Sex-Specific Differences in Life Span Brain Volumes in Multiple Sclerosis

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ABSTRACT

BACKGROUND AND PURPOSE: Numerous sex-specific differences in multiple sclerosis (MS) susceptibility, disease manifestation, disability progression, inflammation, and neurodegeneration have been previously reported. Previous magnetic resonance imaging (MRI) studies have shown structural differences between female and male MS brain volumes. To determine sex-specific global and tissue-specific brain volume throughout the MS life span in a real-world large MRI database.

METHODS: A total of 2,199 MS patients (female/male ratio of 1,651/548) underwent structural MRI imaging on either a 1.5-T or 3-T scanner. Global and tissue-specific volumes of whole brain (WBV), white matter, and gray matter (GMV) were determined by utilizing Structural Image Evaluation using Normalisation of Atrophy Cross-sectional (SIENAX). Lateral ventricular volume (LVV) was determined with the Neurological Software Tool for REliable Atrophy Measurement (NeuroSTREAM). General linear models investigated sex and age interactions, and post hoc comparative sex analyses were performed.

RESULTS: Despite being age-matched with female MS patients, a greater proportion of male MS patients were diagnosed with progressive MS and had lower normalized WBV (P < .001), GMV (P < .001), and greater LVV (P < .001). In addition to significant stand-alone main effects, an interaction between sex and age had an additional effect on the LVV (F-statistics = 4.53, P = .033) and GMV (F-statistics = 4.59, P = .032). The sex and age interaction was retained in both models of LVV (F-statistics = 3.31, P = .069) and GMV (F-statistics = 6.1, P = .003) when disease subtype and disease-modifying treatment (DMT) were also included. Although male MS patients presented with significantly greater LVV and lower GMV during the early and midlife period when compared to their female counterparts (P < .001 for LVV and P < .019 for GMV), these differences were nullified in 60+ years old patients. Similar findings were seen within a subanalysis of MS patients that were not on any DMT at the time of enrollment.

CONCLUSION: There are sex-specific differences in the LVV and GMV over the MS life span.

Keywords: Female, lateral ventricular volume, menopause, MRI, multiple sclerosis, sex.

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Introduction

Multiple sclerosis (MS) is an inflammatory, demyelinating, and neurodegenerative disease of the central nervous system, which presents with intermittent episodes of clinical worsening that are followed by either full or partial recovery. Recent studies have also demonstrated early and continuous underlying neurodegeneration, which is more strongly associated with disability outcomes.1 As presumably an autoimmune disease, MS also demonstrates greater disease susceptibility among the female population.2 This female-to-male ratio has varied from 1.5:1 in the early 20th century and has increased up to almost 3.5:1 in 21st.3,4 For example, a longitudinal study performed through the Canadian health system showed a general increase in MS prevalence as a result of disproportional increment of newly
Furthermore, the Swedish MS registry reported that the female-to-male ratio has increased from 1.7:1 for population born within the 1930s to 2.67:1 for population born in the 1980s. The temporal changes and differences in MS epidemiology are often explained by multitude of environmental factors, inherited immunological sex differences, biologically different structure of the brain, and endocrine fluctuations related to menarche, menopause, or pregnancy. For example, multiple studies have suggested that menopause can contribute toward significant worsening of MS symptoms and hormonal replacement therapy may potentially reverse those changes. As such, established MS mouse models (experimental autoimmune encephalomyelitis) have confirmed the effect of estrogen hormone as a potent neuroprotective, anti-inflammatory, and remyelinating agent. The absence of high estrogen levels within the male MS patients may further provide explanation for the findings of greater rate of global grey matter (GM) volume loss and subcortical GM atrophy. For instance, just after 6 years post their diagnosis, male MS patients display decreased subcortical volumes and lower cognitive performance when compared to their female counterpart. Similarly, a larger cohort of 763 MS patients showed significant sex differences where males demonstrate lower GM volume and more advanced central atrophy.

Based on this background, we aimed at determining sex-specific differences in global and tissue-specific brain volumes in a large real-world magnetic resonance imaging (MRI) database of more than 2,000 samples. Previous sex-based MRI investigations generally utilize smaller controlled sample sizes that may have been underpowered in detecting patterns of brain volume trajectories. Based on preplanned post hoc testing for different age periods, we hypothesized that lifetime periods marked with presumed low estrogen levels will be associated with lower brain volumes. Such sex-based studies have the potential to advance the understanding regarding the relationship between sex effects on the MS pathophysiology.

