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Association of Menopause With Functional Outcomes and Disease Biomarkers in Women With Multiple Sclerosis

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Abstract

Background and Objective

The impact of menopause on the brain is not well understood. Hormonal changes, including puberty and pregnancy, influence the onset and course of multiple sclerosis (MS). After menopause, a worsening of MS disease trajectory measured on the clinician-rated Expanded Disability Status Scale (EDSS) was reported in some, but not all, studies. Evaluating the association between menopause and more objective measures of CNS injury is warranted. This study sought to assess the trajectory of objective functional outcomes and disease biomarkers in women with MS before and after menopause in a longitudinal prospective observational cohort.

Methods

Data were collected prospectively from a longitudinally followed MS cohort, including the performance-based Multiple Sclerosis Functional Composite (MSFC) as the primary functional outcome and the paraclinical marker of neuronal injury serum neurofilament light chain (sNfL) as the primary biomarker outcome. Outcomes were analyzed using segmented linear mixed model regressions adjusted for age, BMI, and tobacco use, with a change in slope at the time of menopause, as the a priori inflection point.

Results

One hundred and eighty-four postmenopausal women met inclusion criteria. Participants were followed for a median of 13 years (interquartile range [IQR] = 4, range: 1–17). The median MS duration was 24 years (IQR = 13, range: 3–64), and the median EDSS score was 2.5 (IQR = 2, range: 0–8). The median age at natural menopause was 50 years (IQR = 5, range: 33–60); 17% of participants used any systemic menopausal hormone therapy. Menopause reflected an inflection point in MSFC worsening (slope difference 0.08, 95% CI 0.01, 0.14, $p = 0.0163$) and increase in serum neurofilament light chain (slope difference −0.95, 95% CI −1.74 to −0.16, $p = 0.0194$) while the opposite was found for EDSS (slope difference 0.05, 95% CI 0.01–0.09, $p = 0.0200$). Findings remained significant after adjustment for multiple covariates. When using additional nonlinear regression modeling, similar inflection points were found (within 3 years of the final menstrual period) for sNfL and EDSS but not MSFC.

Discussion

The menopausal transition may represent an inflection in accumulation of neuronal injury and functional decline in MS.

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Coinvestigators are listed in appendix at the end of the article.

Glossary

DMT = disease-modifying therapy; **EDSS** = Expanded Disability Status Scale; **EPIC** = Expression/genomics, Proteomics, Imaging, and Clinical; **FMP** = final menstrual period; **HT** = hormone therapy; **IQR** = interquartile range; **MS** = multiple sclerosis; **MSFC** = Multiple Sclerosis Functional Composite; **MSSS** = Multiple Sclerosis Severity Score; **PASAT** = Paced Auditory Serial Addition Test; **sNfL** = serum neurofilament light chain.

Introduction

Approximately 43% of all individuals currently living with multiple sclerosis (MS) are premenopausal women, and one-third are postmenopausal women.[1] The potential impact of menopause on the inflammatory and progressive aspects of their disease course warrants study to better understand the effects of gonadal hormones on the CNS.

The immune-mediated demyelination in the CNS characteristic of MS[2] seems to be modulated by reproductive exposures, including puberty and the immunotolerant stage of pregnancy.[3] In addition, a decrease in relapses was reported coinciding with the age at menopause.[4,5] However, the effect of reproductive aging on the progressive aspects of MS, including neuroaxonal degeneration and worsening of neurologic function,[2] is less well understood. In the general population, postmenopausal accelerations in tau,[6] β-amyloid,[7] and white matter hyperintensity[8] accumulation and decreased regional gray matter volume,[7,9] metabolism, and recovery after ischemic stroke[10] were reported, suggesting that menopause may interfere with neural repair mechanisms. In MS, as in other chronic diseases, distinguishing the effects of reproductive and chronological aging is challenging because progression becomes more evident in both men and women after age 45.[11,12] Some, but not all, studies suggest an effect of menopause on clinical disability progression.[4,5,13,14]

