Body mass index, but not vitamin D status, is associated with brain volume change in MS

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Abstract

Objective

To determine whether body mass index (BMI) or vitamin D status is associated with MRI measures of neurodegeneration in a cohort of individuals with relapsing-remitting multiple sclerosis (RRMS) or clinically isolated syndrome (CIS).

Methods

Expression, Proteomics, Imaging, Clinical (EPIC) is a longitudinal multiple sclerosis (MS) cohort study at the University of California, San Francisco. Participants had clinical evaluations, brain MRI, and blood draws annually. We evaluated patients with CIS or RRMS at baseline. In multivariate repeated-measures analyses adjusted for age, sex, ethnicity, smoking status, and use of MS treatments, annual 25-hydroxyvitamin D levels and BMI were evaluated for their association with subsequent brain volumes (normalized gray matter [nGMV], brain parenchymal [nBPV], and white matter volumes, as determined by Structural Image Evaluation using Normalization of Atrophy-X).

Results

Among 469 participants, each 1-kg/m² higher BMI was independently associated with reduced nGMV in multivariate models (−1.1 mL, 95% confidence interval [CI] −1.8 to −0.5, $p = 0.001$). BMI was likewise independently associated with nBPV (nBPV per 1-kg/m² greater BMI: −1.1 mL, 95% CI −2.1 to −0.05, $p = 0.039$). Vitamin D levels did not appear to be meaningfully associated with brain volumes.

Conclusions

Higher BMI appears to be associated with greater reductions in nGMV and nBPV, which is relevant because, in particular, nGMV loss portends greater longer-term disability. Because obesity is modifiable, further studies should explore these relationships in detail, and evaluating the effect of reducing BMI on imaging and clinical outcomes in MS may be warranted.

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Glossary

BMI = body mass index; **CI** = confidence interval; **CIS** = clinically isolated syndrome; **DMT** = disease-modifying therapy; **EDSS** = Expanded Disability Status Scale; **FOV** = field of view; **MS** = multiple sclerosis; **nBPV** = normalized brain parenchymal volume; **nGMV** = normalized gray matter volume; **nWMV** = normalized white matter volume; **RRMS** = relapsing-remitting multiple sclerosis; **SIENAX** = Structural Image Evaluation Using Normalization of Atrophy-X; **TE** = echo time; **TR** = repetition time.

At onset, multiple sclerosis (MS) is usually characterized by relapses of neurologic dysfunction and white matter lesion accrual on MRI. Over time, many patients with MS develop neurologic disability progression, thought to be caused by neurodegeneration; brain volume loss on MRI likely reflects this phenomenon.^{1–6} Normalized gray matter volume (nGMV) loss is particularly associated with subsequent disability^{1,7–12} and may be more meaningfully associated with cognition and quality-of-life declines than white matter or whole-brain volume losses.^{13,14} Studying the relation between candidate prognostic factors and nGMV may provide insight into their roles in longer-term disability.

Established nongenetic MS risk factors include smoking, Epstein-Barr virus seropositivity, obesity, and vitamin D insufficiency.^{15–22} While obesity is associated with lower brain volume in other populations,^{23–27} there are minimal data exploring its role in MS prognosis.²⁸ Lower vitamin D levels are associated with increased MS relapses and brain lesions.^{29–33} Higher 25-hydroxyvitamin D levels are associated with preserved GMV in a shorter study of patients with clinically isolated syndrome (CIS),³⁴ and another study showed similar results for vitamin D levels and overall brain volume,³¹ but the relationship between vitamin D status and GMV in more established MS is unclear. Because vitamin D levels are lower in those with higher body mass index (BMI), evaluating both factors concomitantly is ideal to minimize potential confounding.³⁵

We capitalized on a 5-year cohort study of patients with existing MS/CIS to determine whether BMI or vitamin D status is associated with longer-term MRI measures of neurodegeneration.

