38].

The possible determinants of lower serum 25(OH)D levels among people with MS have not been formally assessed. It is important to understand the reasons why people with MS may have an inadequate vitamin D status so that vitamin D deficiency and its adverse effects on bone health can be avoided. In addition, there is increasing evidence that higher sun exposure and vitamin D may reduce the risk of MS [28, 42], and some evidence to suggest that vitamin D status may influence disease course [7, 13, 17, 25, 27], but further analytical work is required to establish a causal association between vitamin D status and MS progression.

This report aims to: (i) examine the prevalence of vitamin D deficiency and insufficiency in a population-based sample of people with MS and age- and sex-matched community controls under the age of 60 years living in Tasmania (latitude 41–43°S); (ii) describe factors associated with 25(OH)D status in MS cases and controls, and (iii) compare 25(OH)D status between MS cases and controls, taking into account case disability level.

Methods

■ Subjects

The source population consisted of residents under the age of 60 years living in Tasmania, Australia, with at least one grandparent who was born in Tasmania. To recruit cases, multiple population-based strategies were used including information evenings at local MS societies and letters of invitation from neurologists to patients. Eligible cases had cerebral MRI abnormalities consistent with MS [32] and clinically definite MS [33]. Of the 169 patients who responded, 30 did not meet the eligibility criteria, one refused, one deteriorated and was unable to participate and one died. Controls were selected from the roll of voters for compulsory political elections. For each case, two control subjects were randomly selected and matched to the index case on sex and birth year. For the 136 cases included in the study, estimated to comprise at least 79% of eligible cases, 272 eligible controls participated with a response rate of 76%. The project received ethics approval from the Human Research Ethics Committee of the Royal Hobart Hospital.

■ Measurements

Disease characteristics

Each case was assessed by one of the four study neurologists; detailed disease characteristics were available for 134 of the 136 cases. These included age of first symptom, age of diagnosis, type of MS, use of immunomodulatory treatment, expanded disability status scale (EDSS) [23], Scripp's Neurologic Rating Scale [37], and MS Severity Score [34]. The MS Severity Score is based on the EDSS but takes disease duration into account [34]. The EDSS progression index was calculated by dividing the EDSS by disease duration

since first symptom for those with a disease duration greater than 5 years ($n = 108$).

Serum 25(OH)D

Serum 25(OH)D levels were measured with a commercially available radioimmunoassay (Stillwater, Minnesota-DiaSorin Inc.) for 136 cases and 262 controls.

Measures related to sun exposure

Two research assistants conducted all interviews and measurements between March 1999 and June 2001. Participants were asked, for different periods of their lives, about the amount of time they would normally have spent in the sun during weekends and holidays in winter and summer (“time in the sun” question) (< 1 h a day, 1–2 h a day, 2–3 h a day, 3–4 h a day, or > 4 h a day) [42], using a question validated for teenagers in this climate [10]. The weighted κ statistic [8] (95% confidence interval) for the “Time in the sun” question in the last three years on retest after 11 weeks for 52 cases and 52 controls was 0.68 (0.48–0.87), and no difference in this statistic was found between cases and controls. Prior to the interview, subjects were sent a lifetime calendar and asked to fill this out for each year of their life (e.g. place of residence, school, occupation, number of days working). During the interview, subjects answered the “Time in the sun” question for summer for each year of their life and indicated, for each year of occupation, how much time (to the closest 15 minutes) they would spend outside on a working day during working hours. In this paper, sun exposure in the last year or last three years was considered to be recent sun exposure. The standardized questionnaire included a question on occupational sun exposure (mainly indoors, both indoors and outdoors, or mainly outdoors) and the frequency of the use of sunscreen (never/rarely, occasionally, most of the time, or always/almost always). Silicon skin surface casts of the hand, measuring actinic damage, were used as an objective marker of cumulative lifetime sun exposure [42].

Skin type related factors

Skin phenotype was assessed with a spectrophotometer at the upper inner arm and buttock; body sites usually not exposed to sunlight. Cutaneous melanin density was estimated from the skin reflectance of light centered at 400 nm and 420 nm [11]. Research assistants assessed visually the skin color at the upper inner arm (fair, fair/medium, medium/olive, or olive), counted the number of nevi greater than 5 mm on the left arm (two or more, one, or zero), and recorded hair (red, light blonde, “mousey” blonde, light brown, dark brown, or black). The standardized questionnaire included questions on number of lifetime sunburns (> 10 times, 6–10 times, 2–5 times, once, or never), tendency to burn (burn then peel, burn then tan, or tan only), and ability to tan (practically no tan, light tan, medium tan, or dark tan).