**Methods**

**Study Population**

The study population utilized in this analysis was derived by retrospective collection of demographic, clinical, and MRI data from MS patients, which were prospectively followed by the University at Buffalo MS clinic during the period of 2006 and 2016. The inclusion criteria included: (1) being 18 or older at the day of study enrolment, (2) being diagnosed with MS as per 2005- and 2010-revised McDonald criteria or being clinically isolated syndrome (CIS) patient, (3) availability of age, sex, Expanded Disability Status Scale (EDSS) scores, disease duration, and use of disease-modifying treatment (DMT) data, and (4) availability of MRI examination on either 1.5-T or 3-T scanner. Contrarily, the exclusion criteria included: (1) being pregnant or nursing mother and (2) presence of another major neurological disease apart from MS. Based on clinical history and presentation, the MS patients were further classified into relapsing-remitting (RRMS) or progressive (PMS) phenotype. The retrospective study was approved by the University at Buffalo Institutional Review Board (IRB), and given the retrospective nature of the study, the informed consent requirement was waived.

**MRI Acquisition and Analysis**

MRI scans were collected during the period of 2006 and 2016. When there were multiple scans available, the first available scan of the patient was utilized. All patients were scanned using either 1.5-T Signa 4x/Lx or 3T Signa Excite HD MRI scanner (General Electric, Milwaukee, WI, USA) with eight-channel head and neck coil. There were no major hardware changes nor software changes over the study enrollment. The sequences utilized for this analysis included: (1) axial T2-Fluid-Attenuated Inversion Recovery (FLAIR) sequence with echo time (TE)/inversion time (TI)/repetition time (TR) of 120/2,100/8,500 ms, flip angle (FLIP) = 90°, echo train length = 24, with 3-mm slice thickness and no gap; (2) high-resolution 3-dimensional T1-weighted image (WI) with a fast, spoiled, gradient echo, and magnetization prepared inversion recovery pulse (IR-FSPGR), TE/TI/TR = 2.8/900/5.9 ms, FLIP = 10°, and 1-mm slice thickness with no gap.

Global and tissue-specific brain volumes were determined on the 3-dimensional T1-WI utilizing Structural Image Evaluation using Normalisation of Atrophy Cross-sectional (SIENAX) software (version 2.6, FMRIB, Oxford, UK). In short, the brain and skull images are extracted and affine-registered to MNI152 space. A skull-constrained registration was used to calculate a scaling factor to normalize volumes for head size. Normalized whole brain volume (WBV), white matter volume (WMV), and GM volume (GMV) were segmented after application of a lesion in-painting technique, which minimizes the impact of T1 hypointensities on tissue segmentation. Brain scans with insufficient quality and scans that failed the analysis were excluded from the study. In comparison to SIENAX, the lateral ventricular volume (LVV) was determined with previously described Neurological Software Tool for REliable Atrophy Measurement (NeuroSTREAM) on the T2-FLAIR images.

**Statistical Analysis**

All analyses were performed with Statistical Package for the Social Science (SPSS), version 25.0 (IBM, Armonk, NY, USA). Data distribution and homoscedasticity were determined by Kolmogorov test of normality and by visual inspection of Q-Q plots of the standardized residuals. Data comparison was performed with $\chi^2$ (for categorical variables such as race, disease phenotype, scanner use, and DMT use), Student’s $t$-test (used for parametric variables such as age, disease duration, and MRI-derived brain volumes), and Mann-Whitney $U$-test (for nonparametric variables such as EDSS scores). General linear models (univariate analysis of variance) were utilized where the MRI-derived volumes were set as dependent variables and sex, age, SIENAX scaling factor (for NeuroSTREAM-based LVV, whereas SIENAX volumes are normalized), MRI scanner field strength, and an interaction between age and sex as independent predictors. Parameter estimates, standard errors (SE), effect size ($r^2$), and model-derived statistics are reported. The homogeneity of variance was tested with Levene’s test of equality and lack-of-fit tests were also performed. Moreover, models that additionally included DMT use and disease phenotype as new covariates were created and discussed. Due to the co-linearity between age and disease duration, we only include age in the analysis. Graphs with locally estimated scatter-plot smoothing (LOESS) were used to visualize the data. Models that resulted with significant age and sex interaction were
corroborated with post hoc analysis, which utilized Student’s t-test and determined brain volume differences within subgroups of patients with 18-29, 30-39, 40-49, 50-59, and 60+ years old. In order to separate the potential effect of DMT, an age-based post hoc sex comparison of LVV and GMV in MS patients not treated with any DMT was also performed. Effect size was determined by Cohen’s d where <.2 represents no effect, .2-.5 represents small effect, .5-.8 represents medium effect, and >.8 represents large effect. Similarly, ANOVA-based $\eta^2$ of >.1 represents small effect, .01-.06 medium effect, and >.14 large effect. P-values lower than .05 were considered statistically significant.