To date, studies examining the effects of menopause on MS focused on clinician ratings of global disability using the Expanded Disability Status Scale (EDSS). This ordinal scale is heavily weighted toward ambulation, with different rates of progression at different ranges of the scale. The EDSS is also sensitive to "non-MS" changes due to older age and polypharmacy, which may be difficult to distinguish from MS disability.[15] Markers of progression most sensitive to change over short time intervals include the continuous MS Functional Composite (MSFC), which also captures non-ambulatory changes (cognition and dexterity) compared with the EDSS,[16] and the serum biomarker of axonal pathology, neurofilament light chain (NfL), which correlates with both inflammatory activity and neurologic injury and silent progression in MS and is associated with long-term outcomes.[17,18] NfL is influenced by age and, at a lesser scale, basic metabolic index (BMI) and renal function; thus, age-adjusted sNfL may be more specific to MS-driven changes.[19] Other potentially useful markers of progression include the subscales of the MSFC and the Multiple Sclerosis Severity Score (MSSS), which combines the EDSS and disease duration. This study sought to advance our understanding of the impact of menopause on progression by leveraging these objective, prospectively collected functional (primary: MSFC) and paraclinical (primary: sNfL) measures of disability progression.

Methods

Participants and Data Collection

Participants were enrolled in the Expression/genomics, Proteomics, Imaging, and Clinical (EPIC), EPIC2, and ORIGINS longitudinal cohort studies[20] at the University of California, San Francisco (UCSF). Participants with a diagnosis of MS or clinically isolated syndrome based on International Panel Criteria[21] were evaluated annually with measures of neurologic function, standardized MRI, and plasma and serum sampling. Enrollment for EPIC began in 2004, for EPIC2 in 2013, and for ORIGINS in 2015.

Criteria for inclusion in these analyses were (1) sex assigned female at birth and ciswoman gender identity (for brevity, herein referred to as "women") and (2) postmenopausal status with the date of final period known within 1 year. For most participants, reproductive variables were collected using a Gender-Inclusive EPIC Lifestyle Questionnaire, which was administered to participants at a single time point between 2019 and 2022. Variables included were menopausal status (cycling or postmenopausal), and if postmenopausal, date of final menstrual period (FMP), cause of menopause (natural; surgical, i.e., bilateral oophorectomy; or chemotherapy-induced), and use of menopausal hormone therapy (HT), as previously described.[13] These data were validated or updated through electronic chart review, with >80% agreement. Missing data points were obtained through chart review when available. Additional chart review was performed for women older than 45 years in EPIC/ORIGINS who did not complete the questionnaire (42%), and they were included in analyses if menopausal status, reason for menopause, and date of menopause data were available. Date of menopause was defined as the date of final period for natural menopause or of bilateral oophorectomy in surgical menopause; menopausal status for women undergoing hysterectomy with preserved ovaries was based on follicle stimulating hormone (FSH) levels. HT use was categorized dichotomously based on whether systemic (i.e., oral or patch) estrogen was taken within 5 years of menopause. Participants with menopause due to chemotherapy were not included in the sample because

of potential confounding effects of chemotherapy.[13] Women who were amenorrheic because of hormonal intrauterine device use, surgical endometrial ablation, or ovary-sparing hysterectomy were only included in our sample if the date of menopause was available through sequential FSH measurements. Strengthening the Reporting of Observational Studies in Epidemiology cohort reporting guidelines were used.[22]

Outcome Measures

Functional Variables

The primary functional outcome measure was the MS Functional Composite (MSFC).[23] This is an empiric rating scale based on participant performance on cognitive (Paced Auditory Serial Addition Test [PASAT]), fine motor [9-Hole Peg Test (9HPT)], and walking [Timed 25-Foot Walk (T25FW)] tasks. Thresholds for clinically significant worsening were previously defined for each outcome and are defined as change by 20% for the composite score.[17,18] MSFC z-scores were calculated as previously described.[24] Mean performance of the current sample at baseline was used as reference. The secondary functional outcome measure was the clinician-derived EDSS,[25] a measure of 7 functional systems including pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, and cerebral domains. Global scores on the EDSS range ordinally from 0 to 10 in increments of 0.5 (after a score of 1.0) with higher scores indicating worse function. A continuous measure, the MS Severity Score (MSSS), was also calculated using EDSS and disease duration.[18]

Serum NfL

Serum NfL values were measured using homebrew Simoa assay, as described previously.[17,26] Age-adjusted NfL z-scores were calculated using an assay-specific reference database of 485 healthy control samples from the GeneMSA study.[26,27] NfL z-scores are a measure of deviation from values observed in healthy controls: for example, a NfL z-score of 1 means that NfL concentration deviates by 1 SD from values in the reference database (84th percentile) adjusted for relevant physiologic factors.