Dietary intake in the last 12 months and other factors

Participants indicated how often on average they ate fish, eggs, and meat in the last 12 months. They were asked about their milk intake in the last 12 months (< 150 ml per day, 150–300 ml, 301–600 ml, or > 600 ml), and whether or not they used vitamin D supplements in the last 12 months and the type of supplement. It was checked whether the supplement reported contained vitamin D. If it did not, the response was recoded as not using vitamin D supplements.

The standardized questionnaire also included questions on medical history including reported infections and immunizations, exposure to chemicals, exposure to pets and farm animals,

occupation, education, smoking, alcohol intake, whether the subject was breastfed, and reproductive history for women (age of menarche, number of children and breastfeeding). For the timing of exposures, we obtained, in accordance to the recommendations of Boiko [5], either the exact age or the five-year age group at the time of the exposure.

Statistical analysis

The prevalence of vitamin D deficiency and insufficiency was calculated from the crude 25(OH)D levels and from 25(OH)D levels that had the seasonal component removed (deseasonalized). To model seasonal variation, we fitted a sinusoidal model to data on 25(OH)D levels and the month (t) the sample was taken:

$$25(\text{OH})\text{D levels} = \beta_0 + \beta_1 \sin \frac{2\pi t}{12} + \beta_2 \cos \frac{2\pi t}{12}$$

The ANOVA F-test was used to decide whether there was significant seasonal variation and the amplitude was calculated using the formula $\sqrt{\beta_1^2 + \beta_2^2}$. To deseasonalize the data, we subtracted the predicted value from the sinusoidal model for each subject from the actual value and added the overall mean. This was done separately for cases and controls.

To examine predictors of 25(OH)D, Pearson correlation coefficients were calculated, using deseasonalized 25(OH)D values that were log-transformed to reduce skewness. The 25(OH)D values were deseasonalized to reduce the effect of the time of year that the serum sample was taken, producing 25(OH)D values that were more like those that would have obtained if all participants were measured at the same time of year. Through linear regression, tests for interaction were conducted using the coefficient and standard error of a product term. For some analyses, the case group was divided into a group with low disability (EDSS ≤ 3) and a group with higher disability (EDSS > 3) based on the median EDSS score of three. In the figure, EDSS scores were grouped when required to obtain mean values based on groups of more than ten subjects.

To compare cases and their matched controls, matched odds ratios and 95% confidence intervals were estimated by conditional logistic regression. The models were adjusted for the month (t) that the serum sample was taken by using a seasonal term ($\cos \frac{2\pi t}{12}$). Tests for trend of categorical variables were undertaken by replacing the binary predictors with a single predictor taking category rank scores. A test for interaction between 25(OH)D levels and EDSS (> 3 vs ≤ 3) was conducted using the coefficient and standard error of a product term of 25(OH)D and EDSS. Controls were given the EDSS score of their case pair. The contribution of amount of recent sun exposure to the excess of low 25(OH)D status in cases with a high EDSS score (> 3) compared with controls was obtained by comparing the odds ratio for low 25(OH)D status and MS before (OR_b) and after (OR_a) adjustment for recent sun exposure ($([1 - \text{OR}_b] - [1 - \text{OR}_a]) / (1 - \text{OR}_b)$) [35]. Using the lifetime calendar, average sun exposure for different age spans was calculated. For the “Time in the sun” question, categories were assigned rank scores. For occupational sun exposure, average exposure was calculated from the exact time outside during work hours and then categorized in a four level variable (0–1 h a week, 1.01–2.5 h a week, 2.51–7 h a week, > 7 h a week).