**Results**

**Demographic, Clinical, and MRI-Derived Characteristics**

The demographic, clinical, and MRI-derived characteristics of the MS patients are shown in Table 1. The study population consisted of 2,199 MS patients with female/male ratio of 1,651/548, mean age of 46 years old, average disease duration of 11.9 years, and median disability score of 2.5 EDSS (interquartile range 1.5-4.5). Based on the clinical phenotype, the study population consisted of 192 CIS, 1,554 RRMS, and 453 PMS patients. Approximately half of the patients (1,107, 50.3%) were scanned on the 1.5-T MRI scanner, whereas the remaining half (1,092, 49.7%) on the 3-T MRI scanner with no differences between sexes. Overall, the calculation of SIENAX-based brain volume failed in 232 (10.6%) MRI scans. When compared to the entire population, more males failed the SIENAX protocol (female/male ratio of 1,651/548 vs. 158/74, $\chi^2$-test $P = .021$). The demographic differences between the sexes remained the same within the sample that had successful SIENAX analysis as well ($n = 1,967$). There were no differences in age (45.5 vs. 45.8 years old, Student’s t-test $P = .636$), disability (median EDSS 2.5 vs. 3.0, Mann-Whitney U-test $P = .064$), scanner use (756/737 vs. 217/257 for 1.5-T and 3-T, $\chi^2$-test $P = .653$). As previously shown, males from the SIENAX cohort had longer disease duration (Student’s t-test $P = .028$) and had a greater proportion of PMS ($\chi^2$-test $P < .001$). In terms of SIENAX-derived brain volumes, the MS patients had mean WBV of 1,532.2 mL, WMV of 850.1 mL, GMV of 682.7 mL, and NeuroSTREAM-derived LVV of 23.4 mL. Furthermore, sex differences in clinical and MRI-derived volumes are shown in Table 1. There were no differences in age (Student’s t-test $P = .727$), disease duration (Student’s t-test $P = .051$), disability levels (Mann-Whitney U-test $P = .006$), DMT use ($\chi^2$-test $P = .065$), nor in use of different MRI scanners ($\chi^2$-test $P = .074$) between the male and female MS patients. Despite the nonsignificant age and disease duration difference, male MS patients had greater proportion of PMS when compared to female MS patients ($\chi^2$-test $P < .001$). Moreover, male MS patients exhibited significantly smaller normalized WBV (1,515.5 vs. 1,537.5 mL, Student’s t-test $P < .001$), normalized GMV (666.1 vs. 687.9 mL, Student’s t-test $P < .001$), and greater absolute LVV (27.8 vs. 18.2 mL, Student’s t-test $P < .001$).

