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Multiple sclerosis patients have a diminished serologic response to vitamin D supplementation compared to healthy controls

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Abstract

Background—Vitamin D insufficiency is a risk factor for MS, and patients don't always show the expected response to vitamin D supplementation.

Objective—To determine if vitamin D supplementation leads to a similar increase in serum 25-hydroxyvitamin-D (25(OH)D) levels in MS patients and healthy controls (HCs).

Methods—Participants in this open-label study were female, white, aged 18–60 years, had 25(OH)D levels ≤ 75 nmol/L at screening, and had RRMS or were HCs. Participants received 5,000 IU/day of vitamin D_3 for 90 days. Utilizing generalized estimating equations we examined the relationship between the primary outcome (serum 25(OH)D level) and the primary (MS versus HC status) and secondary predictors.

Results—27 MS patients and 30 HCs were enrolled. There was no significant difference in baseline 25(OH)D level or demographics except for higher body mass index (BMI) in the MS group (25.3 vs 23.6 kg/m^2, p=0.035). 24 MS subjects and 29 HCs completed the study. In a

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Conflict of interest statement

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multivariate model accounting for BMI, medication adherence, and oral contraceptive use, MS patients had a 16.7 nmol/L (95%CI: 4.2, 29.2, p=0.008) lower increase in 25(OH)D levels compared to HCs.

Conclusions—MS patients had a lower increase in 25(OH)D levels with supplementation, even after accounting for putative confounders.

Keywords

Multiple Sclerosis; Vitamin D supplementation; Pharmacokinetics; Nutrition

Introduction

Vitamin D insufficiency has been linked to increased risk of several autoimmune disorders, including multiple sclerosis (MS) [1]. Vitamin D is a potent immunomodulator and impacts almost all aspects of the immune system [2]. In an animal model of MS, vitamin D reduced the severity of the disease, which may be linked to its ability to reduce the number of Th1 and Th17 cells [3],[4]. Studies suggest that higher levels of serum 25-hydroxyvitamin D are associated with lower disease activity [5],[6]. A prior report showed difficulty in obtaining adequate serum 25-hydroxyvitamin D (25(OH)D) levels with supplementation in a cohort of MS patients [7], raising the question of whether vitamin D pharmacokinetics differ between patients with MS and healthy individuals. This hypothesis is appealing given that polymorphisms in some genes related to vitamin D metabolism have been associated with MS risk [8]–[11]. While many providers recommend vitamin D supplementation to patients with MS (while awaiting randomized controlled trial evidence) and several randomized controlled trials are now underway to assess the benefit of supplementation of vitamin D on disease activity in MS [12]–[14], the optimal dosing to achieve target serum levels of vitamin D has not yet been established in MS.

The objective of this study was to assess whether MS patients and healthy individuals have a similar change in serum 25(OH)D levels after a course of a fixed dose of vitamin D_3 supplementation. We also evaluated independent determinants of the response to vitamin D supplementation.

Methods

Study design

This was an open-label study in which all subjects received the same dose of oral vitamin D_3 for 90 days.

Standard protocol approvals, registrations and patient consents

This study was approved by the University of California, San Francisco (UCSF) and Johns Hopkins institutional review boards, and all participants provided written informed consent. The study was registered at clinicaltrials.gov (NCT01667796).

Participants

MS patients were recruited from the UCSF (screening started in November 2010) and Johns Hopkins (started in November 2011) MS centers, while healthy controls were recruited from friends and biologically unrelated family members in addition to the Johns Hopkins and UCSF communities; the final participant was screened in December 2013. Participants were recruited only during certain months (screening visits September 1 to March 1) to minimize the likelihood of subjects being exposed to very high ultraviolet index days. This was based on 2008 charts for San Francisco provided by the National Oceanic and Atmospheric Administration [15], as we hypothesized that there may be subtle, unreported discrepancies in sun exposure behaviors during high UV index periods due to avoidance by MS patients.