Results

Prevalence of vitamin D insufficiency and deficiency

Table 1 shows the characteristics of the population-based sample of cases with MS and community

Table 1 Characteristics of the MS cases and controls

	Cases	Controls
Female/Male ratio	92/44	184/88
Mean age, years (SD)	43.5 (9.3)	43.6 (9.2)
Living in Tasmania in the last three years, % (n/N)	97.8 (133/136)	98.9 (269/272)
Mean 25(OH)D level (SD)	51.4 (20.3)	53.1 (21.1)
Mean age at diagnosis, years (SD)	34.6 (9.1)	
Mean age at first symptoms, years (SD)	31.0 (9.1)	
Mean duration of MS since diagnosis, years (SD)	9.4 (7.5)	
Mean duration of MS since first symptoms, years (SD)	12.1 (8.0)	
Mean EDSS score (SD)	3.5 (2.2)	
DRB1*1501—DQB1*0602 haplotype frequency, % (n/N)	53.7 (66/123)	
Type of MS		
Relapsing remitting MS, % (n)	66.4 (89)	
Secondary progressive MS, % (n)	26.1 (35)	
Primary progressive MS, % (n)	7.5 (10)	

controls. Table 2 shows the prevalence of vitamin D deficiency and insufficiency using the crude 25(OH)D levels as well as deseasonalized 25(OH)D levels, because the literature and our data suggest that the prevalence is influenced by the season in which the vitamin D sample was taken. Overall, the crude prevalence of vitamin D deficiency among MS cases was 11.8%. After deseasonalization, the prevalence was 7.4% (Table 2). For controls, the figures were 8.8% and 6.1%, respectively. The deseasonalized prevalence of vitamin D insufficiency (levels between 25 and 50 nmol/l) among cases was 43.3%, and similar for females and males. When comparing cases with controls, MS cases had a higher frequency of vitamin D levels in the range 25–40 nmol/l (Table 2).

■ Disease characteristics and 25(OH)D status

We examined how disease characteristics related to log-transformed 25(OH)D levels among people with MS (Table 3). EDSS (Figure 1, panel A) and Scripps neurological rating scale were most strongly associated with 25(OH)D, with people with a higher disability having lower levels of 25(OH)D (Table 3). The use of immunotherapy was not associated with 25(OH)D. EDSS correlated strongly with other disease characteristics (duration of MS since first symptom, $r = 0.44$; MS Severity Score, $r = 0.83$; EDSS progression index, $r = 0.58$; type of MS, $r = 0.63$) and the associations between 25(OH)D and those characteristics were reduced or disap-

Table 2 The crude and deseasonalized prevalence of vitamin D deficiency and insufficiency in cases with multiple sclerosis and community controls (total sample and by gender)

Serum 25(OH)D level	Total sample			Females			Males		
	n	%	cum %	n	%	cum %	n	%	cum %
A. Crude data									
MS cases									
0–25 nmol/l*	16	11.8	11.8	11	12.0	12.0	5	11.4	11.4
26–40 nmol/l**	30	22.1	33.9	25	27.2	39.2	5	11.4	22.8
41–50 nmol/l**	20	14.7	48.6	11	12.0	51.2	9	20.5	43.3
>50 nmol/l	70	51.5	100	45	48.9	100	25	56.8	100
Community controls									
0–25 nmol/l*	23	8.8	8.8	15	8.6	8.6	8	9.1	9.1
26–40 nmol/l**	38	14.5	23.3	30	17.2	25.8	8	9.1	18.2
41–50 nmol/l**	66	25.3	48.6	44	25.3	51.1	22	25.0	43.2
>50 nmol/l	135	51.5	100	85	48.9	100	50	56.8	100
B. Deseasonalized data									
MS cases									
0–25 nmol/l*	10	7.4	7.4	8	8.7	8.7	2	4.5	4.5
26–40 nmol/l**	35	25.7	33.1	26	28.3	37.0	9	20.5	25.0
41–50 nmol/l**	24	17.6	50.7	15	16.3	53.3	9	20.5	45.5
>50 nmol/l	67	49.3	100	43	46.7	100	24	54.5	100
Community controls									
0–25 nmol/l*	16	6.1	6.1	10	5.7	5.7	6	6.9	6.9
26–40 nmol/l**	50	19.2	25.3	39	22.4	28.1	11	12.6	19.5
41–50 nmol/l**	56	21.5	46.8	41	23.6	51.7	15	17.2	36.7
>50 nmol/l	139	53.3	100	84	48.3	100	55	63.2	100