**Sex-Specific Brain Volume Throughout the MS Life Span**

The effects of sex, age, MRI strength, head size (SIENAX scaling factor), and sex and age interaction on the LVV are shown in Table 2. In addition to the large effects derived from age (Beta $= -.49$, SE $= .05$, $\eta^2 = .099$, F-statistics $= 215.5$, $P < .001$) and head size (Beta $= -30.1$, SE $= 2.6$, $\eta^2 = .066$, F-statistics $= 139.3$, $P < .001$), the interaction between sex and age had an additional significant effect on the resulting LVV (Beta $= -.12$, SE $= .06$, $\eta^2 = .002$, F-statistics $= 4.53$, $P = .033$). The differences in LOESS-derived LVV between male and female MS patients are shown in Figure 1. Comparably, GMV showed similar effect derived from the sex and age interaction (Beta $= -.7$, SE $= .3$, $\eta^2 = .02$, F-statistics $= 4.59$, $P = .032$). Differences in LOESS-derived sex-based GMV are shown in Figure 2. Such interactions were not detected for WBV nor for WMV (results not shown). Additional LVV and GMV models that include DMT use and disease subtype were also created. Within the new LVV model ($R^2 = .218$), no significant main effect of DMT use was noted ($P = .45$), whereas MS subtype was significant contributor (Beta $= 3.0$, SE $= .49$, $\eta^2 = .018$, F-statistics $= 36.4$, $P < .001$). The age and sex interaction was rendered insignificant but retained in the model ($B = -.1$, SE $= .06$, $\eta^2 = .02$, F-statistics $= 3.314$, $P = .069$). Despite the addition of the significant effects of MS subtype ($P = .02$) and DMT use ($P = .006$), the age and sex interaction remained significant (Beta $= -.8$, SE $= .3$, $\eta^2 = .03$, F-statistics $= 6.104$, $P = .003$) in the new GMV model ($R^2 = .317$). Furthermore, sex-based post hoc comparisons are shown in Table 3. There were no statistically significant differences in LVV size between female and male patients in the 18-29 years old age bracket (14.6 vs.17.4 mL, $d = .287$, $P = .089$) and in the 30-39 years old age bracket (17.7 vs. 18.8 mL, $d = .107$, $P = .347$). In comparison, middle age female MS patients had significantly smaller LVV when compared to age-matched male MS patients (for 40-49 age bracket; 21.0 vs. 29.9 mL, $d = .571$, $P < .001$; and for 50-59 age bracket; 23.9 vs. 31.3 mL, $d = .515$, $P < .001$). Last, there was no significant difference in LVV between the aging [60+] female and male patients (32.4 vs. 36.0 mL, $d = .265$, $P = .174$). Similarly, male MS patients had lower total GMV when compared to female MS patients in the first four age brackets; that is, aged 18-29 years old (773.6 vs. 744.2 mL, $d = .416$, $P = .019$), 30-39 years old (725.5 vs. 703.5 mL, $d = .305$, $P = .017$), 40-49 years old (683.9 vs. 656.1 mL, $d = .362$, $P < .001$), and 50-59 years old (659.9 vs. 642.6 mL, $d = .245$, $P < .001$). There was no difference in GMV between the elderly male and female MS patients (60+ years old, 624.8 vs. 620.7 mL, $d = .061$, $P = .729$). The comparison in sex-specific LVV differences is also shown in Figure 3.

Furthermore, comparison of both absolute and normalized NeuroSTREAM-based LVV in all age groups is shown in Table 4. As shown with aforementioned absolute LVV, males had larger normalized LVV at the age of 40-49 (34.8 vs. 29.2 mL, $d = .308$, $P = .001$) and at the age of 50-59 (37.7 vs. 32.4 mL, $d = .298$, $P = .002$). There were no differences in normalized LVV at earlier (18-39 years old) and later age brackets (60+ years old).

Last, a sex-based analysis on MS patients who were not treated with any DMTs at the time of the enrollment ($n = 392$) is shown in Table 5. There were no demographic and clinical differences between the 300 female and 92 male MS patients in age (46.9 vs. 47.9 years old, Student’s t-test $P = .541$), disease duration (12.3 vs. 12.1 years, Student’s t-test $P = .875$), and disability (median EDSS 2.5 vs. 3.5, Mann-Whitney U-test $P = .299$). Untreated male MS patients had greater proportion of PMS subtype ($\chi^2$-test $P < .001$). Overall, the untreated male MS patients had lower GMV (676.6 vs. 700.8 mL, $d = .31$, $P = .01$) and greater LVV (26.7 vs. 21.3 mL, $d = .36$, $P = .002$). As previously shown with the entire age-based analysis, male MS patients had lower GMV (669.5 vs. 705.8 mL, $d = .55$, $P = .017$).
Table 1. Demographic and Clinical Characteristics of the MS Population

