IMPORTANCE Cerebrovascular reactivity (CVR) is an inherent indicator of the dilatory capacity of cerebral arterioles for a vasomotor stimulus for maintaining a spontaneous and instant increase of cerebral blood flow (CBF) in response to neural activation. The integrity of this mechanism is essential to preserving healthy neurovascular coupling; however, to our knowledge, no studies have investigated whether there are CVR abnormalities in multiple sclerosis (MS).

OBJECTIVE To use hypercapnic perfusion magnetic resonance imaging to assess CVR impairment in patients with MS.

DESIGN, SETTING, AND PARTICIPANTS A total of 19 healthy volunteers and 19 patients with MS underwent perfusion magnetic resonance imaging based on pseudocontinuous arterial spin labeling to measure CBF at normocapnia (ie, breathing room air) and hypercapnia. The hypercapnia condition is achieved by breathing 5% carbon dioxide gas mixture, which is a potent vasodilator causing an increase of CBF.

MAIN OUTCOMES AND MEASURES Cerebrovascular reactivity was calculated as the percent increase of normocapnic to hypercapnic CBF normalized by the change in end-tidal carbon dioxide, which was recorded during both conditions. Group analysis was performed for regional and global CVR comparison between patients and controls. Regression analysis was also performed between CVR values, lesion load, and brain atrophy measures in patients with MS.

RESULTS A significant decrease of mean (SD) global gray matter CVR was found in patients with MS (3.56 [0.81]) compared with healthy controls (5.08 [1.56]; P = .001). Voxel-by-voxel analysis showed diffuse reduction of CVR in multiple regions of patients with MS. There was a significant negative correlation between gray matter CVR and lesion volume (R = 0.6, P = .004) and a significant positive correlation between global gray matter CVR and gray matter atrophy index (R = 0.5, P = .03).

CONCLUSIONS AND RELEVANCE Our quantitative imaging findings suggest impairment in functional cerebrovascular pathophysiology, by measuring a diffuse decrease in CVR, which may be the underlying cause of neurodegeneration in MS.
Multiple sclerosis (MS) is considered a chronic autoimmune inflammatory disease. One of the hallmarks, however, is progressive neurodegeneration, which plays a key role in the advancement of neurological disabilities. Little is known of the link between neuroinflammation and neurodegeneration of the disease. Previous biochemical studies have shown elevated levels of nitric oxide in patients with MS produced by the activation of nitric oxide synthase secondary to repetitive proinflammatory cascades. Nitric oxide has a wide range of biological functions, one of which is a strong vasodilator that can manipulate cerebral blood flow (CBF), thus mediating neurovascular coupling that is responsible for increased blood supply during transient neural activation. In healthy volunteers, studies reported an immense increase of blood flow in the visual cortex in response to visual stimuli (an increase of more than 60%-80% compared with the resting state), indicating that intact neurovascular coupling of the brain is essential for instant and rapid oxygen delivery. However, for patients with MS, chronically high levels of nitric oxide may have detrimental effects on the vascular health of the brain; its overproduction may desensitize endothelial and smooth muscle function (vascular habituation), causing decreased vasodilatory capacity and limited blood supply for neurons that perform demanding tasks; thus, this overproduction may create neuronal activity-induced hypoxia.

Several histopathological studies have elucidated hypoxia-like tissue injury in patients with MS, which may help us better understand its pathogenic role in lesion formation and neurodegeneration. This is likely an underlying cause of progressive neurodegeneration secondary to neuroinflammation in MS. Cerebrovascular reactivity (CVR), a useful index of cerebral vascular function, can be used to measure the impaired hemodynamic response to vasodilator stimuli. Although perfusion measurements (e.g., CBF and cerebral blood volume) have been reported in numerous studies, vasoactivity has not yet been investigated in patients with MS.