* Vitamin D deficiency; ** Vitamin D insufficiency

Table 3 Disease characteristics associated with 25(OH)D * status among MS cases

	Unadjusted		Adjusted for EDSS	
	r	p-value	r	p-value
EDSS (0–9)	–0.44	<0.01		
Scripps Neurological Rating Scale (15–100)	0.45	<0.01	0.13	0.15
Duration of MS since first symptom (1–40 yrs)	–0.27	<0.01	–0.10	0.28
Duration of MS since first symptom (0–5 yrs, > 5 yrs)	–0.27	<0.01	–0.19	0.03
MS Severity Score (0–10)	–0.29	<0.01	0.16	0.07
EDSS progression index (0–0.9)	–0.24	0.01	–0.02	0.85
Type of MS (1–3) [#]	–0.34	<0.01	–0.12	0.18
Immunomodulatory treatment (0, 1) [#]	0.12	0.15	0.04	0.64

r = correlation coefficient

* The 25(OH)D levels were deseasonalized and log-transformed

[#] Type of MS: 1 = relapsing remitting, 2 = secondary progressive, 3 = primary progressive; Immunomodulatory treatment: 0 = No, 1 = Yes

peared after adjustment for EDSS (Table 3), indicating that most of the association of these variables with 25(OH)D was explained by the association of EDSS with 25(OH)D. By contrast, the associations between 25(OH)D and EDSS were little changed by adjusting for the other disease characteristics (adjusted for: duration of MS since first symptom, $r = -0.38$ ($p < 0.0001$); MS Severity Score, $r = -0.38$ ($p < 0.0001$); EDSS progression index, $r = -0.38$ ($p < 0.0001$); type of MS, $r = -0.30$ ($p < 0.0001$)). The EDSS and Scripps were closely related with a strong correlation between the two factors ($r = -0.91$), and the correlation between 25(OH)D and EDSS adjusted for Scripps ($r = -0.09$, $p = 0.30$) was similar to the correlation between 25(OH)D and Scripps adjusted for EDSS ($r = 0.13$, $p = 0.15$) (Ta-

ble 3). Importantly, higher disability, measured by a higher EDSS or lower Scripps score, were also strongly associated with lower levels of recent sun exposure (EDSS: $r = -0.39$, $p < 0.0001$ (Figure 1, panel B); Scripps: $r = 0.37$, $p < 0.0001$).

■ Seasonal variation

To explore the seasonal variation in the levels of 25(OH)D, we fitted a sinusoidal model to 25(OH)D levels and the month the serum sample was taken. We observed a significant seasonal pattern ($p < 0.001$) among cases. The maximum fitted value (63.7 nmol/l) was reached in late February, and the minimum fitted value (36.2 nmol/l) was reached in

Fig. 1 Higher disability, measured by EDSS score, among people with MS is associated with lower serum 25(OH)D levels (Panel A), and reduced summer sun exposure in the last year on leisure days (Panel B)