| Demographic and Clinical Characteristics | MS (n = 2,199) | Female (n = 1,851) | Male (n = 548) | Female vs. Male P-Value |
|-----------------------------------------|----------------|-------------------|---------------|------------------------|
| Female, n (%)                           | 1,651 (75.1)   | -                 | -             | -                      |
| Caucasian, n (%)                        | 1,990 (90.5)   | 1,483 (89.8)      | 507 (92.5)    | .311                   |
| African American, n (%)                 | 192 (8.7)      | 154 (9.3)         | 38 (6.9)      |                        |
| Other, n (%)                            | 17 (0.8)       | 14 (0.9)          | 3 (0.6)       |                        |
| Age, mean (SD)                          | 46.0 (11.6)    | 45.9 (11.6)       | 46.2 (11.5)   | .727                   |
| CIS/RMS/PSM                            | 192/1,554/453  | 147/1,185/319     | 45/369/134    | <.001*                 |
| Disease duration, mean (SD)             | 11.9 (10.1)    | 12.2 (10.1)       | 11.2 (9.9)    | .051                   |
| EDSS, median (IQR)                      | 2.5 (1.5-4.5)  | 3.0 (1.5-6.0)     | 3.0 (2.0-6.0) | .906                   |
| 3-T/1.5-T MRI scanner, n                | 1,107/1,092    | 838/813           | 254/294       | .074                   |
| DMT, n (%)                              |                |                   |               |                        |
| Interferon-beta                         | 835 (37.9)     | 621 (37.6)        | 214 (39.1)    | .065                   |
| Glatiramer acetate                      | 387 (17.6)     | 297 (17.9)        | 90 (16.4)     |                        |
| Natalizumab                             | 144 (6.5)      | 104 (6.3)         | 40 (7.3)      |                        |
| Oral DMT                                | 45 (2.0)       | 30 (1.8)          | 15 (2.7)      |                        |
| Off-label medications                   | 60 (2.7)       | 42 (2.5)          | 18 (3.3)      |                        |
| No DMT                                  | 392 (17.8)     | 300 (18.2)        | 92 (16.8)     |                        |
| Unknown                                 | 336 (15.5)     | 257 (15.7)        | 79 (14.4)     |                        |
| MRI-derived brain volumes               |                |                   |               |                        |
| Normalized WBV (n = 1,967)              | 1,532 (104.1)  | 1,537.5 (103.8)   | 1,515.5 (103.2)| <.001*                |
| Absolute LVV (n = 2,199)                | 23.4 (14.7)    | 18.2 (13.8)       | 27.8 (16.3)   | <.001*                 |
| Normalized GMV (n = 1,967)              | 682.7 (84.7)   | 687.9 (86.5)      | 666.1 (76.5)  | <.001*                 |
| Normalized WMV (n = 1,967)              | 850.1 (87.1)   | 850.0 (86.8)      | 850.3 (88.1)  | .954                   |

MS = multiple sclerosis; CIS = clinically isolated syndrome; RRMMS = relapsing-remitting MS; PMS = progressive MS; EDSS = Expanded Disability Status Scale; DMT = disease-modifying treatment; WBV = whole brain volume; LVV = lateral ventricular volume; GMV = gray matter volume; WMV = white matter volume; n = number; SD = standard deviation; IQR = interquartile range.

Table 2. The Effect of Age, Sex, Tesla Strength, and the Age and Sex Interaction on the GMV and LVV in MS Patients

| LVV (adjusted $R^2 = .204$) | Beta     | Standard Error | $r^2$ | F-Statistics | P-Value |
|-----------------------------|----------|----------------|-------|--------------|---------|
| Intercept                   | 42.3     | 4.2            | .206  | 130.2        | <.001   |
| Sex (female as reference)  | 5.4      | 2.8            | .002  | 3.7          | .054    |
| Age                         | 0.49     | 0.5            | .099  | 215.5        | <.001*  |
| MRI tesla strength          | -0.17    | 0.38           | .0    | 200.2        | .653    |
| SIENAX scaling factor       | -30.1    | 2.6            | .066  | 139.3        | <.001*  |
| Sex-age interaction         | -0.12    | 0.06           | .002  | 4.53         | .033*   |

| GMV (adjusted $R^2 = .298$) | Beta     | Standard Error | $r^2$ | F-Statistics | P-Value |
|-----------------------------|----------|----------------|-------|--------------|---------|
| Intercept                   | 742.9    | 14.4           | .78   | 6964.6       | <.001   |
| Sex (female as reference)  | 54.6     | 15.3           | .06   | 12.7         | <.001*  |
| Age                         | -2.97    | 0.3            | .176  | 418.3        | <.001*  |
| MRI tesla strength          | 25.7     | 2.1            | .069  | 144.3        | <.001*  |
| Sex-age interaction         | -0.7     | 0.3            | .02   | 4.59         | .032*   |

MS = multiple sclerosis; MRI = magnetic resonance imaging; LVV = lateral ventricular volume; GMV = gray matter volume; SIENAX = Structural Image Evaluation using Normalisation of Atrophy Cross-sectional. General linear models analyzed the impact of all factors and their interactions on the size of LVV. $P$-value lower than .05 was considered statistically significant and labeled with asterisk (*). ANOVA-based $r^2$ effect size was used. Sex is utilized where female is considered reference.

and greater LVV (33.8 vs. 22.8 mL, $d = .74, P < .001$) during the age of 40-49 and 50-59, respectively. These differences were not present at the 60+ years old subgroup of untreated MS patients.