Methods

Participants

Expression, Proteomics, Imaging, Clinical (EPIC) is an MS cohort study in which patients, recruited from the University of California, San Francisco MS Center, had clinical and MRI evaluations and contributed blood at baseline and annually for 5 years. At baseline, all participants were white, were 18 to 70 years of age, and had an Expanded Disability Status Scale (EDSS) score <8.0. A diagnosis of MS or CIS was required^{36,37}; the brain MRI of those with CIS had to meet 3 of 4 of the dissemination in space 2005 criteria.³⁷ Those who had had a relapse or were treated with corticosteroids within the past

month, were enrolled in a trial of unapproved MS medications, were unable to undergo MRI, had recently abused alcohol or illicit drugs, or had a medical disorder that created potential risks by participating were excluded. The study began in July 2004 with a target enrollment of 500 individuals. Participants were added to compensate for dropouts; those added by September 2005 were considered members of the original cohort. For purposes of this study, all participants with CIS or relapsing-remitting MS (RRMS) who were in the original cohort were included if they had had at least 1 follow-up visit. We also included subsequent enrollees who were scheduled to have had at least 2 follow-up visits by the time the blood samples were prepared for vitamin D measurement (June 2010), corresponding to those who enrolled in EPIC before October 2008.

Standard protocol approvals, registrations, and patient consents

The University of California, San Francisco Committee on Human Research approved the study, and written informed consent was obtained from each participant.

Clinical and laboratory assessments

At baseline, age, ethnicity (Hispanic or not), sex, MS duration, weight, height (self-reported), smoking status (current vs never/former), EDSS score, use of MS therapies and other medications, and additional clinical data were recorded. At follow-up visits, weight (usually self-reported), use of MS therapies and other medications, and EDSS scores were captured. BMI was calculated for each visit in kilograms per meter squared. Those who noted taking calcium supplements without specifically listing that the supplement contained vitamin D were not considered to be taking vitamin D because not all calcium supplements contain it. The month and year at which an MS therapy was started or stopped were recorded; we assigned the 15th of the month as the actual start or stop date if the exact date was not clear.

The 25-hydroxyvitamin D concentration (vitamin D level) was measured by batched chemiluminescent immunoassay (Heartlands Assays, Inc, Ames, IA) from plasma (baseline) or serum (years 1–4)³² because insufficient quantities of serum remained to conduct the baseline measurements and plasma was not available at all follow-up visits. In a subset of 15 participants for whom serum and plasma were available at the same time point, the correlation of 25-hydroxyvitamin D levels in the samples was extremely high (intraclass correlation coefficient 0.95, 95% confidence interval [CI] 0.85–0.98).

MRI protocol

MRI scans were performed and processed by investigators blinded to BMI and vitamin D levels for each study visit at the Advanced Imaging in MS Laboratory of Dr. Daniel Pelletier. On a 3T GE Excite scanner (GE Healthcare Technologies, Waukesha, WI), images were acquired with an 8-channel phased array coil in reception and a body coil in transmission. Each MRI included scout localizers and axial dual-echo spin echo sequences (echo time [TE] 20 and 90 milliseconds, repetition time [TR] 2,000 milliseconds, $512 \times 512 \times 44$ matrix, $240 \times 240 \times 132$ -mm³ field of view [FOV], slice thickness 3 mm, interleaved). An inversion-recovery gradient-echo T1-weighted isotropic, volumetric sequence (3-dimensional inversion-recovery prepared spoiled gradient echo, $1 \times 1 \times 1$ mm³, 180 slices) was also performed (TE/TR/inversion time 2/7/400 milliseconds, flip angle 15°, $256 \times 256 \times 180$ matrix, $240 \times 240 \times 180$ -mm³ FOV, number of excitations 1) at high resolution. Five minutes after administration of a single dose (0.1 mmol/L/kg) of contrast agent (TE/TR = 8/467 milliseconds, $256 \times 256 \times 44$ matrix, $240 \times 240 \times 132$ -mm³ FOV, number of excitations 1), conventional spin echo, T1-weighted images were acquired.

Brain lesions were identified by viewing high-resolution T1-weighted, T2-weighted, and proton density-weighted images simultaneously. Regions of interest were drawn that were based on a semiautomated threshold with manual editing with in-house software, and T1- and T2-lesion masks were created as previously described.³⁸ Both intraobserver and interobserver variability analyses were performed to ensure the accuracy of data acquired. T2-lesion volumes from region-of-interest selections were calculated by multiplying the area of the lesion by the slice thickness and the number of slices penetrated with in-house software. A qualitative analysis for the presence of gadolinium enhancement was performed (by D.T.O. and D.P.) on postcontrast T1-weighted images.

