Serum 25-Hydroxyvitamin D Levels and Risk of Multiple Sclerosis

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Context Epidemiological and experimental evidence suggests that high levels of vitamin D, a potent immunomodulator, may decrease the risk of multiple sclerosis. There are no prospective studies addressing this hypothesis.

Objective To examine whether levels of 25-hydroxyvitamin D are associated with risk of multiple sclerosis.

Design, Setting, and Participants Prospective, nested case-control study among more than 7 million US military personnel who have serum samples stored in the Department of Defense Serum Repository. Multiple sclerosis cases were identified through Army and Navy physical disability databases for 1992 through 2004, and diagnoses were confirmed by medical record review. Each case (n=257) was matched to 2 controls by age, sex, race/ethnicity, and dates of blood collection. Vitamin D status was estimated by averaging 25-hydroxyvitamin D levels of 2 or more serum samples collected before the date of initial multiple sclerosis symptoms.

Main Outcome Measures Odds ratios of multiple sclerosis associated with continuous or categorical levels (quantiles or a priori–defined categories) of serum 25-hydroxyvitamin D within each racial/ethnic group.

Results Among whites (148 cases, 296 controls), the risk of multiple sclerosis significantly decreased with increasing levels of 25-hydroxyvitamin D (odds ratio [OR] for a 50-nmol/L increase in 25-hydroxyvitamin D, 0.59; 95% confidence interval, 0.36-0.97). In categorical analyses using the lowest quintile (<63.3 nmol/L) as the reference, the ORs for each subsequent quintile were 0.57, 0.57, 0.74, and 0.38 (P=.02 for trend across quintiles). Only the OR for the highest quintile, corresponding to 25-hydroxyvitamin D levels higher than 99.1 nmol/L, was significantly different from 1.00 (OR, 0.38; 95% confidence interval, 0.19-0.75; P=.006). The inverse relation with multiple sclerosis risk was particularly strong for 25-hydroxyvitamin D levels measured before age 20 years. Among blacks and Hispanics (109 cases, 218 controls), who had lower 25-hydroxyvitamin D levels than whites, no significant associations between vitamin D and multiple sclerosis risk were found.

Conclusion The results of our study suggest that high circulating levels of vitamin D are associated with a lower risk of multiple sclerosis.

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MULTIPLE SCLEROSIS (MS) IS among the most common neurological diseases in young adults, affecting 350 000 individuals in the United States and 2 million worldwide.[1] Prevailing thought is that MS is an autoimmune disorder whereby an unknown agent or agents triggers a T cell–mediated inflammatory attack, causing demyelination of central nervous system tissue.[2]

A striking feature of the global distribution of MS is a multifold increase in incidence with increasing latitude, both north and south of the equator.[3] Genetic predisposition contributes to this variation,[4] but the change in MS risk with migration among people of common ancestry[5] strongly supports a role for environmental factors. One potential factor may be vitamin D,[6-9] a potent immunomodulator that in its hormonal form can prevent experimental autoimmune encephalomyelitis (EAE), an animal model of MS.[10] Because food provides little vitamin D, the major source for most people is through skin exposure to sunlight.[11] At latitudes of 42° or more (eg, Boston, Mass), in winter most UV-B radiation is absorbed by the atmosphere, and even prolonged sun exposure is insufficient to generate vitamin D.[12] As a result, seasonal vitamin D deficiency is common.[11]

A protective effect of vitamin D on MS is supported by the reduced MS risk associated with sun exposure[13,14] and use of vitamin D supplements,[15] but evidence remains inconclusive. In the present study, we examined prospectively for the first time whether high blood levels of 25-hydroxyvitamin D, a good marker of vitamin D availability to tissues,[11] predict a lower risk of MS.

See also Patient Page.

METHODS

This study has been approved by the institutional review boards of the Harvard School of Public Health and the Walter Reed Army Institute of Research, both of which waived the need for informed consent to use

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archived blood products and medical records.