**Discussion**

We utilized three different statistical approaches in order to determine potential sex and age interactions on the brain volumes of MS patients throughout their life span. The findings of this study are twofold. First, and based on the post hoc comparisons, middle age male MS patients exhibit greater ventricular volume and lower central and GMVs when compared to age-matched female MS patients. These findings remained significant after adjusting for the head size and in subpopulation of patients not treated with any DMT. However, these differences are not significant by the age of 60 years old. Second, LOWESS-plotted estimated sex-based trajectories demonstrate shift toward accelerated LVV loss in females older than 50 years old. This finding can also be appreciated by the changes in normalized LVV from 29.2 mL at 40-49 years old to 32.4 mL at 50-59 years old (10.9% increase) and to 42.4 mL at the 60+ years old (30.9% increase). No significant positive skew is present because the
60+ age group had only 23 subjects that were older than 70 years old.

The sex discrepancy favoring greater female MS prevalence has been slowly increasing over the 20th century. Additionally, the female-to-male ratio has been largely driven by the youngest age groups, a category that is highly susceptible to lifestyle and hormonal changes. A recent large cohort study that enrolled almost 16,000 definitive MS patients showed significant widening of the sex gap. The observed epidemiological characteristics of greater female MS susceptibility can be potentially explained by experimental studies performed at a cellular level. For example, local differences in hormonal expression, balance, and production seen in MS-specific lesions and in the normal-appearing WM can account for potential sex discrepancy in the process of the demyelinating lesion formation. Increased progesterone synthesis within the normal-appearing WM of female patients can serve as an endogenous protective mechanism. Therefore, the remyelinating protective effect of estrogen (or the lack of) can be utilized as potentially explanation regarding findings that although males have lower amount of gadolinium-enhancing lesions, there is a greater chance of hypointense T1 lesions (black holes) transformation. Recent findings of greater and accelerated central brain atrophy within aging MS patients may be a result of the more prevalent and female-driven population.

The hormonal influence on MS outcomes has been recently shown in several observational and controlled clinical trial
Table 3. Post Hoc Analysis of Absolute LVV and Normalized GMV for Sex- and Age-Specific MS Groups

| Age Bracket | Sex   | Sample Size | Absolute LVV | Normalized GMV | LVV Cohen’s d | LVV P-Value | GMV Cohen’s d | GMV P-Value |
|-------------|-------|-------------|--------------|----------------|--------------|-------------|--------------|-------------|
| 18-29       | Female| n = 156     | 14.6 (9.1)   | 773.6 (77.9)   | .29          | .089        | .42          | .019*       |
|             | Male  | n = 51      | 17.4 (10.4)  | 744.2 (62.5)   |              |             |              |             |
| 30-39       | Female| n = 332     | 17.7 (10.6)  | 725.5 (78.8)   | .11          | .347        | .31          | .017*       |
|             | Male  | n = 100     | 18.8 (9.3)   | 703.5 (64.9)   |              |             |              |             |
| 40-49       | Female| n = 482     | 21.0 (12.6)  | 683.9 (78.1)   | .57          | <.001*      | .36          | <.001*      |
|             | Male  | n = 164     | 29.9 (18.1)  | 656.1 (72.4)   |              |             |              |             |
| 50-59       | Female| n = 485     | 23.9 (13.5)  | 659.9 (72.4)   | .52          | <.001*      | .25          | .011*       |
|             | Male  | n = 175     | 31.3 (15.2)  | 642.6 (68.6)   |              |             |              |             |
| 60+         | Female| n = 196     | 32.4 (18.0)  | 624.8 (73.9)   | .21          | .174        | .06          | .729        |
|             | Male  | n = 58      | 36.0 (17.1)  | 620.7 (59.7)   |              |             |              |             |

MS = multiple sclerosis; LVV = lateral ventricular volume; GMV = gray matter volume; n = number.
Student’s t-test was used. P-value lower than .05 was considered statistically significant and labeled with asterisk (*).

Fig 3. Bar plot representation of the differences in LVV and GMV between males and females at different age brackets. Both LVV and GMV are represented as milliliters and the age is represented in years. LVV = lateral ventricular volume; GMV = gray matter volume.