Neuroimaging Variables

MRI scans were collected annually for all participants on the same research 3T scanner and analyzed by the EPIC neuroimaging team with a series of semiautomated pipelines. T1 sequences with and without gadolinium-diethylenetriamine pentaacetic acid and T2 sequences were acquired. T2 lesion volume was calculated using semiautomated lesion segmentation software (Amira [FEI, Hillsboro, OR] and Lesion Segmentation Toolbox [Structural Brain Mapping Group, Jena, Germany]).[20]

Covariates

Covariates for the primary analyses were selected a priori based on their hypothesized role as confounders: age at examination, BMI,[30] and tobacco use.[31] We created a directed acyclic graph after analysis to represent the hypothesized relationship between these variables (Figure 1). Additional demographic, clinical, and reproductive variables were selected for inclusion in exploratory sensitivity analyses, based on their previously reported association with either MS functional status and disease progression (disease duration,[28] disease-modifying therapy (DMT) efficacy,[29] relapses in the past year) or their effect on systemic estrogen levels (HT use,[32,33] reason for menopause[34]). There was no clinical or statistically significant effect of vitamin D levels in a similar cohort, so this was not included.[13] Smoking status, date of birth, and date of symptom onset were collected at baseline. MS phenotype, DMT use, relapses in the past year, and BMI were reassessed at each visit. DMTs were categorized by efficacy: modest (glatiramer, interferons), moderate (fumarates, fingolimod, teriflunomide, cladribine), and high (e.g., natalizumab, alemtuzumab, and anti-CD20 B-cell–depleting therapies: rituximab, ocrelizumab, ofatumumab).[11]

Standard Protocol Approvals, Registrations, and Patient Consents

The research protocol was approved by the institutional review board at UCSF (IRB11-05903, IRB14-15278). All participants provided informed consent in accordance with the Declaration of Helsinki.

Data Availability

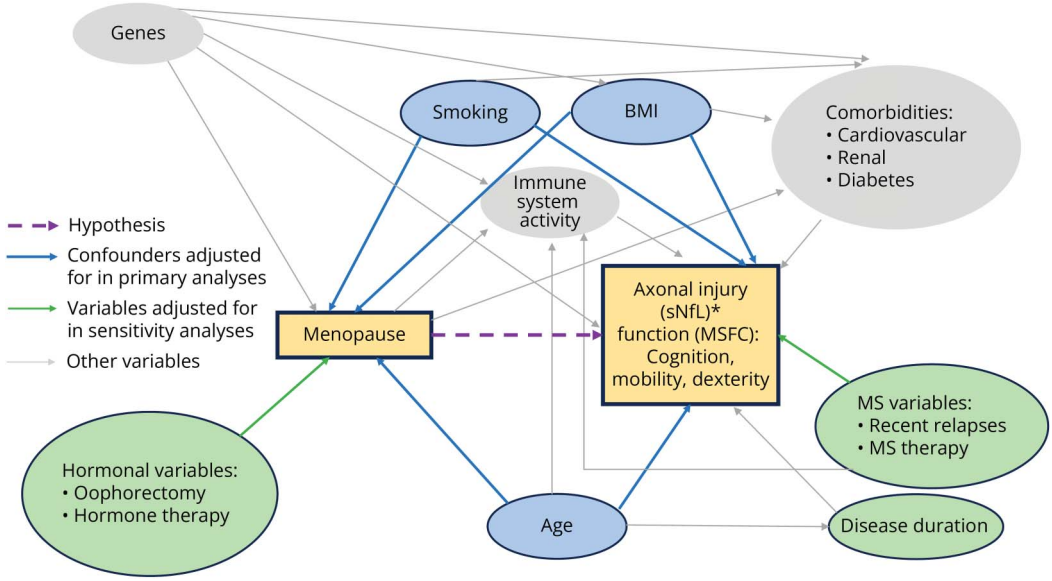
Deidentified data will be shared with qualified investigators on reasonable request.