Study Population

The study population includes more than 7 million active-duty US military personnel who have at least 1 serum sample stored in the Department of Defense Serum Repository (DoDSR). Since 1985, the DoDSR has collected and stored more than 30 million serum samples leftover from routine human immunodeficiency virus and worldwide deployment-related blood tests.[16] Personnel generally provide 1 sample at entry into the military and, on average, every 2 years thereafter. All samples are cataloged and stored at –30°C.[16]

Case and Control Ascertainment

Multiple sclerosis case ascertainment within the military has been previously described.[17] Briefly, active-duty personnel in the US Army and the US Navy (which includes the Marines) who were evaluated by their respective Physical Evaluation Boards for a diagnosis of MS between 1993 and 2004 (Army) or 1992 and 2004 (Navy) were identified by searching the Physical Evaluation Boards' databases for members with the Veterans Administration Schedule for Rating Disabilities code for MS (code 8018). This search identified 515 potential MS cases. Medical records of the potential cases were reviewed and abstracted by 2 trained study personnel.

Cases included in this study were classified as either definite or probable MS. A case was definite if the final diagnosis in the medical record was made by a neurologist and specified as definite, clinically definite, or laboratory-supported definite MS,[18] or if there was a history of 2 or more neurological attacks, a magnetic resonance imaging result consistent with MS, and a diagnosis of MS made by a neurologist. A case was considered probable if there was a neurologist's diagnosis of probable, clinically probable, or laboratory-supported probable MS[18] or at least 2 of the following: clinical history of 2 or more attacks, magnetic resonance imaging findings consistent with MS, and a diagnosis of MS made by a neurologist. Of the 515 cases reviewed, 315 had definite (n=237) or probable (n=78) MS and had at least 1 serum sample collected prior to their date of onset (the date of first neurological symptoms attributable to MS noted in the medical record)[19]; 83 of these 315 cases were included in our previous study on Epstein-Barr virus (EBV) and MS among Army personnel.[17] For each case, we obtained up to 4 serum samples: 3 before the date of onset (the earliest and latest available, as well as a third sample collected between those 2) and 1 after the date of MS onset (the earliest available).

Controls were randomly selected from the DoDSR population, and 2 controls were matched to each case by age (±1 year), sex, race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other), dates of sample collection (±30 days, except for the sample collected after the date of MS onset), and branch of military service (Army, Navy, or Marines). Controls had to be on active duty on the date of onset of the matched case. Appropriate controls could not be found for 10 cases, which were therefore excluded from further analysis. Race/ethnicity status was provided by the Army Medical Surveillance Activity, based on categories defined by the Department of Defense independent from the investigators. Cases and controls were matched on race/ethnicity to control for confounding—blacks have a lower risk of MS than whites, likely because of genetic factors,[20] and lower 25-hydroxyvitamin D levels, mostly because of darker skin pigmentation, which decreases UV-B–induced subcutaneous production of vitamin D.[21]

Covariates

In addition to the matching factors, information was collected on latitude of place of residence at time of entry into the military. As in previous studies, latitude was attributed as follows: northern latitudes were states approximately 41° to 42° latitude or higher; middle latitudes, states between 37° and 41° latitude; southern latitudes, states approximately 37° latitude or lower; and outside of the continental United States (including Alaska, Hawaii, and Puerto Rico).[22] We also created a UV index variable from the state of residence at entry into the military using the average UV index by state for 1995 (the earliest available year) from the National Oceanic and Atmospheric Administration[23] and categorized as less than 5, 5 to less than 6, and 6 or higher. For consistency with the latitude variable, Alaska, Hawaii, and Puerto Rico were not included in the UV index.