A number of vasoactive stimuli have been explored for CVR assessment, including caffeine, carbon dioxide (CO₂), and injection of acetazolamide. CO₂ is the most commonly used and most appropriate vasodilator for CVR measurement owing to its practicality, reliability, and ability to be standardized. Using a fixed CO₂ gas mixture (5% by volume), previous studies have reported CVR impairment using blood oxygen level-dependent functional magnetic resonance imaging (fMRI) for several neurological diseases (e.g., Alzheimer disease and stroke). However, blood oxygen level-dependent functional MRI measures CBF changes indirectly because it relies heavily on the blood oxygenation level. The modulation of physiological blood flow can be evaluated using arterial spin labeling (ASL) perfusion MRI, which measures CBF noninvasively by magnetically labeling arterial blood water as an endogenous tracer. A few studies have shown that ASL-based techniques can reliably measure CVR (see, e.g., Tancredi et al). Our hypothesis is that patients with MS have significant abnormalities in CVR that may be due to the presence of chronically high nitric oxide levels, and such vascular hemodynamic response deficits may be a significant underlying cause of diffuse and progressive neurodegeneration. In the present study, we used a robust and well-validated pseudocollateral ASL (pCASL) perfusion MRI acquisition at normocapnia (room air) and at hypercapnia (5% CO₂) conditions to evaluate CVR in patients with MS, compared with healthy controls, and to correlate CVR and brain atrophy measures.

**Methods**

**Participants**

Nineteen patients with clinically definite MS (17 with relapsing-remitting MS and 2 with secondary progressive MS), with a mean (SD) age of 42.8 (10.2) years (10 women and 9 men), and 19 healthy control volunteers, with a mean (SD) age of 39.6 (12.9) years (6 women and 13 men), were included in our study. The protocol was approved by the institutional review board and research ethics committee at New York University School of Medicine and NYU Langone Medical Center. After receiving an explanation of the study protocol, all participants provided written informed consent and were financially compensated for participation. Patients and controls with a prior diagnosis of cardiovascular, pulmonary, cerebrovascular, or other neurological disease, as well as active smokers, were excluded. All patients had varied symptoms that included visual disturbances, numbness and tingling, weakness or fatigue, pain or sensitivity to heat, dizziness, bowel or bladder problems, or difficulty thinking clearly. All patients received 1 or more immunosuppressive or immunomodulatory drugs in the 3 years prior to the examination. Among them, 6 patients received natalizumab (Tysabri; Biogen Idec) and glatiramer acetate (Copaxone; Teva Neuroscience), 4 received interferon beta-1a (Rebif [EMD Serono/Pfizer] and Betaseron [Bayer HealthCare]), 3 received fingolimod (Gilenya; Novartis), 2 received interferon beta-1a (Avonex; Biogen Idec), and 1 received rituximab (Rituxan; Genentech/Biogen Idec). None of these medications are known to cause changes in vascular function. All participants were instructed to refrain from drinking coffee for at least 4 hours before the examination. The patients had a mean (SD) disease duration of 10.4 (7.6) years (range, 2.2-24 years) and a mean (SD) Expanded Disability Status Scale score of 2.9 (1.5) (range, 1-6).

**Magnetic Resonance Imaging**

The MRI data were obtained by use of a 3-T whole-body MR scanner (Siemens Tim Trio; Siemens Healthcare) with a 12-channel head coil, following our standard protocol for intracranial lesion assessment. This includes nonenhanced T₁-weighted (repetition time/echo time: 600/14 milliseconds), fluid-attenuated inversion recovery images (repetition time/echo time: 9010/134 milliseconds; inversion time: 2500 milliseconds) and T₂-weighted images (repetition time/echo time: 3400/119 milliseconds) covering the whole brain. Postcontrast T₁-weighted imaging (repetition time/echo time: 600/14 milliseconds) was acquired to evaluate enhancing lesions. In addition to conventional routine sequences, CBF was measured using pCASL perfusion MRI, based on single-shot gradient-echo planar imaging with the following para-
impaired cerebrovascular reactivity in MS

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meters: repetition time/echo time: 3950/17 milliseconds; labeling duration: 1470 milliseconds; postlabeling delay: 1230 milliseconds; slice thickness: 5 mm; 32 transverse slices; field of view: 22 cm; 64 × 64 in-plane matrix; and a total of 52 measurements with 26 pairs of label and control images. Labeling was performed at 97 mm below the center-of-imaging volume such that it was approximately perpendicular to the internal carotid and vertebral arteries. We used a generalized autocalibrating, partially parallel acquisition factor of 2 with a scan duration of 3 minutes and 35 seconds.