Brain segmentation and normalization were performed at each time point with Structural Image Evaluation Using Normalization of Atrophy-X (SIENAX; Image Analysis Group, Oxford, UK), a fully automated technique.³⁹ T1-lesion masks (described above) were presented to the SIENAX program to correct for misclassifications of parenchymal tissue. After skull stripping, cross-sectional brain images were registered to MN1152 space to obtain the volumetric scaling factor, which is used to correct for head size. Tissue-type segmentation was then carried out on T1-weighted images, including separate estimates of whole brain, gray matter, white matter, and CSF volumes. The lesion masks overrode all SIENAX tissue classifications. Normalized tissue volumes were calculated by adding the lesion-corrected partial volume estimate maps, multiplied by the volumetric scaling factor calculated by the SIENAX program, yielding the normalized brain parenchymal volume (nBPV), nGMV, and normalized white matter volume (nWMV).

Statistical analyses

Statistical analyses were performed with Stata (version 14.2; StataCorp, College Station, TX). Repeated-measures analyses were performed with generalized estimating equations with robust standard errors and an exchangeable correlation matrix. The primary outcome for the study was determined a priori as nGMV given its more consistent association with longer-term disability. Secondary outcomes included nWMV and nBPV. The 2 predictors of interest were the 25-hydroxyvitamin D level, assessed in 10-ng/mL (25-nmol/L) increments, and BMI, assessed in increments of 1 kg/m², of the prior visit.

In addition to univariate models, multivariate models were created and included age at visit, sex, ethnicity, baseline smoking status (binary measure), and any exposure to disease-modifying therapy (DMT) in the time interval preceding the outcome measure. To assess linearity, a covariate of BMI $\times$ BMI or vitamin D level $\times$ vitamin D level was added to the models. DMT was also evaluated as a categorical variable (none, first-line [interferon beta, glatiramer acetate, and steroids], and second line [chemotherapy, monoclonal antibodies, and fingolimod]). To account for concerns that newly initiated DMT may influence brain volume, we performed sensitivity analyses to exclude MRI scans that were obtained within 6 months of initiating DMT so as to minimize any influence of pseudoatrophy.⁴⁰ We also performed a sensitivity analysis in which MRIs performed after an interval during which DMT exposure was not constant for the whole interval (whether on or off DMT) were removed. We explored whether adding baseline disability, disease duration, or *HLA-DRB1*1501* status (1 or 2 copies of the *1501 allele vs none, assessed as previously reported)⁴¹ to the models changed the results. We also evaluated models with new T2 lesions or baseline T2 lesion volume as a covariate, and we explored for interactions between new T2 lesions and vitamin D levels or BMI. We assessed for interactions between vitamin D and *HLA-DRB1* genotype based on a report of a vitamin D response element in *HLA-DRB1*15* haplotypes.⁴² Finally, although results were already published for vitamin D,³² exploratory analyses evaluating the association of BMI with EDSS score (clinical disability) were conducted.

Data availability

The EPIC MS dataset used in this analysis is not sharable publicly. The acquisition of the brain MRIs in the EPIC MS dataset was funded by investigator-initiated studies sponsored by pharmaceutical companies.

Results

After the removal of 72 patients without a diagnosis of RRMS/CIS at baseline, 469 participants had RRMS/CIS and were included in these analyses. Seventy-four percent ($n = 349$) of the total group (79% of the original cohort

RRMS/CIS participants) completed a year 5 visit, with a mean of 4.1 brain MRIs performed across the group. Patient characteristics at baseline were presented in a previous publication³² and are available in table 1. Patients were largely female (n = 330; 70%) with an average age of 42 ± 10 years and median disease duration of 5 years (interquartile range [IQR] 0–35 years). At baseline, the mean 25-hydroxyvitamin D level was 27.8 ± 10.7 ng/mL, mean BMI was 25.3 ± 4.9 kg/m² (median 24.2 kg/m², IQR 17.9–41.7), and median EDSS score was 1.5 (IQR 0–5.0). A test for trend did not demonstrate a meaningful change in BMI during the course of the study (p = 0.41), but as previously reported,³² mean 25-hydroxyvitamin D levels and self-reported vitamin D supplementation increased during the follow-up period (p < 0.0001 for both tests for trend). Baseline and follow-up segmented brain volumes are presented in table 2.