Statistical Analyses

A Priori Analyses

To examine the effects of menopause on MS progression, the primary measures were MSFC z-score (functional outcome) and sNfL levels (paraclinical MS outcome). The EDSS score was a secondary outcome. Outcomes representing a subscale or adjusted version of a primary or secondary outcome were designated exploratory and included the subitems of the MSFC, the MSSS (EDSS adjusted for disease duration[35]), and age-adjusted sNfL. Total T2 lesion volume was included as a measure of overall disease burden, with change in T2 lesion volume over time used as an approximation of neuroinflammation. Segmented regression with a change in the slope at the time of menopause, our a priori biological inflection point, was fit using a linear mixed-effect regression model (assumes normal distribution) separately for each numeric outcome to account for multiple observations per participant over time. We estimated the slopes before and after menopause using a separate term in the model through an indicator term (before or after menopause) and time (years) as continuous. Using the SAS v9.4 estimate statement, we assessed the difference in slopes between them. Random intercepts and separate slopes before and after menopause were fit with the unstructured variance covariance matrix, except for MSFC where the variance component was used. We also assessed whether the log transformation improved

Figure 1 Hypothesized Relationship Between Covariates and Outcome Measures



* Note that age is corrected for in the exploratory outcome, age-adjusted sNfL. sNfL = serum neurofilament light chain.

the fit of the models, except for those outcomes already transformed using z-scores, and we found that the results were similar and, therefore, present the untransformed results. All models were adjusted for age (years), BMI (lbs), and tobacco use (current/former/never), except for "age-adjusted sNfL," which was not adjusted for "age."

Mixed model analyses account for both within-subject and between-subject variations. They also adjust for differential loss to follow-up and produce unbiased estimates even when some individuals have missing observations.

No formal sample size was generated, given that the sample size was fixed based on the available participants from UCSF. This approach was used in comparable studies and yielded statistically significant results with smaller sample sizes.[13] We used 184 participants for each model so that the same participants were represented.

We summarized demographic and clinical characteristics of participants at study visit using medians, means (SD), and interquartile range (IQR) for numeric variables and frequency and percentage for categorical. SAS v.9.4 was used for analyses. Findings were considered statistically significant after Holm-Bonferroni correction for our 2 primary outcome measures (MSFC and sNfL) and secondary outcome (EDSS), otherwise 2-sided $p < 0.05$ for our exploratory outcomes, which were not adjusted for multiple testing.

Sensitivity Analyses

A number of sensitivity analyses were performed to investigate the effect of other covariates on our outcomes and to evaluate the robustness of FMP as an inflection point. Additional regression analyses of our main outcomes were performed with the exploratory covariates listed above. The inflection point was artificially varied from −4 years before menopause and 4 years after menopause.[36] Segmented regression models were fit with an unknown inflection point. The inflection point was estimated using nonlinear regression modeling.[37] For the MSFC model, the results did not converge regarding determining an inflection point, and a segmented regression was, therefore, not included but rather a standard linear mixed model with random intercepts. The main effects included years from FMP adjusted for age (years), BMI, and tobacco use. The models were also evaluated using a quadratic model (years from FMP^2) and cubic model (years from FMP^3) while including the lower order terms in the model. The -2 log likelihood between nested models (using maximum likelihood) was used to assess the best fit.

Results

Participants

The final sample included 184 participants (eFigure 1). This included those who were enrolled in EPIC/ORIGINS premenopausally and were followed through their menopausal transition (n = 100), those who were enrolled in EPIC/ORIGINS postmenopausally (n = 70), and those who were enrolled in EPIC/ORIGINS premenopausally and for whom postmenopausal data were not available (n = 14). NfL data were analyzed in batch, before enrollment of some participants, and were, therefore, available for 161 (89%) of 184 participants included in our analyses. Data were obtained using a questionnaire with chart

validation for 80% of the sample and directly by chart review for 20%.

Demographic, Clinical, and Menopausal Characteristics of Participants

Demographic and Clinical Characteristics at the Most Recent Study Visit

The median age at first MS symptom onset was 37 years (IQR = 10, range: 16–61). At the most recent visit, the median age was 63 years (SD = 8); 69% of participants had relapsing-remitting MS. The median MS duration was 24 years (IQR = 13, range: 3–64), and the median EDSS score was 2.5 (IQR = 2, range: 0–8). The median years of study enrollment were 13 (IQR = 4, range: 1–17). Most participants were non-Hispanic White (83%). Characteristics of the subset of individuals with NfL data are presented in eTable 1 and Table 1.