Two pCASL sequences were recorded, the first sequence under normocapnia, in which the patient breathed room air, and the next sequence under hypercapnia, in which the patient breathed a 5% CO2 gas mixture (5% CO2, 21% oxygen gas, and 74% nitrogen gas) from a Douglas bag. The participants were tightly fitted with a nose clip to restrict breathing to the mouth. During both sequences, end-tidal CO2 (ETCO2) was monitored and measured at a 2-second sampling rate, using the MR-compatible Medrad 9500 MRI Multigas Monitor. After the normocapnia scan, the hypercapnia gas was switched on, and a 1-minute, 18-second, T1-weighted anatomical imaging (repetition time/echo time: 320/2.42 milliseconds; voxel resolution size: 0.9 × 0.9 × 1 mm3) with identical imaging orientation, resolution, and slice number as the pCASL was performed for optimal image registration. This time permitted ETCO2 to reach a new steady state, allowing the second pCASL sequence to be run under the hypercapnia condition. Three-dimensional magnetization-prepared rapid acquisition with gradient echo (MPRAGE), high-resolution, T1-weighted imaging was performed for brain tissue segmentation (repetition time/echo time: 2300/2.98 milliseconds; inversion time: 900 milliseconds; voxel resolution size: 1 × 1 × 1 mm3).

Image Processing and Evaluation

The processing of images was conducted using Statistical Parametric Mapping (University College London) software in order to perform motion correction for both pCASL sequences and in order to register the geometrically matched 2-dimensional spin-echo T1-weighted images and 3-dimensional high-resolution MPRAGE anatomical images. For both pCASL sequences, the difference between the average labeled image and the control image was corrected for the differences in the acquisition delay time of the slices. The CBF maps were generated for each condition by conducting CBF calibration using

\[
\text{CBF} (\text{mL/100 g/min}) = \frac{60 \times 100 \Delta M \times \lambda}{2 \alpha \times M0 \times T1 \times (e^{-\frac{\Delta M}{T1}} - e^{-\frac{\Delta M}{T2}})}
\]

where \(\Delta M\) is the difference signal between the control and labeling states; \(\lambda = 0.9 \text{ g/mL}\) which is the blood/tissue water partition coefficient; \(\alpha = 0.86\), which is used empirically for the inversion tagging efficiency of pCASL at 3 T \(^{20,22}\); \(M0\) is the equilibrium magnetization of brain tissue determined from a whole-brain region of interest during the control condition, after accounting for T1 relaxation; \(T1 = 1.6\) seconds, which is the conventional T1 constant of blood at 3 T \(^{23}\); \(w\) is the postlabeling delay, which is different for individual slices (1.23 seconds + slice acquisition delay\(^{23}\); and \(\tau\) is the labeling duration (1.47 seconds in our data).

The resulting maps were subsequently transformed into the Montreal Neurological Institute template of 52 space masking brain-only regions and were spatially smoothed using a gaussian kernel (full-width at half-maximum = 8 mm). A CBF difference map (hypercapnia – normocapnia) was divided at each voxel by the average gray matter (GM) partial volume-adjusted normocapnia CBF to reflect the CBF percent increase. This was performed by assuming that GM perfusion is 2.5 times white matter (WM) perfusion per unit volume. \(^{24}\) These maps were normalized by individual ETCO2 increases, to adjust for participant-specific variation, resulting in CVR maps. Thus, CVR (calculated as the percent increase of normocapnic to hypercapnic CBF normalized by the change in ETCO2) represents the percent change of CBF per unit of ETCO2 (mm Hg) change. Global GM and WM CBF values were computed by overlaying the tissue mask (defined as >70% probability of being the tissue type) on the normalized CBF maps and calculating the mean value within. These values were used to calculate global CVR according to

\[
\text{CVR} (\% / \text{mm Hg}) = \frac{100 \times (\text{CBF}_{\text{hypercapnia}} - \text{CBF}_{\text{normocapnia}})}{\text{ETCO2}_{\text{hypercapnia}} - \text{ETCO2}_{\text{normocapnia}}}
\]

FireVoxel software (https://files.nyu.edu/hr18/public/\(^{25}\)) was used for semiautomatic MS lesion load quantification, after intensity uniformity correction of the fluid-attenuated inversion recovery images. Lesions were visually inspected to correct errors. The brain was segmented into GM, WM, and cerebrospinal fluid (CSF) regions based on the 3-dimensional, high-resolution, T1-weighted MPRAGE images using the Brain Extraction Tool and the Automated Segmentation Tool from the FMRIB (Functional Magnetic Resonance Imaging of the Brain) Software Library. \(^{4,26}\) Results were visually inspected and manually corrected. The fractional brain parenchymal volume, an index of brain atrophy, was computed as the ratio of brain parenchymal volume (GM and WM) to total intracranial volume (GM, WM, and CSF). Fractional GM (fGM) volume was computed as the ratio of GM volume to total intracranial volume.