In terms of clinical disability, higher BMI was associated with greater subsequent disability in multivariate models, a result that was statistically, but not clinically, significant (per 5 kg/m², the EDSS score was 0.13 points higher, 95% CI 0.04–0.22, p = 0.004). There was no evidence of nonlinearity (p = 0.14). These results were not meaningfully altered when disease duration, baseline T2 lesion load, new T2 lesions, or *HLA-DRB1* status was added to the original multivariate model; when treatment was classified as categorical; or when prespecified treatment-related sensitivity analyses were performed.

Normalized gray matter volume

Body mass index

In cross-sectional univariate linear regression models at baseline, each 1-kg/m² greater BMI was correlated with lower nGMV (−2.5 cm³, 95% CI −3.6 to −1.4, p < 0.001).

Table 1 Baseline characteristics (n = 469)

Feature	
Age, mean (±SD), y	42 ± 10
Female, n (%)	330 (70)
Hispanic, n (%)	24 (5)
Positive for <i>HLA-DRB1</i> , n (%)	214 (46)
Current smoker, n (%)	56 (12)
Body mass index, mean ± SD, kg/m ²	25 ± 5
25-Hydroxyvitamin D level, mean ± SD, ng/mL	27.8 ± 10.7
Vitamin D supplement use, n (%)	40 (9)
Multiple sclerosis duration, median (interquartile range), y	5 (0–35)
Expanded Disability Status Scale score, median (±interquartile range)	1.5 (0–5)
Use of multiple sclerosis treatment in past year, n (%)	298 (64)

Longitudinally, each 1-kg/m² higher BMI was associated with reduced nGMV in univariate (−1.6 mL, 95% CI −2.4 to −0.8, p < 0.001) and multivariate (−1.1 mL, 95% CI −1.8 to −0.5, p = 0.001; table 3) models. There was some evidence of nonlinearity (p = 0.048). We thus modeled BMI as normal or underweight (<25 kg/m²), overweight (≥25–<30 kg/m²), class 1 obesity (≥30–<35 kg/m²), class 2 obesity (≥35–<40 kg/m²), and class 3 obesity (≥40 kg/m²). Strikingly, when BMI was evaluated categorically, nGMV was much more substantially reduced with greater levels of categorically classified BMI (figure), with those ≥40 kg/m² realizing a nearly 30-mL lower nGMV over the course of follow-up (−29.2 mL, 95% CI −46.8 to −11.7, p = 0.001). The p value for the test for trend across the weight categories was 0.018. To evaluate the consistency of these findings with more even distributions of patients per group, we also evaluated BMI as a dichotomous predictor (e.g., ≥25 vs <25 kg/m²; ≥30 vs <30 kg/m²) and found similar results: there did not appear to be an association evident in those overweight or greater (≥25 kg/m²) vs normal/underweight (nGMV −1.8 mL, 95% CI −6.4 to 2.7, p = 0.43), whereas dichotomizing BMI at higher cut points led to progressively more substantial relationships (for class 1 obesity or greater vs BMI <30 kg/m²: nGMV −8.7 mL, 95% CI −17.3 to −0.08, p = 0.048; for class 2 obesity or greater vs BMI <35 kg/m²: nGMV −10.4 mL, 95% CI −19.2 to −1.5, p = 0.02; for class 3 obesity or greater vs BMI <40 kg/m²: nGMV −22.0 mL, 95% CI −36.5 to −7.4, p = 0.003).

Adding baseline EDSS score, disease duration, baseline T2 lesion load, new T2 lesions, or *HLA-DRB1* status to the original multivariate model; classifying treatment as categorical; or performing the prespecified treatment-related sensitivity analyses did not meaningfully change the results. There was no apparent interaction of BMI with new T2 lesions (p = 0.82).

Vitamin D

In univariate models, higher vitamin D levels were associated with lower nGMV (nGMV per 10-ng/mL higher vitamin D: −2.6 mL, 95% CI −4.5 to −0.6, p = 0.009), but this association was not apparent in multivariate models (table 3). There was no evidence of nonlinearity (p = 0.22). Adding baseline EDSS score, disease duration, baseline T2 lesion load, new T2 lesions, or *HLA-DRB1* status to the multivariate model; classifying treatment as categorical; or performing the prespecified treatment-related sensitivity analyses did not meaningfully change the results.