Although the latitude gradient in MS risk could be a result of a protective effect of vitamin D, latitudes at birth or in early childhood also correlate with socioeconomic status and age at infection with common viruses, which are potential risk factors for MS[24] and, thus, could confound the association of 25-hydroxyvitamin D and MS. In our study population, place of birth or residence in childhood was not generally available. However, 25-hydroxyvitamin D levels reflect recent UV exposure, and adjustment for latitude at entry into the military would be expected to remove any correlation that may exist between 25-hydroxyvitamin D levels and latitude at earlier ages.

The validity of the information on latitude of residence at entry into the military is supported by its expected correlation with 25-hydroxyvitamin D levels in samples collected prior to entry into the military (that is, at the time of application or initial screening), when the service member was likely to be residing in his/her state of entry. In these samples, among white controls (n=87), mean 25-hydroxyvitamin D levels increased from 74.4 nmol/L in the northern latitudes to 81.4 nmol/L in the middle latitudes and to 90.6 nmol/L in the southern latitudes and from 71.3 nmol/L to 79.7 nmol/L and 89.9 nmol/L for UV index ratings of less than 5, 5 to less than 6, and 6 or higher, respectively. All the analyses presented were therefore adjusted for latitude of resi-

dence at entry. Adjusting for UV index as either a categorical or a continuous variable did not materially change the results.

Laboratory Analyses

25-Hydroxyvitamin D levels were measured in the laboratory of B.W.H., as previously described.[25] Briefly, 25-hydroxyvitamin D was extracted from each serum sample using acetonitrile, and a radioimmunoassay with an iodine I 125–labeled tracer was used to measure the amount of 25-hydroxyvitamin D.[25] The serum samples were randomly sorted within each matched case-control triplet, and the laboratory was blinded to the case/control status of the samples. The intra-assay coefficient of variation, determined from blind quality control samples included with the study samples, ranged from 4.5% to 7.9% in different batches.

Statistical Analyses

All analyses were stratified by race/ethnicity because, as expected, whites had much higher 25-hydroxyvitamin D levels than blacks (see "Results" section). Because of small numbers, we combined Hispanic and other race/ethnicity determinations into 1 group. To remove extraneous variation in 25-hydroxyvitamin D due to season of blood collection and other sources, we regressed the 25-hydroxyvitamin D levels on the periodic function $-\sin(2\pi X/12)-\cos(2\pi X/12)$, where X is month of sample collection,[26] age at sample collection, sex, and laboratory assay batch. The residuals from this model were added to the sex-specific 25-hydroxyvitamin D means derived from the model to create an adjusted 25-hydroxyvitamin D measurement.

To obtain an integrated measure of long-term, preclinical 25-hydroxyvitamin D level for each individual, we calculated the average of these adjusted 25-hydroxyvitamin D levels from all the available samples, except for those collected after the onset of MS among cases. Because a single measurement of serum 25-hydroxyvitamin D may not fully reflect long-term vitamin D status, the analyses were restricted to the 257 cases and 514 matched controls who had at least two 25-hydroxyvitamin D measurements before MS onset.

Conditional logistic regression analysis, adjusting for latitude of residence at entry into the military, was used to estimate odds ratios (ORs).[27] We modeled 25-hydroxyvitamin D level both as a continuous variable, to estimate its association with MS risk under a linear assumption, and in quantiles, to explore the dose-response relationship. Quintiles among whites and tertiles (because of the smaller sample size) among blacks were determined based on the distributions of average 25-hydroxyvitamin D levels among their respective controls. In tests for trend, the medians of the quintiles or tertiles were modeled as continuous variables.

We also conducted analyses classifying individuals into 5 a priori–defined categories of 25-hydroxyvitamin D by 25-nmol/L increments (<25, 25 to <50, 50 to <75, 75 to <100, or ≥ 100 nmol/L). However, because of small sample sizes in the lower 25-hydroxyvitamin D categories among the white and Hispanic/other groups, the first 3 categories were collapsed and used as the referent in those analyses, and because few blacks had 25-hydroxyvitamin D levels higher than 75 nmol/L, the category of 50 to less than 75 nmol/L was used as the referent in the black race–specific analysis. Repeated-measures linear models were used to compare changes in 25-hydroxyvitamin D level over time among cases. The statistical significance level was set at $P<.05$ for 2-tailed tests.