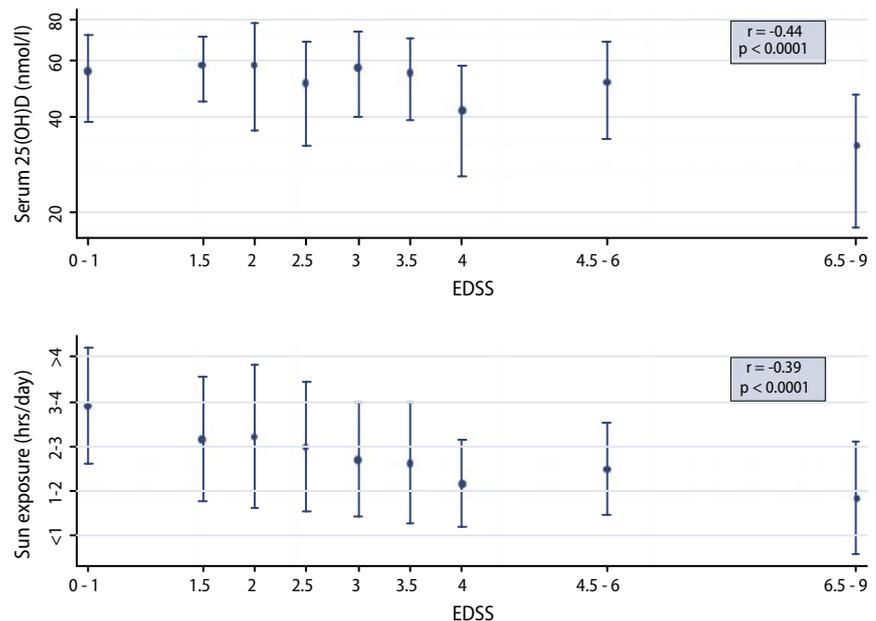


Table 4 Factors associated with 25(OH)D status* among MS cases and controls

Factor	Correlation coefficient (p-value)		
	MS cases		Controls
	High EDSS(> 3) n = 56	Low EDSS(≤ 3) n = 78	n = 262
Age (years)	−0.34 (0.01)	−0.07 (0.54)	−0.06 (0.37)
Female	−0.22 (0.10)	−0.07 (0.54)	−0.06 (0.32)
<u>Measures related to recent sun exposure</u>			
Time in the sun in <u>summer</u> on leisure days in the last 3 years (5 levels)	0.39 (< 0.01)	0.07 (0.51)	0.16 (0.01)
Time in the sun in <u>winter</u> on leisure days in the last 3 years (5 levels)	0.35 (< 0.01)	0.07 (0.51)	0.14 (0.03)
Time in the sun in <u>summer/winter</u> on leisure days in the last 3 years** (5 levels)	0.39 (< 0.01)	0.19 (0.09)	0.15 (0.02)
Time in the sun in <u>summer</u> on leisure days in the last year (C) (5 levels)	0.40 (< 0.01)	0.12 (0.28)	0.23 (< 0.01)
Time in the sun in <u>summer</u> during <u>work</u> hours in the last year (C)*** (4 levels)	−0.05 (0.84)	0.17 (0.25)	0.08 (0.27)
Use of sunscreen in the last 3 years (4 levels)	0.01 (0.96)	−0.15 (0.20)	−0.03 (0.59)
<u>Measures related to past sun exposure</u>			
Average time in the sun in summer on leisure days at age 6–15 yrs (5 levels)	0.23 (0.09)	−0.02 (0.86)	0.09 (0.15)
Average time in the sun in summer on leisure days prior to disease onset# (5 levels)	−0.07 (0.60)	0.26 (0.03)	0.04 (0.56)
Job for the longest period of life (3 levels)	0.11 (0.44)	0.18 (0.11)	0.09 (0.14)
Marked actinic damage (grade 3 to 6)	0.03 (0.95)	−0.04 (0.76)	0.11 (0.11)
<u>Skin type related factors (from light to darker complexion)</u>			
Skin melanin density at upper inner arm, %	0.40 (< 0.01)	0.28 (0.01)	0.16 (0.01)
Skin melanin density at buttock, %	0.19 (0.24)	0.23 (0.05)	0.10 (0.15)
Skin color by nurse observation (4 levels)	0.11 (0.42)	0.21 (0.06)	0.14 (0.02)
Ability to tan (4 levels)	0.09 (0.51)	0.24 (0.03)	0.14 (0.02)
Tendency to burn (3 levels)	0.15 (0.27)	0.02 (0.86)	0.06 (0.35)
Hair color (6 levels)	0.07 (0.60)	−0.04 (0.65)	−0.07 (0.25)
Lifetime severe sunburns (5 levels)	−0.06 (0.63)	0.01 (0.90)	−0.03 (0.59)
Number of naevi > 5 mm on left arm (3 levels)	−0.17 (0.24)	0.03 (0.78)	0.05 (0.38)
<u>Dietary intake in the last 12 months</u>			
Vitamin D supplementation (No/Yes)	−0.03 (0.84)	0.08 (0.48)	−0.02 (0.74)
Fish consumption (times per month)	0.22 (0.10)	0.13 (0.24)	0.05 (0.42)
Milk consumption (4 levels)#	−0.11 (0.42)	−0.01 (0.96)	−0.05 (0.41)
Egg consumption (eggs per month)	−0.06 (0.66)	0.11 (0.32)	−0.004 (0.95)
Meat consumption (times per month)	−0.01 (0.92)	−0.04 (0.76)	0.09 (0.16)

* The 25(OH)D levels were deseasonalized and log-transformed

** For subjects who had their serum sample taken in the summer and autumn months, sun exposure in summer was used and for subjects who had their serum sample taken in the winter and spring months, sun exposure in winter was used.*** Among those who worked (cases high EDSS n = 18, cases low EDSS n = 46, controls n = 185)

For controls, the age of onset was used of the case that they were matched to.## None of the common milk brands in Tasmania are fortified with vitamin D. (C) Measure obtained from the lifetime calendar

late August. The amplitude of this fitted model, half this range, was 13.7 nmol/l. When we compared the seasonality among cases with a higher EDSS score (> 3) with those with a low EDSS score, we noticed a weaker seasonality among those with a higher EDSS score (amplitude 10.3, $p = 0.04$, vs amplitude 18.2, $p < 0.001$ for low EDSS, test for interaction $p = 0.15$). This finding seems to be in line with a more constant sun exposure behavior among cases with a high EDSS score. Among cases with a higher EDSS score, 73.2% reported a similar amount of sun in summer and winter, while 21.4% reported more sun in summer and 5.4% more sun in winter. Among cases with a low EDSS score, only 46.2% reported a similar amount of sun in summer and winter, while 35.9% reported more in summer and 17.9% more in winter. The patterns for controls were similar to that of the cases with a low EDSS (data not shown).