To understand potential confounding related to the use of vitamin D supplements on the primary outcome results, post hoc sensitivity analyses were performed in which those who were taking extra vitamin D were excluded. In univariate models, which included 422 participants, there was no apparent association between vitamin D levels and nGMV (nGMV per 10-ng/mL higher vitamin D: −0.8 mL, 95% CI −3.5 to 2.0, p = 0.60); the same was true in multivariate

Table 2 Summary statistics for brain volume measures at each annual brain MRI scan^a

Volumes	Baseline (n = 469), mL	Year 1 (n = 447), mL	Year 2 (n = 407), mL	Year 3 (n = 353), mL	Year 4 (n = 345), mL	Year 5 (n = 349), mL
nGMV	849 ± 61	842 ± 58	836 ± 57	831 ± 58	820 ± 56	815 ± 55
nWMV	690 ± 42	688 ± 41	685 ± 43	683 ± 41	708 ± 51	686 ± 47
nBPV	1,544 ± 89	1,535 ± 86	1,527 ± 87	1,520 ± 87	1,506 ± 86	1,506 ± 88

Abbreviations: nBPV = normalized brain parenchymal volume; nGMV = normalized gray matter volume; nWMV = normalized white matter volume.

^a Missing scans at each time point for nGMV, nBPV, and nWMV: baseline = 12, year 1 = 13, year 2 = 10, year 3 = 11, year 4 = 7, and year 5 = 9.

models (nGMV per 10-mL higher vitamin D: −0.4 mL, 95% CI −2.8 to 2.0, $p = 0.77$).

There was no apparent interaction of vitamin D levels with new T2 lesions ($p = 0.70$), but the p value for the interaction of vitamin D levels with *HLA-DRB1* status was 0.067; while vitamin D was not meaningfully associated with nGMV in *DRB1*− participants (per 10-ng/mL higher vitamin D level, nGMV 0.6 mL, 95% CI −1.8 to 3.0, $p = 0.61$), there was a possible association in *DRB1*+ participants (per 10-ng/mL higher vitamin D, nGMV 2.7 mL lower; 95% CI −5.4 to −0.06, $p = 0.045$). However, the apparent interaction between vitamin D levels and *DRB1* status did not appear to be present in the post hoc analyses that included only the patients who did not report use of vitamin D supplements (p for interaction term = 0.32).

nBPV and nWMV

Body mass index

As was seen for the nGMV models, BMI was longitudinally associated with nBPV in univariate models (nBPV per 1-kg/ m^2 greater BMI: −1.7 mL, 95% CI −2.9 to −0.6, $p = 0.004$) and was independently associated with lower nBPV in longitudinal multivariate models (nBPV per 1-kg/ m^2 greater BMI: −1.1 mL, 95% CI −2.1 to −0.05, $p = 0.039$; table 3). The results were similar when *HLA-DRB1*1501* status, baseline

EDSS score, disease duration, or baseline or new T2 lesions were added to the model. There was no evidence of non-linearity ($p = 0.69$). Additional methods of modeling DMT, including sensitivity analyses, did not lead to a meaningful change in the associations. There was no apparent interaction of BMI with new T2 lesions ($p = 0.46$).

Overall, BMI did not appear to be associated with nWMV in univariate (−0.9 mL, 95% CI −2.5 or 0.8, $p = 0.30$) or multivariate models (table 3). There was no meaningful influence of adding *HLA-DRB1* status, baseline T2 lesion load, new T2 lesions, baseline EDSS score, or disease duration to the multivariate models, nor were the results meaningfully different when treatments were modeled categorically or in prespecified treatment-related sensitivity analyses. There was no apparent nonlinearity of BMI ($p = 0.82$) or interaction of BMI and new T2 lesions ($p = 0.32$).