To limit inter-individual variability, participants enrolled in the study were white, non-Hispanic females who were aged 18 to 60 years with screening 25(OH)D level ≤ 75 nmol/L (30 ng/mL, conversion factor: 1 ng/ml=2.496 nmol/L) and screening body mass index (BMI) of 18–30 kg/m^2. Participants had to be willing to avoid tanning beds and additional multivitamins or cod liver oil for the duration of the study. Participants were not pregnant or nursing and had to be willing to avoid pregnancy for the study period. Individuals with known gastrointestinal disorders or who were on a non-fat diet were excluded to avoid the potential impact on vitamin D absorption. Those with a known history of kidney or liver disease, nephrolithiasis, hypercalcemia or hypercalciuria, hyperthyroidism, *Mycobacterium* infection, sarcoidosis or other serious chronic illness (cardiac disease, cancer, or HIV) were excluded. Use of heparin, low-molecular weight heparin, thiazide diuretics, digoxin, diltiazem, verapamil, cimetidine, or medication associated with malabsorption also made participants ineligible. Participants were likewise excluded if screening serum calcium >2.5 mmol/L (10 g/dL, conversion factor: 1 mg/dL=0.25 mmol/L) or screening hemoglobin was < 110 g/L (11.0 g/dL). Finally, subjects were excluded if in the month prior to screening, they had used illicit drugs, smoked cigarettes, or taken non-topical steroids. MS patients had relapsing-remitting disease by 2005 McDonald criteria [16] with Expanded Disability Status Scale scores ≤ 3.0 [17] and did not endorse significant sun avoidance; these criteria were chosen to minimize differences in vitamin D levels related to sun exposure behaviors. The initial inclusion criteria stipulated that patients were taking either no MS medication or glatiramer acetate (GA) in order to minimize heterogeneity associated with medications; however, due to slow recruitment, allowances were later made to include patients on interferon beta and later, to those receiving natalizumab.

Study visits

At the screening visit, participants provided written informed consent and then underwent screening procedures, including a physical (and, for MS patients, a neurologic) examination and serum 25(OH)D level. Participants who met inclusion/exclusion criteria were seen at a baseline visit within 10 days of screening and were provided with the study drug and a medication alarm watch. They also underwent blood collection and completed a Block 2005 Food Frequency Questionnaire [18], Block Dietary Fat Screener [19], Block Calcium/Vitamin D Screener [20], and a sun exposure questionnaire (which includes questions about sunscreen usage) [21]. Follow-up visits were conducted at the study mid-point and at the end

of the study and included blood collection, adherence assessment, Block Calcium/Vitamin D and Dietary Fat Screeners, and the sun exposure questionnaire.

Intervention

All participants were given 90 capsules (5,000 IU; 125 μg) (purchased from Tishcon Corporation, Westbury, NY) at the baseline study visit. Participants were asked to take one capsule every day with a meal.

Adherence and safety

Participants were provided with a medication alarm watch and were reminded of the importance of adhering to the study drug at each visit, as well as by phone contact at week 3. Adherence was expressed as a ratio of the amount of medication consumed divided by the duration of possession of the medication and was based on the pill counts at the mid-study and final visits.

Outcomes

The primary outcome of this study was the serum 25-hydroxyvitamin D level at the mid-study and final study visits. The primary predictor was disease status (MS versus HC). Secondary predictors included age, BMI, sun exposure, dietary vitamin D intake, dietary fat intake, adherence to study drug, and other concomitant medications. 25(OH)D levels were measured from all samples in a single batch at Heartland Assays, LLC (Ames, IA) using 0.3 cc of serum by liquid chromatography/mass spectrometry (LC/MS). The intra-assay coefficient of variation for this assay is 6.5%.