Menopausal Characteristics

Of the 184 postmenopausal participants included, 85% of the sample had natural menopause and 15% surgical menopause after bilateral oophorectomy. The median age at natural menopause was 50 years (IQR = 5, range: 33–60). The median age at surgical menopause was 45 years (IQR = 10, range: 30–63). Overall, 17% of participants used estrogen-containing systemic menopausal HT for at least 1 year. At menopause, the median EDSS score was 2.5 (IQR = 2) and the median disease duration was 13 years (IQR = 15).

Changes in Markers of Progression After Menopause

Functional Outcomes

Menopause reflected an inflection point in the primary functional outcome, the MSFC, with accelerated worsening observed after menopause (slope difference 0.08 (95% CI 0.01–0.14), $p = 0.0163$, Holm-Bonferroni adjusted $p = 0.0489$) in analyses adjusted for age, BMI, and tobacco use (Figure 2A). For all individual MSFC components, the slope difference indicated worsening after menopause (PASAT slope difference 0.06 (95% CI −0.10 to 0.23), $p = 0.45$, and 9HPT slope difference −0.23 (95% CI −0.49 to 0.03), $p = 0.09$), with a significant effect for the Timed 25-Foot Walk (slope difference −0.46 (95% CI −0.78 to −0.15), $p = 0.0043$). However, for the EDSS, there was a statistically significant deceleration in worsening after menopause (slope difference 0.05 (95% CI 0.01–0.09), $p = 0.0200$) (eFigure 2). Changes in the MSSS were not statistically significant (slope difference 0.03 (95% CI −0.04 to 0.08), $p = 0.45$). Results were similar with adjustment for MS variables (e.g., DMT efficacy and disease duration, in eTable 2). Full model results are given in Table 2.

Table 1 Study Sample Characteristics

	N = 184
Age at MS onset (median, IQR)	37 (10)
Recent age (median, IQR)	63 (8)
Relapsing-remitting MS (%)	69
Recent EDSS score (median, IQR)	2.5 (2)
Disease duration (median, IQR)	24 (13)
Non-Hispanic White (%)	96
Natural menopause (%)	85
Age at menopause (median, IQR)	50 (5)
Menopausal hormone therapy use (%)	17

Abbreviations: EDSS = Expanded Disability Status Scale; IQR = interquartile range; MS = multiple sclerosis.

Serum Biomarker

Menopause also reflected an inflection point for our primary biomarker outcome, sNfL, with more rapid increase in sNfL observed after menopause (slope difference −0.95 (95% CI −1.74 to −0.16), $p = 0.0194$, Holm-Bonferroni adjusted $p = 0.0388$). This was similarly observed using age-adjusted sNfL (slope difference −0.06 (95% CI −0.09 to −0.02), $p = 0.0019$) (Figure 2B). Results were similar with adjustment for MS variables (e.g., DMT efficacy and disease duration, in eTable 2).

Neuroinflammation

Statistical significance was not reached for the change in the slope of accrual of total lesion volume after menopause (slope difference 0.23 (95% CI −0.25 to 0.71), $p = 0.35$). This indicates that menopause does not reflect an inflection point in inflammatory activity.