■ Factors associated with 25(OH)D status among cases with MS and controls

We next examined which factors were associated with log-transformed 25(OH)D levels among cases with a higher EDSS score, cases with a low EDSS score, and controls (Table 4). Recent sun exposure during leisure time was strongly associated with vitamin D status among cases with a higher EDSS score, and moderately associated among controls. For cases with a low EDSS score, there was only a moderate association with recent sun exposure during leisure time when we aligned the season that the vitamin D sample was taken with the appropriate sun exposure question (summer or winter). Differences in effect for males and females might partly explain the weaker associations between recent sun exposure and vitamin D status among cases with a low EDSS score. We found a strong positive correlation between vitamin D status

and recent sun exposure during leisure time for females with a low EDSS score ($r = 0.26$, $p = 0.05$), while no association was found for males with a low EDSS score ($r = 0.01$, $p = .98$, test for interaction $p = 0.35$). For males with a low EDSS score, sun exposure during work seemed much more important (males: $r = 0.62$, $p = 0.03$; females: $r = -0.03$, $p = 0.87$, test for interaction $p = 0.03$). In all groups, melanin density (at the upper inner arm and buttock) or darker skin color was positively associated with 25(OH)D (Table 4), and these associations persisted after adjustment for recent sun exposure and use of sunscreen/clothing. In this sample, a weak non-significant association was observed between sun exposure and melanin or skin type for cases ($r = 0.11$, $p = 0.21$ for melanin at upper inner arm), and no association for controls ($r = 0.01$, $p = 0.87$). Age was negatively associated with 25(OH)D among cases with a higher EDSS, but not among cases with a low EDSS or controls. Part of the association was explained by EDSS ($r = -0.25$, $p = 0.06$ after adjustment for EDSS). Vitamin D supplementation was used by 15.4% (12/78) of cases with a low EDSS, 7.1% (4/56) of cases with a higher EDSS and 8.1% (22/272) of the controls and was not found to be associated with 25(OH)D. Fish consumption was associated, although not significantly, with 25(OH)D status among people with a higher EDSS, but not among those with a low EDSS or controls. The following factors, examined but not reported in Table 4, did not relate to 25(OH)D status: - season of birth (Jan-March, April-June, July-Sept, Oct-Dec), history of being breastfed as an infant, consumption of fish, milk, eggs or vitamin D supplements at age 10–15 years, level of education, and whether or not subjects currently smoked tobacco. The main factors associated with 25(OH)D were not dependent on the method of using deseasonalized

25(OH)D values. For example, the correlations between non-deseasonalized 25(OH)D and recent time in the sun in summer was 0.31 ($p = 0.02$) for cases with a high disability and 0.22 ($p < 0.01$) for controls, and the correlations between non-deseasonalized 25(OH)D and melanin density at the upper inner arm was 0.39 ($p < 0.01$) for cases with a high disability and 0.24 ($p < 0.01$) for controls.

■ Comparison of vitamin D status in MS cases and age- and sex-matched controls

Compared with those with 25(OH)D levels > 40 nmol/l, having lower levels of 25(OH)D was moderately associated with MS (OR 1.62 (0.96, 2.74) after adjustment for the month the serum sample was taken). When we stratified by EDSS score, we observed a strong positive association among MS cases (and their matched controls) with a higher EDSS, while no or at best a weak negative association among those with a low EDSS (test for interaction, $p = 0.04$) (Table 5). Thus, the risk of becoming more vitamin D insufficient or deficient than controls seems to occur among people with MS that have a higher disability.