Statistical analysis

The sample size of the study was based on previous data that showed that a dose of 5,000 IU daily of vitamin D_3 led to an increase of 91.3 nmol/L with a standard deviation of 9.4 nmol/L [22]. To detect a difference of 10% between the two groups, with an alpha of 0.05 and power of 90%, the calculated sample size was 24 in each group. To account for potential drop-outs, a target sample size of 30 in each group was chosen. Baseline demographic characteristics were compared using a two-tailed t-test for normally distributed variables and a Mann-Whitney U-test for non-normally distributed variables. A chi-square test was used for categorical variables. Generalized estimating equations (GEE) with an autoregressive with lag one correlation matrix were used to compare the serially-measured serum 25(OH)D levels between MS patients and HCs to take into account repeated measures and within-subject correlations. GEE models assuming different correlation matrices, including exchangeable and unstructured covariance matrices, were also assessed. We included an interaction term between visit time and disease status, with both coded as categorical variables. Covariates that were considered included age, adherence, BMI, oral contraceptive (OCP) use, fat intake (per Block Dietary Fat Screener covering study period), sun exposure during study period, dietary vitamin D intake during study period, and month of enrollment (based on UV index – group 1: November, December, group 2: October, January, group 3: September, February, group 4: March) to determine if they altered the association between disease status and the serum 25(OH)D levels during the study. Each of these putative

covariates was evaluated for its association with 25(OH)D levels in univariate GEE models; those that were significantly associated were carried forward to the final “base” multivariate model in which the primary predictor was MS versus HC status. However, to assess if any of the other potential covariates impacted the results of the base model, we evaluated the effect of adding in each covariate one at a time; we also built a large model incorporating all of the potential confounders. We also utilized a mixed effects model with a random intercept and random slope with serum 25(OH)D as the dependent variable, and other covariates selected from the GEE method as described above, to corroborate our findings.

Since previous studies have suggested interactions between vitamin D levels and interferon-beta [23], we performed a sensitivity analysis excluding the MS patients on interferon-beta. Additionally, since the final 8 participants enrolled received a second batch of vitamin D_3 (the first had expired), we performed a sensitivity analysis excluding those patients to evaluate if the results were meaningfully different.

Results

Participants and characteristics

A total of 57 participants (30 HCs and 27 MS) were enrolled in the study, of whom 29 HCs and 24 MS subjects completed the study. A total of 108 participants (62 HCs and 46 MS) were screened for the study, of these 19 MS participants screened out of the study (serum 25(OH)D > 75 nmol/L – n=9, BMI>30 kg/m^2 – n=2, hemoglobin < 110 g/L – n=1, EDSS > 3.0 – n=3, calcium > 2.5 mmol/L –n=1, participant opted out–n=1, non-Caucasian – n=2) while 32 HCs were excluded (serum 25(OH)D > 75 nmol/L – n=24, BMI>30 kg/m^2 – n=3, hemoglobin < 110 g/L – n=2, calcium > 2.5 mmol/L – n=4, participant opted out –n=1). Table 1 lists the baseline characteristics of both groups; the only difference between the groups was that there was a slightly higher BMI in the MS subjects as compared to HCs.

During the course of the study, adherence was high; in MS patients, the median adherence was 96.9% (interquartile range: 94.5–100%), while in healthy controls (one of whom stopped the supplements), it was 98.9% (interquartile range: 96.8–100%). Of the subjects with MS that completed the study, 16 were on glatiramer acetate, four were on interferon beta-1a (two on the weekly intramuscular formulation and two on the three times weekly, subcutaneous version), one was on natalizumab, and three were not on a disease-modifying therapy.

Effect of vitamin D supplementation on serum 25(OH)D levels

Following vitamin D_3 supplementation, there was a significant increase in vitamin D levels in both groups. In the HC group, the change in 25(OH)D over the course of the study was 82.4 ± 5.2 nmol/L, which was significantly higher than the change in the MS group, which was 65.9 ± 5.9 nmol/L (mean ± SEM, two-tailed t-test: p=0.039) (Figure 1). Utilizing the univariate GEE model, a difference in change in 25(OH)D level of 21.9 nmol/L (95% CI: 8.5 to 35.4, p=0.001) was noted between the HC and MS group by the end of the study (Table 2). In univariate GEE models, the other covariates that independently predicted change in 25(OH)D levels included BMI, adherence to study medication and OCP use.