Vitamin D

In univariate models, vitamin D levels were not meaningfully associated with nBPV over the course of follow-up (nBPV per 10-ng/mL higher vitamin D: −1.6 mL, 95% CI −4.9 to 1.8, $p = 0.36$). The results were similar in the basic multivariate model (table 3) and when *HLA-DRB1*1501* status, baseline or new T2 lesions, baseline EDSS score, or disease duration was added to the model. There was no evidence of non-linearity ($p = 0.38$). Sensitivity analyses and additional

Table 3 Multivariate models depicting association of vitamin D levels and BMI with brain volume measures

	SIENAX nGMV, mL	SIENAX nWMV, mL	SIENAX nBPV, mL
25-Hydroxyvitamin D level (per 10 ng/mL greater)	−0.9 (−2.7 to 1.0) $p = 0.36$	0.7 (−3.4 to 4.9) $p = 0.73$	0.6 (−2.7 to 3.9) $p = 0.74$
BMI (per 1 kg/ m^2 greater)	−1.1 (−1.8 to −0.5) $p = 0.001$	−0.7 (−2.4 to 1.0) $p = 0.42$	−1.1 (−2.1 to −0.05) $p = 0.039$
Age (per year greater)	−4.1 (−4.5 to −3.8) $p < 0.001$	−1.4 (−1.8 to −0.9) $p < 0.001$	−5.2 (−5.7 to −4.6) $p < 0.001$
Female sex	24.2 (15.2 to 33.2) $p < 0.001$	−5.2 (−20.3 to 9.9) $p = 0.50$	12.7 (−1.4 to 26.7) $p = 0.078$
Hispanic ethnicity	−14.2 (−33.7 to 5.3) $p = 0.15$	−9.8 (−32.7 to 13.0) $p = 0.40$	−9.5 (−41.4 to 22.4) $p = 0.56$
Smoker at baseline	−9.5 (−23.0 to 4.1) $p = 0.17$	−11.5 (−24.5 to 1.6) $p = 0.085$	−14.3 (−34.6 to 5.9) $p = 0.17$
Use of any DMT	−2.9 (−8.9 to 3.1) $p = 0.34$	−6.5 (−23.2 to 10.2) $p = 0.44$	−6.3 (−16.1 to 3.4) $p = 0.20$

Abbreviations: BMI = body mass index; DMT = disease-modifying therapy; nBPV = normalized brain parenchymal volume; nGMV = normalized gray matter volume; nWMV = normalized white matter volume; SIENAX = Structural Image Evaluation Using Normalization of Atrophy-X. Values in parentheses are 95% confidence intervals.

Figure Change in nGMV by BMI quintile

methods of modeling DMT did not meaningfully change the associations. There was no evidence for a meaningful interaction of vitamin D status with new T2 lesions ($p = 0.48$); the p value for the interaction term of vitamin D status with *HLA-DRB1* was 0.037. Subgroup analyses showed that per 10-ng/mL higher vitamin D, among *DRB*⁻ participants, nBPV was 3.5 mL greater (95% CI -1.1 to 8.0, $p = 0.14$), while among *DRB*⁺ participants, nBPV was 3.0 mL lower (95% CI -7.1 to 1.1, $p = 0.15$). When the post hoc analyses were restricted to those not taking extra vitamin D, the primary model results were not meaningfully changed, but the apparent interaction between vitamin D levels and *DRB1* status was attenuated (p value for interaction term = 0.10).

Vitamin D status did not appear to be associated with nWMV in univariate models (each 10-ng/mL higher vitamin D associated with 1.4-mL higher nWMV, 95% CI -1.6 to 4.4, $p = 0.35$). The results were similar in multivariate models (table 3), and these associations were not meaningfully changed when baseline EDSS score, disease duration, *HLA-DRB1* status, baseline T2 lesion load, or new T2 lesions were added to the model. The results were also similar when treatments were modeled categorically and in prespecified treatment-related sensitivity analyses. There was no apparent nonlinearity ($p = 0.45$) or interaction between vitamin D status and new T2 lesions ($p = 0.83$). The p value for the interaction of vitamin D status with *HLA-DRB1* was 0.012. Each 10-ng/mL higher vitamin D level was associated with 4.6-mL greater nWMV (95% CI 1.0–8.2, $p = 0.012$) in *DRB1*⁻ participants but did not appear to be associated with nWMV in *DRB1*⁺ participants (-3.4 mL, 95% CI -9.6, 2.8, $p = 0.28$). Results of the post hoc analyses that included only those not taking extra vitamin D were similar in all models.