Epstein-Barr virus antibody titers were strongly associated with risk of MS in analyses conducted in a subset of 83 cases and 166 controls from this population[17] but were not correlated with 25-hydroxyvitamin D levels (data not shown) and, thus, are unlikely to confound the association between 25-hydroxyvitamin D levels and MS. For this reason and because EBV serologic results were unavailable for most cases and controls in the present study, EBV antibody titers are not included in this report. Results of analyses restricted to definite cases are materially identical to those including all cases and also are not shown.

Analyses were conducted using SAS software, version 9.1 (SAS Institute Inc, Cary, NC).

RESULTS

The main characteristics of the cases and controls are shown in the TABLE. Multiple sclerosis cases were, on average, 28.5 years old (age range, 18-48 years) at symptom onset. The initial disease course was relapsing-remitting in 73%, primary progressive in 7%, and uncertain in the remaining 20%. The average time between collection of the first and last samples before MS symptom onset was 4.4 years (range, <1-11.8 years) and between the first sample and MS symptom onset was 5.3 years (range, <1-13 years). The average serum 25-hydroxyvitamin D level among whites (mean [SD], 75.2 [28.1] nmol/L) was 29.7 nmol/L higher than that among blacks (mean [SD], 45.5 [21.2] nmol/L; $P<.001$), and was 8.6 nmol/L higher than that of the Hispanic/other group (mean [SD], 66.6 [25.4] nmol/L; $P<.001$). The mean for each group is consistent with levels in the general US population.[28]

White Race/Ethnicity

Among whites, there was a 41% decrease in MS risk for every 50-nmol/L increase in 25-hydroxyvitamin D (OR, 0.59; 95% confidence interval [CI], 0.36-0.97; $P=.04$), and there was no significant difference by sex (men: OR, 0.60; 95% CI, 0.33-1.10; women: OR, 0.53; 95% CI, 0.22-1.29; $P=.90$ for interaction). In analysis by quintiles, MS risk was highest among individuals in the bottom quintile and lowest among those in the top quintile of 25-hydroxyvitamin D levels (OR for top vs bottom quintile, 0.38; 95% CI, 0.19-0.75; $P=.006$). Risks in quintiles 2 through 4 were intermediate, and the overall trend across quintiles was significant (FIGURE). Results based on the a priori–defined categories of 25-hydroxyvitamin D were similar: using individuals with

25-hydroxyvitamin D levels of less than 75 nmol/L as the reference (69 cases and 114 controls) there was a nonsignificant reduction in risk among those with 25-hydroxyvitamin D levels of 75 to less than 100 nmol/L (62 cases and 124 controls; OR, 0.83; 95% CI, 0.54-1.29; $P = .41$) and a significant 51% reduction among those with 25-hydroxyvitamin D levels of 100 nmol/L or higher (17 cases and 58 controls; OR, 0.49; 95% CI, 0.27-0.91; $P = .02$).

Adolescence appears to be a crucial exposure period for MS.[5] Therefore, we further examined whether serum 25-hydroxyvitamin D concentrations before age 20 years predict MS risk. One of 39 cases and 16 of 76 controls (2 of the 78 matched controls were 20 years old at time of blood collection and were excluded) had 25-hydroxyvitamin D levels of 100 nmol/L or higher, resulting in an OR of 0.09 (95% CI, 0.01-0.75; $P = .03$) compared with levels less than 100 nmol/L.