Following adjustment for these factors in the base model, the difference between the two groups was still significant: MS patients had 16.7 nmol/L (95% CI: 4.2 to 29.2, p=0.008) lower change in 25(OH)D level.

Utilizing the exchangeable or unstructured correlation matrix did not meaningfully alter the results. Adjusting one at a time or in a large model for other factors that could possibly affect the change in vitamin D levels, such as dietary fat intake, sun light exposure, month of enrollment, and dietary vitamin D intake, did not change the results meaningfully (Table 2). Utilizing a mixed effects model resulted in similar findings. In sensitivity analyses excluding MS patients on interferon, the change in 25-hydroxyvitamin D levels was 15.2 nmol/L lower in the MS group compared to HCs adjusting for BMI, adherence to study medication and OCP use (95% CI: 2.0 to 28.7 nmol/L, p=0.025). Excluding patients not on disease-modifying therapy, the change in 25(OH)D levels was 20.7 nmol/L lower in the MS group compared to the HCs, adjusting for BMI, adherence to study medication and OCP use (95% CI: 8.5 to 32.9 nmol/L, p=0.001). Similarly, while the confidence intervals widened, as expected, when the 8 participants who received the second batch of vitamin D_3 were removed, the direction of the association remained the same.

As in the univariate models, BMI (2.2 nmol/L less for every 1kg/m^2 greater, 95%CI: 0.3 to 4.2 nmol/L, p=0.03), adherence (1.0 nmol/L greater for every 1% increase, 95%CI: 0.5 to 1.5 nmol/L, p<0.001) and concomitant OCP use (19.0 nmol/L greater in those taking OCPs, 95%CI: 7.0 to 30.9 nmol/L, p=0.002) remained independent predictors of change in vitamin D status in the multivariate model.

Safety outcomes

There were no serious adverse events in either group. The serum calcium levels at mid-study (2.34 ± 0.08 mmol/L) and final (2.34 ± 0.08 mmol/L) visits were within normal limits. One HC discontinued vitamin D after developing palpitations with an unremarkable cardiac workup except for rare premature atrial and ventricular contractions; however, the person then reported a remote history of atrial fibrillation (that had not been disclosed at the screening visit). This was not deemed to be related to vitamin D supplementation.

Discussion

Compared to healthy individuals, participants with multiple sclerosis have a lesser average increase in serum 25(OH)D levels following oral vitamin D_3 supplementation despite having similar starting concentrations (which was expected since the study only included subjects who were insufficient in 25(OH)D). This difference in response persisted following adjustment for multiple putative confounders.

We demonstrate a difference in the response to vitamin D supplementation between MS patients and HCs that has relevance to clinical practice as well as trial design. First, for clinical practitioners who are recommending vitamin D supplementation to MS patients, it may be necessary to track the serum levels of 25(OH)D to document adequate response to supplementation rather than prescribing a fixed dose. This is furthermore reasonable since compared with the medication we used in this trial, patients may purchase and take vitamin

D from a variety of manufacturers. In the trial setting, our findings may translate to lower-than-expected increases in serum 25(OH)D levels, perhaps attenuating study power and thus, the ability to detect a difference between treatment groups.