Discussion

We demonstrate that higher BMI was independently associated with lower nGMV and nBPV in follow-up, with a statistically significant, albeit not clearly clinically meaningful, association of higher BMI with greater disability. These results are of interest because obesity has now been identified as a risk factor for MS^{21,22} and because obesity-related comorbid conditions have been associated with worse prognosis for people with MS.^{43,44} One prior study did not show an association between BMI and clinical disability,²⁸ as measured by the EDSS, but it is possible that this relates to the known limitations of the EDSS as a measure of disability, particularly over short periods of time, rather than to a truly absent association. Another study evaluated the association between obesity and related diseases and found that the presence of these conditions, in various combinations, was correlated with reduced brain volume measures in people with MS, but the study was cross-sectional, making inferences about the directionality of the correlation difficult.⁴⁵ Longer, larger studies are needed to investigate the strength of the relationship between BMI and clinical disability demonstrated here.

There was no evidence in our analyses that accounting for the development of new T2 lesions attenuated the association of BMI with nGMV loss, nor was there a suggestion that the relationship between obesity and brain volume differed in magnitude or direction on the basis of the lack or presence of new T2 lesions. These observations reduce the likelihood that the results reported here are due solely to obesity-induced acceleration of inflammatory demyelinating events and subsequent neurodegeneration. Nonetheless, the obese state is associated with a state of systemic inflammation, and systemic inflammation is known to promote the formation of reactive oxygen species, which in turn promote mitochondrial dysfunction, long thought to play an important role in the

pathogenesis of MS-related neurodegeneration.⁴⁶ Several cross-sectional and longitudinal studies have demonstrated that obesity in otherwise healthy mid-life and aging populations appears to have an association with lower brain volume and that BMI is associated over time with dementia risk.^{23–27} Furthermore, in individuals with existing Alzheimer disease or mild cognitive impairment, those who were obese had greater reductions in brain volume than those with normal BMIs.⁴⁷ It is thus unclear whether the association of obesity and neurodegeneration in people with MS that we ascertained in this study is due to the promotion of MS-specific pathophysiologic processes or if the impact of obesity on the brain is a more generalized process that nonetheless compounds or accelerates the rate of gray matter and overall brain volume loss that is already at play in those with MS.

Regardless of the mechanisms underlying the findings, because obesity and related comorbid conditions are modifiable, if this result is confirmed in an independent dataset, particularly at the within-person level assessing change in BMI over time, it may be worthwhile to evaluate whether optimization of body habitus beneficially minimizes brain volume loss and thus disability progression in people with MS. Indeed, in a small study of morbidly obese people undergoing bariatric surgery, the weight loss that followed bariatric surgery was accompanied by widespread increases in white matter and regional increases in gray matter density compared to pre-operative MRI scans.⁴⁸ Because 1 study suggested that the strongest association between obesity and brain volume loss may be in mid-life, it may be that the time to act for overweight or obese people with MS is early in the course, when there is still a reserve of brain parenchyma available for preservation.⁴⁹ Finally, because the analyses suggested that the association of BMI with neurodegeneration is nonlinear and because those who met the actual definition of obesity class 2 (BMI ≥ 30 kg/m²) had particularly lower subsequent nGMV, this significant proportion of the MS patient population (17% of our population at baseline) may represent the group most optimal for studying the imaging and clinical effects of obesity reductions.

Contrary to results from 2 previous publications of patients with recent-onset CIS, in this 5-year longitudinal study of nearly 500 individuals with existing MS or CIS, we were unable to detect a meaningful association between circulating vitamin D levels and relevant brain volume measures in multivariate models.^{31,34} There may be several reasons for this discrepancy. First, it is plausible that the previous publications had type I errors and that there is no association of vitamin D status and brain volume. On the other hand, there were substantial differences in the included populations that may explain the discrepancy. For example, whereas in the previous studies enrollees all were within 60 or 90 days of their first clinical MS event, the baseline median disease duration in the EPIC study was 5 years, with some individuals having a much longer duration. It also may be that those in the prior studies had much more inflammatory disease and that, in this context,

vitamin D status is meaningful with respect to gray matter loss (via anti-inflammatory action and the downstream effect of neuronal injury), whereas it is less likely to occur later in the disease.⁵⁰ That being said, in these analyses, we found no confounding by or interaction with new T2 lesions, and restricting analyses to those with <5 years' disease duration at onset did not reveal a relationship between vitamin D levels and gray matter volume (data not shown).