After adjustment for amount of recent sun exposure in cases with a higher EDSS compared with their matched controls, the odds ratio for low 25(OH)D decreased from 3.07 (1.37, 6.90) to 2.44 (0.98, 6.04), and an additional adjustment for winter sun exposure in the last 3 years reduced the odds ratio to 2.13 (0.84, 5.37). This indicates that a material part (45%) of the association between low 25(OH)D status and MS appears to be due to the fact that cases with a higher disability had had less recent sun exposure. Additional adjustments for the use of sunscreen, melanin density at the upper inner arm and fish intake did not change the association (odds ratio 2.13 (0.54, 8.46)).

Table 5 The distribution of serum 25(OH)D for MS cases and controls with associated odds ratios and 95% confidence intervals by EDSS score

Serum 25(OH)D level	Case EDSS score > 3			Case EDSS score ≤ 3		
	Cases n (%)	Controls n (%)	Adjusted Odds ratio* (95% CI)	Cases n (%)	Controls n (%)	Adjusted Odds Ratio* (95% CI)
>60 nmol/l	11 (19.6)	28 (25.7)	1	27 (34.6)	50 (33.6)	1
51–60 nmol/l	9 (16.1)	25 (22.9)	0.85 (0.26, 2.73)	23 (29.5)	31 (20.8)	1.36 (0.64, 2.92)
41–50 nmol/l	12 (21.4)	32 (29.4)	0.93 (0.36, 2.43)	7 (9.0)	33 (22.1)	0.40 (0.16, 1.04)
26–40 nmol/l	15 (26.8)	14 (12.8)	2.86 (0.90, 9.15)	15 (19.2)	23 (15.4)	1.13 (0.45, 2.85)
0–25 nmol/l	9 (16.1)	10 (9.2)	2.89 (0.79, 10.58)	6 (7.7)	12 (8.1)	0.80 (0.27, 2.65)
Linear trend			$p = 0.04$			$p = 0.45$
Test for interaction with EDSS				$p = 0.04$		
Dichotomized						
> 40 nmol/l	32 (57.1)	85 (78.0)	1	57 (73.1)	114 (76.5)	1
≤ 40.00 nmol/l	24 (42.9)	24 (22.0)	3.07 (1.34, 7.01)	21 (26.9)	35 (23.5)	1.12 (0.55, 2.29)
Test for interaction with EDSS				$p = 0.07$		

* Matched odds ratios adjusted for the month the serum sample was taken using a seasonal term

Discussion

In this population-based sample of people with MS under the age of 60 years living at latitudes 41–43°S, more than half had vitamin D insufficiency (serum 25(OH)D: 50.7% \leq 50 nmol/l). Increasing disability was strongly associated with lower levels of 25(OH)D and with lower levels of sun exposure. People with a higher disability also had more often low levels of vitamin D (\leq 40 nmol/l) compared with their age and sex matched controls, while people with a low disability had similar levels of vitamin D compared with controls. Nearly half of the excess risk of low vitamin D among people with a higher disability compared with controls was statistically attributable to the lower sun exposure.

One of the strengths of this study is that it is a population-based sample rather than a hospital sample, where patients generally tend to have a higher disability and longer disease duration. It is conceivable that we have missed some people with a high disability, although many would have been older than 60 years and would therefore fall outside the study source population. Even though the reliability of self-reported sun exposure was similar for cases and controls, the measures of self-reported sun exposure are likely to have more random misclassification than does serum 25(OH)D. Therefore, the potential contribution of recent sun exposure to the excess of low 25(OH)D status among disabled cases may even be higher than estimated in this study. This study could not examine vitamin D status as a causal factor of MS, because prevalent, not incident, cases were studied, and disease related changes in outdoor behavior would have influenced vitamin D status.

We found a high prevalence of vitamin D insufficiency in this population-based sample with MS, particularly among those with a higher disability. This is in line with the higher prevalence of vitamin D insufficiency that was found among (more disabled) women with MS recruited from a tertiary care hospital in New York (latitude 40°N) (69% $<$ 50 nmol/l, mean EDSS score 6.9) [29]. Our study, indeed, indicates that disability was strongly associated with 25(OH)D levels. In addition, disability was strongly associated with reduced sun exposure, and sun exposure levels explained nearly half of the excess risk of low vitamin D found among cases with a higher disability compared with age- and sex-matched controls. Thus, increasing disability may reduce outdoor behavior and sun exposure and, in turn, increase the risk of vitamin D insufficiency. Prospective studies are required to examine whether low vitamin D among higher disability cases reflects not only disease related changes in outdoor behavior (influencing vitamin D status) but also a direct effect of low vitamin D on disability.