We cannot definitively comment on the immunological or clinical effects of the difference in the rise of 25(OH)D levels between the two groups in this study. However, our group recently reported on the immunological effects of vitamin D supplementation in a cohort of patients with RRMS and demonstrated that when the change in the 25(OH)D level exceeded 45 nmol/L, each additional 12.5 nmol/L increase in vitamin D level was associated with a 1% absolute decrease in IL-17 producing CD4+ T cells [24]. Extrapolating from this study, we would expect to note 1.4 % lower absolute reduction in IL17 producing CD4+ T cells in the MS group compared to the controls when both were given 5,000 IU of vitamin D_3 daily. While such a change is of pathophysiological interest, the true clinical relevance of the lower rise of serum 25(OH)D must be assessed in the form of clinical trials that incorporate relapses and disability as clinical measures. It is not clear what the explanation for the discrepancy in vitamin D response is. One possibility may be a difference in gut absorption. Some studies have demonstrated alteration in gut motility in subjects with MS, which may lead to changes in absorption of various nutrients [25]. However, other studies have shown no abnormalities in the absorption of dietary fat in MS patients [26]. Another possibility may be the alteration of the gut microbiota in patients with MS. Changes in the microbiota can alter bile acid metabolism [27], ultimately impacting the absorption of fat-soluble vitamins such as vitamin D [28]. As more information emerges about the alterations in the microbiome in MS it may help lend credence to this theory. Studies have shown that polymorphisms in genes related to vitamin D metabolism are also related to the risk for developing MS[8]–[10], although other studies have not replicated some of these relationships[29]. It is not clear if these polymorphisms underlie the findings noted in our study.

The other factors that appeared to influence the change in serum 25(OH)D levels in our study included BMI, adherence to study drug, and OCP use. BMI has been linked to 25(OH)D levels, as well as to the response to oral vitamin D supplementation [30],[31]. Previous studies, as well as our results, suggest that subjects with a higher BMI have lower increase in vitamin D levels and may require higher vitamin D dosing [30]–[32]. Improved adherence, as expected, led to a better response to supplementation, and patients being supplemented with vitamin D who do not have an adequate response in 25(OH)D levels should be questioned about adherence prior to modifying vitamin D dosage. There appeared to be a significantly greater increase in serum 25(OH)D levels in subjects on OCPs. This seems consistent with the fact that previous non-MS studies have shown that women on OCPs had higher serum levels of 25(OH)D as well as higher levels of 1,25-dihydroxyvitamin D [33]–[35]. Thus, the concomitant use of OCPs or hormone replacement therapy may need to be taken into account when interpreting changes in serum 25(OH)D levels.

We also adjusted for other factors that could significantly affect vitamin D levels, such as fat intake, sun exposure and dietary vitamin D intake, utilizing standardized questionnaires as well as month of year enrolled. In this study, none of these factors was significantly

associated with change in vitamin D levels. It is important to note that the current subjects may differ from the source population since they were enrolled only during specific times of the year, did not avoid the sun substantially and were consuming a diet with at least some fat in it. Other factors that are determinants of serum 25(OH)D levels, such as race and ethnicity, were controlled for by the study's strict enrollment criteria.

The study does have some limitations. The fact that only female non-Hispanic Caucasians were included in this study, which was stipulated so as to reduce heterogeneity that might have clouded the results due to the sample size, means the generalizability of the findings of this study has to be further tested. In addition, the sole use of pill count as a measure of adherence may not have been completely accurate. Though only including MS patients not on disease-modifying therapies would have been ideal, that decision may have biased the sample in that individuals not receiving treatment for their MS may be different than those who do. A minor limitation of this study was the use of serum calcium levels as a safety outcome, as a rise in urinary calcium excretion is seen prior to a rise in serum calcium levels; urinary calcium thus might have been a more useful measure. Participant may have been using alternative or complimentary medications that could have unknown effects on vitamin D metabolism. Although very few participants reported using vitamin D supplements at the time of the screening visit, it is possible that the results may have been impacted if MS patients and controls differentially utilized vitamin D supplementation prior to the screening visit. We hope to replicate the findings in a future study and to conduct genotyping of the subjects to assess if variants in vitamin D metabolism genes, particularly those already associated with MS, can account for the findings.