We noted that in some of the analyses, higher vitamin D levels appeared to have a numerical trend or statistically significant association with lower brain volume measures. While the total 25-hydroxyvitamin D level accounts for vitamin D from all sources, we hypothesized that perhaps the unexpected associations noted in some of the models herein were related to unmeasured confounding; e.g., perhaps those patients who began using vitamin D supplements were otherwise different than those who did not. Thus, we performed post hoc sensitivity analyses as described herein, removing the <50 participants who reported use of vitamin D supplements; in this context, the associations were no longer present. These results suggest that the putative associations could be due to the hypothesized unmeasured confounding (e.g., perhaps those patients who were getting worse, not well detected clinically, were more likely to take supplements). Such a hypothesis should be tested in future datasets, ideally taking into account the dosages of vitamin D supplements, which were not captured in this cohort. Further requiring exploration in future datasets is the possibility of an interaction between vitamin D status and *HLA-DRB1* genotype. While an apparent interaction was no longer present in our nGMV models that removed those taking extra vitamin D, in the nWMV models, this interaction was present even with these sensitivity analyses, with higher vitamin D levels being associated with greater nWMV in *HLA-DRB1*-negative patients and the opposite in *DRB1*-negative patients. This may be a spurious result but again should be evaluated carefully in other prospective studies.

Our study has some limitations. First, much of the BMI information represents between-person differences, which may be subject to unmeasured confounders, and the reduced variation in BMI over time in the EPIC cohort limits the ability to evaluate for within-person change in BMI as the predictor of interest. The number of patients in the highest BMI categories was smaller, leading to widening of the CIs; the analyses would benefit from confirmation in a larger cohort. This cohort may not be representative of the broader population of people with MS, particularly because only those who were white were enrolled; furthermore, this analysis focused on those with RRMS/CIS. The cohort was heterogeneous with respect to age, disease duration, and baseline disability; although they did not appear to confound our results, larger studies might evaluate whether these and other demographic variables are effect modifiers. Although it may help minimize heterogeneity in the analyses, not all of the currently available MS DMTs were available at the time of this

study. Height and, at some time points, weight were also self-reported, so there may have been some inaccuracies, although BMI was relatively stable across the follow-up period. The lack of data about obesity-related comorbid conditions makes it difficult to further explore the mechanisms by which higher BMI may be associated with reductions in brain volume. Vitamin D supplementation was self-reported, as discussed above, and dosage was not recorded, so we were unable to assess whether the source of the vitamin D level (e.g., sunlight or supplementation) was relevant.

Overall, while our results raise the question of whether vitamin D supplementation will help to prevent or slow down neurodegeneration in established MS, the associations between BMI and gray matter and brain parenchymal volume loss we report here are noteworthy. Further investigations should evaluate how minimizing obesity and related comorbid conditions alters MS-related imaging and clinical outcomes; it may well be that such interventions could be complementary approaches to US Food and Drug Administration–approved MS DMTs in preventing long-term disability accumulation.

Author contributions

Dr. Mowry participated in study concept or design, analysis or interpretation of data, and drafting/revising manuscript for content. She also participated in statistical analyses and obtained funding for a portion of this study. Dr. Azevedo participated in analysis or interpretation of data and drafting/revising manuscript for content, as well as in acquisition of data. Dr. McCulloch participated in analysis or interpretation of data and drafting/revising manuscript for content, as well as in statistical analysis. Dr. Okuda participated in drafting/revising the manuscript and acquisition of data. Ms. Lincoln participated in drafting/revising the manuscript and acquisition of data. Dr. Waubant participated in study concept or design and drafting/revising of the manuscript. Dr. Hauser participated in study concept or design, obtaining funding, acquisition of data, study supervision or coordination. Dr. Pelletier participated in study concept or design, drafting/revising the manuscript, analysis or interpretation of data, obtaining funding, contributing vital tools, study supervision or coordination.

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