Sensitivity Analyses

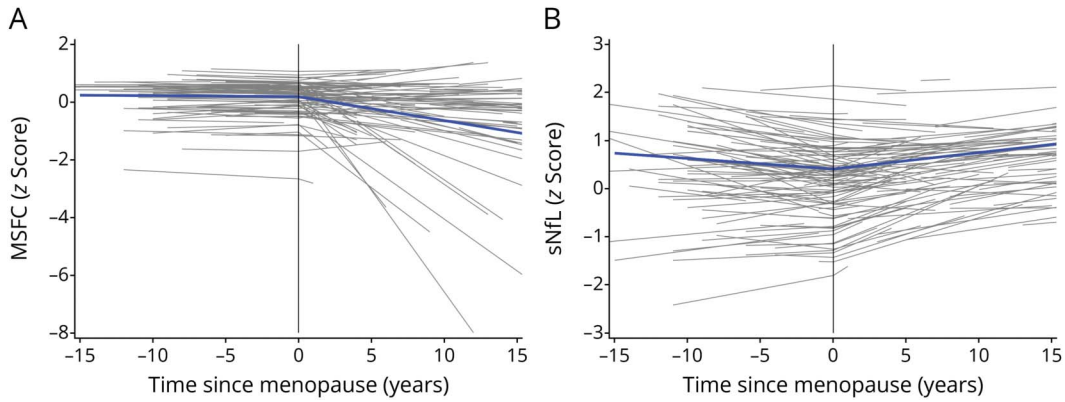
Covariates

Adjusting for the covariates of age, disease duration, DMT efficacy, relapses in the past year, BMI, smoking status, HT use, and reason for menopause did not meaningfully change the statistical significance of the results in any of the main outcome measures (eTable 3).

Alternative Inflection Point Models

The inflection point was determined using nonlinear regression modeling. For sNfL (raw), it was approximately 2.0 years before menopause; for sNfL (age adjusted), it was approximately 0.7 years before menopause; and for EDSS, it was approximately 3.3 years after menopause (Table 3, eFigure 3). For the MSFC model, we were not able to find an inflection point. The best fitting mixed model was the cubic model (eFigure 3, eTable 4). After artificial adjustment of the inflection point relative to menopause, the magnitude of the estimated slope difference was greatest at −3 years from FMP for MSFC, +3 years for sNfL, +4 years for age-adjusted sNfL, and at FMP for EDSS while the CIs of our estimated slope differences were narrowest at or within 3 years of menopause (eTable 5).

Figure 2 Worsening Trajectory Observed in Markers of MS Progression After the Final Menstrual Period (N = 184 Women)



(A) Changes in MS Functional Composite z-scores. (B) Changes in age-adjusted sNfL z-scores. Gray lines represent the slope before and after menopause for individual participants, and blue lines reflect the average slopes across the sample. MS = multiple sclerosis; sNfL = serum neurofilament light chain.

Discussion

Accelerated worsening in objective markers of neurodegeneration was observed after the FMP in this well-characterized cohort of 184 women with MS followed prospectively through their menopausal transition. This includes increase in our primary biomarker outcome, sNfL, a marker of axonal injury that correlates with silent progression in MS, and performance on our primary functional outcome, MSFC, with tasks encompassing cognition, ambulation, and upper extremity function. By contrast, menopause did not represent an inflection in accumulation of total T2 lesion volume, indicating that its association was stronger with markers of secondary progression than with those of neuroinflammation.

By evaluating continuous, objective indicators of neurologic worsening in women before and after their menopausal transition, this study improved significantly on previous work largely focused on EDSS. An inflection point in the slope of EDSS worsening was reported in some studies[4,5,13,14]; however, in this study, the opposite pattern was observed. The EDSS has known limitations including interobserver variability[38] and bias toward ambulation. A further explanation for the heterogeneous findings may be that the EDSS is an ordinal

Table 2 Postmenopausal Changes in Markers of MS Burden

	Outcome	Slope before	Slope after	Difference	95% CIs	*p* Value	Corrected *p* value[a]	Direction
Functional outcomes								
MS Functional Composite	Primary	−0.01	−0.09	0.08	0.01 to 0.14	0.0163	0.0489	Worse
PASAT3	Exploratory	−0.23	−0.29	0.06	−0.10 to 0.23	0.45	—	Worse
Timed 25-Foot Walk	Exploratory	−0.01	0.45	−0.46	−0.78 to −0.15	0.0043	—	Worse
9-Hole Peg Test	Exploratory	0.07	0.30	−0.23	(−0.49 to 0.03)	0.09	—	Worse
9-Hole Peg Test—reciprocal	Exploratory	0.00	0.00	0.00	−0.0001 to 0.0003	0.25	—	Worse
EDSS	Secondary	0.03	−0.02	0.05	0.01 to 0.09	0.0200	0.0200	Better
MS Severity Score	Exploratory	0.04	0.01	0.03	−0.04 to 0.08	0.45	—	Better
Biomarker outcomes								
sNfL (pg/mL)	Primary	−0.35	0.60	−0.95	−1.74 to −0.16	0.0194	0.0388	Worse
Age-adjusted sNfL (z-score)	Exploratory	−0.05	0.01	−0.06	−0.09 to −0.02	0.0006	—	Worse
T2 lesion volume	Exploratory	0.19	0.03	0.23	−0.25 to 0.71	0.35	—	Better

Abbreviations: EDSS = Expanded Disability Status Scale; MS = multiple sclerosis; PASAT = Paced Auditory Serial Addition Test; sNfL = serum neurofilament light chain.
Analyses adjusted for age, BMI, and tobacco use, except for "age-adjusted sNfL," which was not adjusted for age. Statistical significance was set at $p < 0.05$.
[a] Holm-Bonferroni correction for multiple comparisons for 2 primary outcomes and one secondary outcome.