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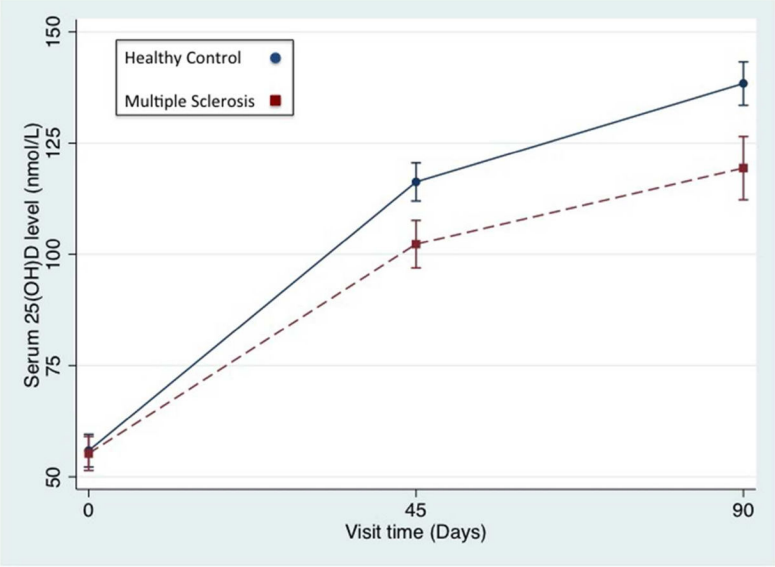

Figure 1. Serial serum 25(OH)D levels by group (MS or HC)

The figure displays the serum 25(OH)D levels in the two groups over the course of the study. The error bars represent the standard error of the mean (SEM) Though both groups begin at the same 25(OH)D level at the first study visit, clear separation is noted between the groups at the final study visit.

Table 1

Baseline characteristics of enrolled subjects

Characteristic*		Multiple sclerosis (n=27)	Healthy controls (n=30)	p-value
Age (years)		40.2 ± 9.2	37.9 ± 12.1	0.44
Body mass index (kg/m^2)		25.3 ± 2.9	23.6 ± 2.9	0.03
Serum 25-hydroxyvitamin D (nmol/L)		55.2 ± 19.7	55.9 ± 20.0	0.91
Calcium (mmol/L)		3.7 ± 0.1	3.8 ± 0.1	0.14
Oral contraceptive use, n (%)		4 (14.8)	9 (30)	0.17
Dietary vitamin D intake (IU/day)		117.8 ± 184	77.3 ± 97	0.31
Dietary calcium intake (mg/day)		340.3 ± 221.5	417.3 ± 235.3	0.20
Total daily fat intake (grams)		85.2 ± 17.4	86.4 ± 14.3	0.79
Sun exposure (hours per week)		12 ± 10.6	9.3 ± 5.8	0.23
Sunscreen use, n (%)	Seldom or never	10 (37)	17 (57)	0.28
	Occasionally or Usually	17 (63)	13 (43)	

* presented as mean ± standard deviation unless otherwise noted

Table 2

Predictors of change in serum 25(OH)D levels

Variable*	**Univariate model**	**Base model**	**Base model + age, fat intake, sun exposure, dietary vitamin D, month of enrollment**
MS versus healthy control	−21.9 (−35.4, −8.5) p=0.001	−16.7 (−29.2, −4.2) p=0.008	−17.0 (−29.2, −5.0) p=0.006
BMI (per kg/m^2 greater)		−1.7 (−3.5, 0.02) p=0.05	−2.2 (−4.2, −0.3) p=0.03
Adherence (per 1% greater)		0.7 (0.5, 1.2) p<0.001	1.0 (0.5, 1.5) p<0.001
Oral contraceptive use		14.7 (3.0, 26.7) p=0.02	19.0 (7.0, 30.9) p=0.002
Age (per 5 years greater)			2.2 (−0.2, 4.7) p=0.06
Fat intake (per 1 point greater)			−0.2 (−1.0, 0.7) p=0.76
Sun exposure (per 5 hours greater)			0.07 (−3.5, 3.7) p=0.97
Dietary vitamin D intake (per 100 IU greater)			1.5 (−2.2, 5.0) p=0.43
Month of enrollment (per unit change in group, based on UV index)			3.0 (−2.2, 8.2) p=0.27

*Presented as mean (95% confidence intervals), all values in nmol/L