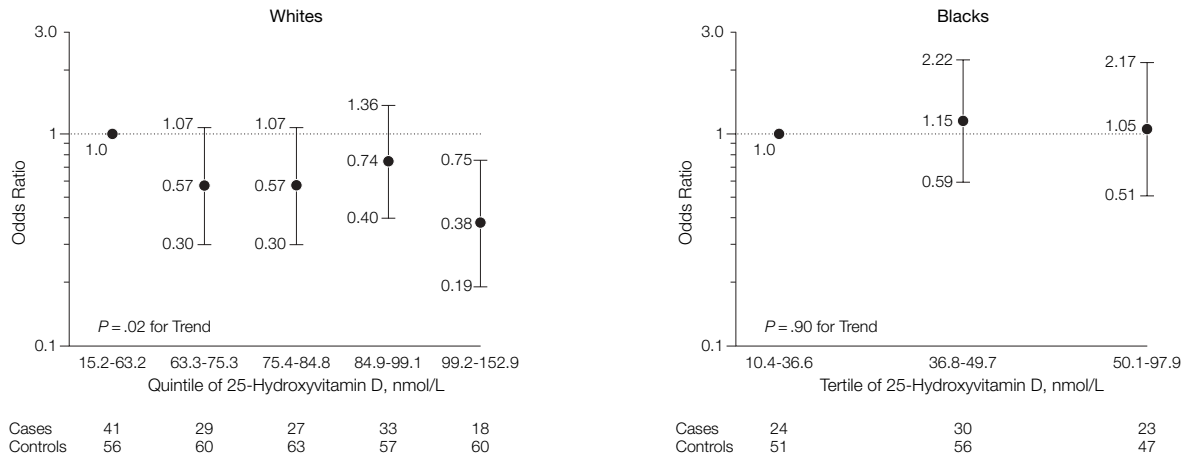
We also were concerned that our results could reflect an effect of MS on 25-hydroxyvitamin D levels rather than an effect of 25-hydroxyvitamin D levels on MS risk. Multiple sclerosis could affect 25-hydroxyvitamin D levels either by some as yet unknown effect on vitamin D metabolism or, more likely, by changes in behavior—because heat commonly exacerbates MS symptoms, individuals with MS tend to avoid sun exposure and, thus, may have lower 25-hydroxyvitamin D levels than healthy individuals.[29] If heat intolerance and sun avoidance preceded the neurological symptoms recognized as the first onset of MS, higher serum levels of 25-hydroxyvitamin D would spuriously appear to be protective. To address this possibility, we examined the temporal relationship between serum 25-hydroxyvitamin D concentrations and the date of onset of MS symptoms among white cases. Average 25-hydroxyvitamin D levels among individuals who developed MS were stable during the years preceding symptom onset ($P = .42$ for trend) but significantly decreased after onset of symptoms ($P = .002$). Mean 25-hydroxyvitamin D levels were 71.8 nmol/L more than 6 years before symptom onset (51 cases), 71.6 nmol/L between 4 and 6 years (51 cases), 73.5 nmol/L between

Table. Selected Characteristics of Multiple Sclerosis Cases and Matched Controls*

Characteristics	Cases (n = 257)	Controls (n = 514)
Male	174 (68)	348 (68)
Race		
White, non-Hispanic	148 (57.6)	296 (57.6)
Black, non-Hispanic	77 (30)	154 (30)
Hispanic/other	32 (12.5)	64 (12.5)
Latitude of residence at entry into the military†‡		
Northern	42 (16.3)	104 (20.2)
Middle	97 (37.7)	156 (30.4)
Southern	101 (39.3)	205 (39.9)
Outside continental United States	3 (1.2)	6 (1.2)
UV index of residence at entry into the military†		
<5	10 (3.9)	32 (6.2)
5 to <6	124 (48.3)	221 (43.0)
≥6	106 (41.3)	212 (41.3)
No. of serum samples available		
2	81 (32)	172 (33.5)
3	176 (68)	342 (66.5)
Age at first serum sample collection, mean (SD) [range], y	23.3 (5.3) [16-40]	23.3 (5.3) [17-41]

*Data are expressed as No. (%) unless otherwise indicated.
†Does not total to 100% because of missing information on place of residence at entry into the military.
‡See "Methods" section of text for definitions of northern, middle, and southern latitudes.