Table 3 Estimated Inflection Point Using Nonlinear Regression Models for Each Outcome

Outcome	Yrs from FMP
MSFC	NA
sNfL (raw)	−2.0
AA-sNfL (z-score)	−0.7
EDSS	3.3

Abbreviations: EDSS = Expanded Disability Status Scale; FMP = final menstrual period; MSFC = Multiple Sclerosis Functional Composite; sNfL = serum neurofilament light chain.
Negative number indicates a statistical inflection point before the final menstrual period.
NA: an inflection point was not found for the MSFC.

measure, and differences between each unit in the global score may not reflect linear increases in disability, with progression in the EDSS known to occur more slowly as disease advances, making it difficult to assess changes in the rate of progression over time.[16] Another possibility is that objective measures—such as task performance and biomarkers—are sensitive to different aspects of progression than global clinician-rated measures, which can also be confounded by "progression independent of MS."[15] Changes observed may also be influenced by processes other than secondary progression of MS that accelerate after menopause, for example, vascular disease.

Distinguishing the effects of reproductive aging from those of chronological aging is notoriously difficult in a condition like MS. Reproductive aging is itself age-dependent, and there is a general trend toward accelerated disease progression after the age of 45 (i.e., perimenopausal age) in both men and women with MS, with similar trajectories seen in men and women with late-onset MS.[39] Furthermore, defining the menopausal transition based on the final menstrual cycle is confounded, and there is known fluctuation in gonadal hormone production before the FMP.[40] Quantitative markers of ovarian reserve such as AMH, which is itself correlated with neurologic aging in our MS cohort,[41] are depleted even before the premenopausal stage of reproductive aging[40] and, therefore, cannot be used to assess the menopausal transition. Alternative approaches to evaluating the effect of menopause on MS trajectories are also challenging. For example, comparing men and women would be inappropriate because of gender differences in MS clinical course and sex-specific changes in men (andropause). Comparing separate cohorts of premenopausal and postmenopausal women would introduce an even greater confounder of chronological age. These analyses considered within-subject change and were adjusted for age, BMI, and tobacco use, with significant effect of menopause persisting.

These findings motivate further research into the mechanistic effects of estrogen and other gonadal hormones within the CNS. In human females, most studies focused on estrogen that is believed to be neuroprotective through anti-inflammatory and antiapoptotic effects, among others.[10,42] In early clinical trials, exogenous estrogens reduced inflammatory activity in nonpregnant women with MS.[43,44] However, testosterone could play a less well-understood role.[3,45] There is increasing evidence that gonadal hormones affect neural repair mechanisms, and further understanding of their role may illuminate new therapeutic advances in age-related illnesses.[45] In addition, this study could not adequately evaluate impact of HT because HT use was infrequent. HT use did not show consistent benefits in previous studies[33,46]; however, there may be important differences in effect based on estrogen type (e.g., Premarin vs synthetic estradiol) that are not yet understood.

Strengths of this study include its deep phenotyping, large sample size, and long duration of follow-up. This study's focus primarily on objective measures of function and paraclinical markers represents a significant advance over previous studies using EDSS. There was a larger sample size than other comparable studies that found statistically significant results[13] and with a long period of follow-up. In addition, self-report of menopausal variables was validated against the medical record to ensure robust assessment of menopausal age and type. Finally, possible covariates such as DMT strength, HT use, smoking status, and BMI were accounted for.

Potential improvements to the study include analysis of additional hormonal variables (e.g., FSH, estradiol, and testosterone) and MS biomarkers (e.g., regional MRI gray matter volume and glial fibrillary acidic protein) and prospective collection of menopausal variables. In addition, future studies would benefit from adjustment for other possible confounders (e.g., genetic factors), which we did not have available to analyze in this study. The results also show that age-adjusted sNfL may be an effective primary outcome in future studies. In addition, future research would benefit from looking separately at individuals undergoing surgical menopause at an age younger than is typical for natural menopause, as has been performed previously, to better distinguish the effects of menopause from aging. Because this cohort included only 14 such individuals, we were insufficiently powered to perform subgroup analyses.