Table 4. NeuroSTREAM-Based LVV Adjusted for SIENAX Scaling Factor

| Age Bracket | Sex   | Sample Size | Absolute LVV | Normalized LVV | Absolute LVV Cohen’s d | Absolute LVV P-Value | nLVV Cohen’s d | nLVV P-Value |
|-------------|-------|-------------|--------------|----------------|------------------------|----------------------|---------------|-------------|
| 18-29       | Female| n = 156     | 14.6 (9.1)   | 19.8 (10.7)    | .29                    | .089                 | .17           | .309        |
|             | Male  | n = 51      | 17.4 (10.4)  | 21.8 (12.4)    |                        |                      |               |             |
| 30-39       | Female| n = 332     | 17.7 (10.6)  | 24.9 (14.1)    | .11                    | .347                 | .05           | .636        |
|             | Male  | n = 100     | 18.8 (9.3)   | 24.2 (11.5)    |                        |                      |               |             |
| 40-49       | Female| n = 482     | 21.0 (12.6)  | 29.2 (16.7)    | .57                    | <.001*               | .31           | .001*       |
|             | Male  | n = 164     | 29.9 (18.1)  | 34.8 (19.5)    |                        |                      |               |             |
| 50-59       | Female| n = 485     | 23.9 (13.5)  | 32.4 (17.4)    | .52                    | <.001*               | .29           | .002*       |
|             | Male  | n = 175     | 31.3 (15.2)  | 37.7 (18.2)    |                        |                      |               |             |
| 60+         | Female| n = 196     | 32.4 (18.0)  | 42.4 (23.3)    | .21                    | .174                 | .07           | .706        |
|             | Male  | n = 58      | 36.0 (17.1)  | 41.0 (18.7)    |                        |                      |               |             |

LVV = lateral ventricular volume; nLVV = normalized lateral ventricular volume.
The normalized LVV is derived from the SIENAX head scaling factor. P-value lower than .05 was considered statistically significant and labeled with asterisk (*).

Studies. Foremost, a randomized, placebo-controlled trial utilized oral estriol supplementation (estrogen unique to pregnancy) in women with RRMS and demonstrated significantly reduced relapse rates when compared to approved DMT use alone. Furthermore, the post hoc MRI analysis of this trial showed lower cortical GM atrophy in the estriol group compared to comparators. Despite the lower level of relapse activity, patients with no gadolinium-enhancing lesions within the treatment arm demonstrated lower cortical atrophy, indicating a primary neuroprotective effect independent of the potential anti-inflammatory properties of estriol. A recent large multi-centric observational study followed 148 female MS patients for a mean of 3.5 years before and after the menopause onset. In comparison to the significant reduction in annualized relapse rate after menopause, the patients exhibited almost a doubled rate of disability progression (from +.2 EDSS score in the period leading to menopause to +.4 EDSS score in the period after the menopause). Effects of menopause were further explored by an online reproductive survey that showed several emerging themes including perimenopausal onset of MS symptoms, MS exacerbations triggered by menopause-induced hot flashes, or escalation/worsening in the disease course. Moreover, a longitudinal study followed postmenopausal MS women for over 10 years and showed more rapid change in clinical severity measures such as EDSS scores. The negative outcomes following menopause demonstrated in the aforementioned MS population fall in line with the vast literature of observational studies, which suggests that early and prolonged loss of ovarian estradiol levels lead to a twofold increase in lifetime risk for developing dementia and fivefold increased risk for mortality from neurological disorders. Interestingly, female MS patients diagnosed before the age of 50 had delayed time to reach EDSS disability milestones when compared to the ones diagnosed after the age of 50. Moreover, females diagnosed after age of 50 have similar disability trajectories to...
the age-matched male MS patients. The findings are further emphasized by similar volumetric brain results. The comparably lower brain volume loss in female is no longer present if both sexes are diagnosed after the age of menopause. The ability of estrogen in attenuating the disease progression can be also seen through studies of female MS patients that use oral hormonal contraceptives. Periods of pill-free, low-estrogen/progesterone phase are associated with more pronounced MS symptoms. Users of oral contraceptives also had significantly lower relapse rates and less severe disease progression when compared to females that had never used hormonal contraception.

We also performed additional models that accounted for the higher rate of progressive phenotype within the male MS population and the resulting differences in DMT use. Although the age and sex interaction effect was rendered insignificant, the prevalence of PMS subtype in males in itself represents a clinical feature that would significantly contribute to greater neurodegenerative differences. A similar argument can be made for the effects of DMT use. MS patients that would present with worse clinical and MRI-based features would therefore be more likely to be prescribed more potent medications.