Figure. Odds Ratios of MS by Quantile of Serum 25-Hydroxyvitamin D Among Whites and Blacks



Error bars indicate 95% confidence intervals.

2 and 4 years (87 cases), 70.3 nmol/L between 1 and 2 years (136 cases), and 63.3 nmol/L after symptom onset (128 cases). These results argue against the possibility that the low preclinical 25-hydroxyvitamin D levels among individuals with MS are a consequence rather than a cause of the disease, although this possibility cannot be completely excluded.

Black and Hispanic Race/Ethnicity

Among blacks, the overall association between 25-hydroxyvitamin D levels and MS risk was not significant (OR for 50-nmol/L increase in 25-hydroxyvitamin D, 0.66; 95% CI, 0.24-1.78; $P=.41$), and there was no significant interaction by sex ($P=.70$). The OR for MS did not appreciably change by 25-hydroxyvitamin D tertile (Figure). Because there were no black cases or controls with 25-hydroxyvitamin D levels of 100 nmol/L or higher and all but 1 case and 5 controls had levels less than 75 nmol/L, we could not assess whether high levels of 25-hydroxyvitamin D in blacks are associated with reduced MS risk.

Among Hispanics and those of other race/ethnicity, the OR associated with a 50-nmol/L increase of 25-hydroxyvitamin D was 0.97 (95% CI, 0.28-3.33; $P=.96$). Because this group is small, we did not conduct a quantile analysis; in categorical analyses, the OR among individuals with 25-hydroxyvitamin D levels of 100 nmol/L or more (3 cases and 8 controls) compared with individuals with levels of less than 75 nmol/L (18 cases and 39 controls) was 0.61 (95% CI, 0.13-2.93; $P=.54$).

COMMENT

In this large prospective study, we found that the risk of MS decreased with increasing serum levels of 25-hydroxyvitamin D. Although this association was not seen among blacks, their smaller sample size and substantially lower 25-hydroxyvitamin D levels may have reduced the power to detect an association in this group.

Our results converge with a growing body of evidence supporting a protective role for vitamin D in MS development. Vitamin D is a potent immunomodulator,[10] and several studies have shown that administration of the biologically active hormone 1,25-dihydroxyvitamin D prevents EAE onset and progression in mice.[30,31] The exact mechanisms of this protection are unknown, but evidence suggests an indirect effect, possibly mediated by regulatory T cells.[10,32] Of interest, regulatory T cells have been shown to be suppressed in individuals with MS.[33] Physiological blood levels of 1,25-dihydroxyvitamin D, however, are tightly regulated and are not measurably affected by exposure to sunlight or dietary vitamin D.[34]

In contrast, circulating levels of 25-hydroxyvitamin D are sensitive to both factors. Therefore, an important question is whether 25-hydroxyvitamin D has a role in regulating immune responses. Serum levels of 25-hydroxyvitamin D were recently shown to control the Toll-like receptor–mediated generation of the microbicide cathelicidin by human monocytes and macrophages in response to *Mycobacterium tuberculosis* challenge, suggesting that nutritional vitamin D status could be key in innate immune response.[35] An inhibitory effect of levels of 25-hydroxyvitamin D in autoimmune reactions is consistent with the accelerated onset of EAE[31] and experimental type 1 diabetes in vitamin D–deficient mice.[36] This effect could be mediated by local synthesis of 1,25-dihydroxyvitamin D from 25-hydroxyvitamin D by activated macrophages expressing 1-α-hydroxylase. If sufficient 1,25-dihydroxyvitamin D is produced, it may exert paracrine effects on surrounding T lymphocytes, thereby regulating the tissue-specific immune responses.[10] Some support for this hypothesis comes from recent experiments showing that mice fed diets high in vitamin D had significantly fewer clinical and pathological signs of EAE than mice fed a vitamin D–deficient diet.[37] Central nervous system levels of 1,25-dihydroxyvitamin D, but not blood levels, were higher in supplemented mice than in vitamin D–deficient mice and correlated inversely with disease severity.