Limitations of the study include the imprecision of using FMP as an approximation of loss of gonadal estrogen. While our findings substantiate the hypothesis that menopause represents a general inflection point in accumulation of disease burden, we do expect some individual variation around this time point. There is significant hormonal fluctuation during the perimenopausal period, which begins approximately 3 years before menopause.[40] Thus, the date of FMP only approximates when we might expect the neuroprotective effects of estrogen and other gonadal hormones to diminish. In addition, it is not well understood on what time

line the absence of these hormones might translate into changes in functional or biomarker outcomes. Indeed, we did see variation when we calculated the best statistical inflection point for each outcome, although altogether most of our findings were within the perimenopausal window. Thus, caution must be used in interpreting our results, particularly for the MSFC, where a statistical inflection point was not found. We instead found that a linear mixed regression model was the best fit, showing that the MSFC scores decreased over time in a linear fashion relative to years from FMP (eFigure 4).

Additional limitations of the data set include the comparatively mild disability in our sample (median EDSS score of 2.5 at menopause), likely reflective of long-term treatment, which may influence observations regarding disease progression. In addition, while age and BMI were controlled for in sNfL analyses, other age-related conditions (e.g., renal disease) may contribute to sNfL worsening (Figure 1).[19] It is similarly possible that inflammatory activity, known to affect sNfL, contributed to our findings; however, inflammatory activity is known to diminish with age, and we did not find an inflection point in T2 lesion volume accumulation at the time of menopause. Another limitation is that not all participants completed the Lifestyle Questionnaire, and for 20% of the sample, data were obtained exclusively through chart review, thus introducing a potential source of bias. However, for participants who did complete the questionnaire, data in their medical record were highly concordant (>80%). In addition, unless sequential FSH levels were available, the specific date of menopause was not known for individuals who were not menstruating during their time of menopause—because of either hysterectomy, endometrial ablation, or use of hormonal intrauterine device; thus, we were unable to include them in our analyses. Furthermore, the cohort was racially and ethnically homogeneous. While more recently enrolled participants in the EPIC2 and ORIGINS cohorts reflect more diverse ancestries (i.e., less than 70% of participants are non-Hispanic White[47]), the current mostly White cohort followed since before 2010 likely reflects both earlier biases in research inclusion and, perhaps, differences in MS incidence or ascertainment. Because various sociocultural factors can influence the age and experience of menopause, prospective studies are needed in more diverse populations in the future. The results also cannot be generalized to transgender men or nonbinary individuals with ovaries, who may experience different trajectories because of use of gender-affirming hormonal therapies. Finally, as with many observational studies, caution must be used in interpreting the results, especially given the relatively small sample size of the data set and the lack of one or more replication cohorts.

These findings suggest that further inquiry into the possible neurobiological effects of this major hormonal transition is warranted. Indeed, our results imply that changes in gonadal hormones may be associated with changes in functional and biological markers in MS. Further research should investigate possible clinical implications, including need for more targeted disease, symptom, and rehabilitation management around the time of menopause.[48] Menopause is a clinically meaningful transition and an important topic of study in neurologic diseases.

Author Contributions

H.E. Silverman: drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data. A. Bostrom: analysis or interpretation of data. A.N. Nylander: drafting/revision of the manuscript for content, including medical writing for content. A. Akula: analysis or interpretation of data. A.A. Lazar: drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data. R. Gomez: major role in the acquisition of data. A. Santaniello: major role in the acquisition of data. A. Renschen: major role in the acquisition of data. M.M. Harms: major role in the acquisition of data. T.P. Cooper: major role in the acquisition of data. R. Lincoln: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. S. Poole: analysis or interpretation of data. A. Abdelhak: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. R.G. Henry: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. J. Oksenberg: drafting/revision of the manuscript for content, including medical writing for content. S.L. Hauser: drafting/revision of the manuscript for content, including medical writing for content. B.A.C. Cree: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. R. Bove: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data.

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Disclosure

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