An important methodological aspect derived from this study should be emphasized. The LVV derived by the newly proposed NeuroSTREAM software is not significantly influenced by the differences in the scanners utilized (1.5-T or 3-T). This may be explained by the central location of the ventricles and thus being less susceptible to field distortions. Furthermore, the contrast of the ventricle-to-brain border can be easily distinguished on both types of scanners, producing similar results. Therefore, when compared to SIENAX and FIRST protocols, the LVV measure derived in such manner may prove itself as a more reliable brain atrophy proxy that can be utilized on a wider array of imaging circumstances.

Despite the brain structure in question, such effects derived from field strength differences (either from 1.5-T to 3-T or from different scanner manufacturers) have been previously demonstrated in the literature. For example, WBV measurements acquired at 1.5-T scanner may significantly overestimate the total WBV. More so, the field strength changes do particularly affect the GMV areas with particular overestimation present at the interface between the gyral surface and the CSF space. These differences can be appreciated even in specialized tertiary centers that undergo all necessary steps of multi-site MRI harmonization. That being said, our MS patients demonstrated distinctly greater LVV when compared to healthy controls described in the literature (healthy control LVV of 13.6, 14.0, 17.3, 17.0, and 20.4 mL for 20-29, 30-39, 40-49, 50-59, and 60-69 years old, respectively).

### Table 5. Post Hoc Analysis of Sex-Based Differences in LVV and GMV in MS Patients Not Treated with Any DMT

| Age Bracket | Sample Size | Absolute LVV | Normalized GMV | LVV Cohen’s d | LVV P-Value | GMV Cohen’s d | GMV P-Value |
|-------------|-------------|--------------|----------------|---------------|-------------|---------------|-------------|
| 18-29 Female | n = 31      | 12.5 (5.9)   | 788.3 (76.1)   | 0.07          | <.001*      | 0.84          | <.001*      |
| 18-29 Male   | n = 7       | 12.2 (2.6)   | 732.0 (57.3)   |              |             |               |             |
| 30-39 Female | n = 52      | 19.3 (13.9)  | 743.2 (85.5)   | 0.12          | <.001*      | 0.32          | <.001*      |
| 30-39 Male   | n = 19      | 17.9 (7.6)   | 717.9 (71.0)   |              |             |               |             |
| 40-49 Female | n = 74      | 20.4 (15.7)  | 705.8 (79.5)   | 0.46          | <.001*      | 0.55          | <.001*      |
| 40-49 Male   | n = 23      | 28.1 (17.8)  | 669.5 (48.3)   |              |             |               |             |
| 50-59 Female | n = 97      | 22.8 (12.5)  | 674.2 (70.4)   | 0.74          | <.001*      | 0.15          | <.001*      |
| 50-59 Male   | n = 26      | 33.8 (17.0)  | 663.9 (68.1)   |              |             |               |             |
| 60+ Female   | n = 46      | 27.4 (14.5)  | 638.7 (79.2)   | 0.18          | <.001*      | 0.05          | <.001*      |
| 60+ Male     | n = 17      | 29.9 (13.6)  | 635.0 (54.6)   |              |             |               |             |

MS = multiple sclerosis; DMT = disease-modifying treatment; LVV = lateral ventricular volume; GMV = gray matter volume; n = number. Student’s t-test was used. P-value lower than .05 was considered statistically significant and labeled with asterisk (*).
Another limitation to mention is that the male MS patients demonstrate an opposite-sided inflection (slowing down) in the GM-LOESS analysis. These changes should be carefully interpreted and can potentially be explained by either plateauing brain volume loss or due to significantly smaller male sample sizes within the 60+ years old group within this analysis. Given that hormones may have a significant effect on lesion accumulation and/or repair, future similar sex-based analysis of lesion trajectories is warranted.

In conclusion, our study showed sex-specific differences in LVV and GMV over the life span. The potentially accelerated central atrophy seen in elderly female MS patients may be influenced by onset of menopause and drop of estrogen levels. The interaction between sex and age remained significant contributor to MS brain volumes after adjusting for the main effects of age, head size, disease subtype, DMT use and MRI scanner. The results coincide with previous findings of perimenopausal reduction in both WMV and GMV and greater risk of neurodegenerative diseases associated with menopause.445 Contrarily, age-matched male MS patients present with significantly greater LVV and lower GM during the early and midlife period. These findings were further replicated within subpopulation of MS patients that were not treated with any DMT at the time of study enrollment. Future longitudinal studies should determine the sex-specific atrophy rate seen in elderly MS population when compared to age-matched healthy population and determine if male MS patients would require more effective early treatment.

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