Although the results of our study support a direct role of vitamin D in MS prevention, other potential explanations should be considered. Although unlikely, a genetic predisposition to both MS and circulating low 25-hydroxyvitamin D levels could appear as a protective effect of vitamin D on MS in our study. Additionally, we cannot exclude the possibility that some other effect of exposure to UV light, rather than vitamin D production, contributes to protection. Serum levels of 25-hydroxyvitamin D largely reflect differences in exposure to UV radiation from sunlight. Whole-body UV light exposure has been shown to suppress EAE in mice[38]; it also enhances regulatory T-cell function and increases production of the immunosuppressive cytokines interleukin 4 and interleukin 10.[39] The relative importance of direct vs vitamin D–dependent effects of UV light at the level of exposure typical of human populations is uncertain,[40] but our previous finding of a lower MS risk among women taking vitamin D supplements[15] supports a specific role for vitamin D.

In most migration studies, the change in MS risk among migrants is stronger when migration occurs in childhood and tends to decrease with increasing age at migration.[5] These results suggest that vitamin D levels earlier in life may be critical in conferring protection for MS and our finding of a strong protective effect of 25-hydroxyvitamin D levels of 100 nmol/L or higher before age 20 years supports this view. Vitamin D supplementation in infancy seems to exert a strong protective effect against the autoimmune disease type 1 diabetes,[41] and vitamin D levels in early childhood could also have an impact on the risk of MS. Although there are no data on vitamin D levels earlier in life and risk of MS, the strong inverse association between MS risk and 25-hydroxyvitamin D levels at ages 16 to 19 years suggests that levels in late adolescence are likely to be important.

A key question is whether it may be possible to reduce the incidence of MS in populations at high risk by increasing circulating levels of 25-hydroxyvitamin D. Almost half of white and two thirds of black adults in the United States have 25-hydroxyvitamin D levels below 70 nmol/L.[28] Although levels above 25 nmol/L have traditionally been considered normal and almost everyone in this study had measurements above this level, much higher levels may be required for bone mineralization and prevention of fractures. According to a recent review, the best serum 25-hydroxyvitamin D concentrations are between 90 and 100 nmol/L.[42] Adolescents have somewhat higher levels than adults, but few have levels higher than that associated with a reduced risk of MS in our study.[43] If the association reported here reflects a true protective effect of vitamin D, increasing the vitamin D levels of adolescents and young adults could result in an important reduction in MS incidence. Such an increase could be achieved by using vitamin D supplements.[44,45] Although the current Institute of Medicine adequate intake of vitamin D is 200 U/d for adults younger than 50 years,[46] and the highest dose that is considered safe is 2000 U/d,[46] adverse effects have been reported only at intakes several-fold higher.[45]

A broad recommendation for a several-fold increase in vitamin D intake among adolescents and young adults requires stronger evidence than that provided by observational studies alone. First-degree relatives of individuals with MS are at a higher risk of developing MS,[47] and a prevention trial among this population would be possible and timely. Meanwhile, use of vitamin D supplements for MS prevention should not be undertaken until efficacy is proven.

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Study concept and design: Levin, Ascherio.
Acquisition of data: Munger, Levin, Howard, Ascherio.
Analysis and interpretation of data: Munger, Levin, Hollis, Ascherio.
Drafting of the manuscript: Munger, Hollis, Ascherio.
Critical revision of the manuscript for important intellectual content: Munger, Levin, Hollis, Howard, Ascherio.
Statistical analysis: Munger, Ascherio.
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Writing criticism is to writing fiction and poetry as hugging the shore is to sailing in the open sea.
—John Updike (1932-)