Age and sex matched community controls also had a concerning high prevalence of vitamin D insufficiency (46.8% \leq 50 nmol/l) with more than half of those with insufficiency having levels \leq 40 nmol/l. Again, recent sun exposure was a strongly associated with serum 25(OH)D.

The findings of a large environmental influence (sun exposure) on case vitamin D status and similar (albeit low) levels of vitamin D among low disability cases and controls indicates that people with MS are unlikely to have a low 25(OH)D status that is predominantly genetically determined or is the result of having had the initial MS onset. However, the bio-availability of the active hormone, 1,25(OH)₂D₃, will still depend on constitutional differences such as allelic variation in the vitamin D receptor (VDR) gene. Allelic VDR status has been associated with MS in a Japanese [16, 30] but not in a Canadian population [39]. Here, in this Caucasian population, cases and controls more often had low vitamin D when they had a fairer skin type. This finding is, however, in contrast with the observation that fairer-skinned people absorb more UVR for a given level of ambient UVR compared with darker-skinned people [21], leaving them less prone to vitamin D deficiency [18]. While fairer-skinned people tend to avoid the sun compared with darker-skinned people [12], this association was not observed in our sample. As a result, the associations between vitamin D and skin type did not reduce after adjustment for sun exposure and use of sunscreen and clothing. Also, sun avoidance behavior is difficult to measure precisely and we can not rule out residual confounding.

We also observed that there is less seasonal variation among those with a higher disability, likely to be the result of a more constant sun exposure behavior. This is in agreement with Nieves et al. who found no seasonality in serum 25(OH)D in their sample of women with a relatively high level of disability [29]. People with a higher disability might not increase their sun exposure in summer as the heat of the sun may exacerbate their symptoms. Heat intolerance is a well-recognized feature of MS, which could lead to an avoidance of the sun. In a recent survey, the high correlation between people with MS reporting that high temperatures made their MS worse and that sun exposure made their MS worse may be related to solar heat [36]. Again, prospective data are required to unravel the nature of the association between sun exposure and heat intolerance. A study on fatigue showed that people with a higher disability are more often fatigued, and that heat makes the symptoms of fatigue worse, which occurred more often in those with more severe fatigue [1]. Thus, in people with MS, solar light is likely to be of clinical benefit, while solar heat may have adverse effects on MS symptoms.

In general, most vitamin D is produced through exposure to UVR, while the contribution of dietary vitamin D is low [18]. This might be the reason why vitamin D supplements or foods containing vitamin D did not predict 25(OH)D levels in controls and cases with a low disability. In cases with a higher disability, where 25(OH)D status and sun exposure levels were particularly low, we did find a positive association between fish intake and serum 25(OH)D. Additional reasons for not finding an association between vitamin supplementation and serum 25(OH)D status could include measurement error, insufficient use or dosage to produce a substantial effect, and the use of vitamin D₂ in Australia which seems not as effective as vitamin D₃ [41]. The low influence of vitamin D supplementation on 25(OH)D status highlights previous recommendations [19] that clinicians should not merely recommend vitamin D supplementation, but rather should monitor and treat vitamin D insufficiency until adequate vitamin D levels are achieved. Therapeutic options which increase the amount of exposure to UVR while avoiding adverse heat-related effects should be considered as well as oral vitamin D supplementation.

There is substantial evidence that vitamin D insufficiency increases the risk of falls and fractures [3, 15]. This seems to apply to MS, as a lower bone mass and an increased number of fractures has been observed in MS patients compared with age- and sex-matched controls [9, 29]. Therefore, preventing vitamin D insufficiency among people with MS is warranted, even if the possible adverse effects of

vitamin D insufficiency on MS progression are not conclusive. Here, the cross-sectional design was not appropriate to examine MS progression. A longitudinal study with serial vitamin D and progression measurements or a randomized clinical trial would be the preferred epidemiological designs to answer whether sun exposure or vitamin D status might influence MS progression. In a recent paper, vitamin D status has also been found to be associated with neuropsychological function [22].

In conclusion, a high prevalence of vitamin D insufficiency was found in this population-based sample of people with MS. People with a higher disability seemed especially prone to insufficient levels of vitamin D, probably as a result of low levels sun exposure. Active detection of vitamin D insufficiency among people with MS and intervention to restore vitamin D status to adequate levels should be considered as part of the clinical management of MS.

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