Statistical Analysis

The CVR map for each participant was used for voxel-based regional analyses between patients and controls. Group statistics were calculated using the Statistical Parametric Mapping software on voxel-by-voxel bases. A 2-sample \(t\) test was used to identify clusters with significantly different CVR values in the MS group compared with the control group, set at a familywise error-corrected threshold of \(P < .05\). Voxels were automatically labeled using the pre-registered Montreal Neurological Institute brain template and the PickAtlas software. \(^{72,26}\) Global CVR values of the patients and controls were compared using a 2-sample \(t\) test. For regression analysis, the Pearson correlations between GM CVR and lesion load, fractional brain parenchymal volume (ie, brain atrophy index), Expanded Disability Status Scale score, and disease duration were evaluated for patients with MS.
Results

All participants completed the scan comfortably. Compared with the control group, the MS group showed a significant decrease of global GM CVR (Figure 1). Global GM CBF and mean ETCO₂ values are listed in Table 1, with no significant difference between the MS group and the control group shown for either measure at normocapnia or hypercapnia. There was a significantly smaller mean CBF percent increase (31.7%) from normocapnia to hypercapnia in the MS group than in the control group (44.8%) (P = .01). However, the ETCO₂ increase was almost identical for both groups (9.2 mm Hg for the MS group vs 8.9 mm Hg for the control group; P = .73). Figure 2 presents the mean (SD) GM CVR values in the control group (5.08 [1.56]) and the MS group (3.56 [1.2]) measured as the percent change of CBF per unit change of ETCO₂ (P = .001) (Figure 2A) and the widespread CVR deficit distribution along the cortical surface between the patients with MS and the controls (corrected P < .05) as a result of a voxel-by-voxel comparison of CVR maps (Figure 2B). The mean (SD) global WM CVR was 6.1 (3.0) for the control group and 4.0 (1.2) for the MS group, showing a significant difference (P = .007). Further analysis was restricted to GM because the pCASL signal is less reliable in WM.

Quantitative analysis of the voxel-based group comparison shows a significant reduction in CVR in more than 10% of the GM volume of the temporal, parietal, sublobar, and limbic regions and in less than 10% of the GM volume of the frontal and occipital lobes. Specifically, GM CVR deficits are seen in more than 25% of the superior temporal gyri, supramarginal gyri, rolandic opercula, Heschl gyri, anterior cingulate gyri, and lenticular nuclei. Table 2 summarizes the lobes and contained subregions where more than 10% of GM volume shows a significant decrease in CVR.

The mean (SD) fractional brain parenchymal volume was decreased in the MS group compared with the control group (0.79% [0.04%] vs 0.82% [0.02%]; P = .02), as was the mean (SD) fGM volume (0.41% [0.03%] vs 0.44% [0.02%]; P = .008). The mean (SD) lesion size in the MS group is 14.3 (12.7) cm³. Figure 3 shows the significant negative correlation of GM CVR with lesion volume (R = 0.6, P = .004), as well as the significant positive correlation of GM CVR with the fGM atrophy index (R = 0.5, P = .03). There was no significant correlation between GM CVR and Expanded Disability Status Scale score or disease duration. No enhanced lesions were visible in the postcontrast T1-weighted image.

Discussion

Neurovascular coupling is a complex and important physiologic mechanism during which neuronal activation leads to
increased local cortical blood flow resulting from activity-induced vasodilation. This prompt response is critical for maintaining normal brain function by fulfilling instantaneous energy demand, through oxygen and glucose delivery. Such an intrinsic mechanism can be estimated with CVR, which defines the ability to change CBF in response to vasodilatory stimuli. In our study, we found that global GM CVR (by CO₂ challenge) significantly decreases and correlates with brain atrophy and lesion volume in patients with MS. The pathophysiological mechanism of such deficits of vascular hemodynamic response in MS may be best explained by chronically high concentrations of nitric oxide (a potent vasodilator causing vascular habituation) secondary to repetitive vascular inflammatory cascades,²⁹ as opposed to other postinflammatory by-products. Over time, inadequate blood flow to neurons due to impaired CVR can cause a chronic state of hypoxia leading to neurodegeneration, especially affecting otherwise healthy neurons. We have provided the first direct evidence that CVR, which is essential for neurovascular coupling, is impaired in MS and may be accountable for neurodegenerative changes often seen in patients with MS.

There is abundant evidence showing higher levels of nitric oxide (and its nitroderivatives in CSF) in patients with MS.²⁹ However, nitric oxide has a very short lifetime and is difficult to use clinically for CVR assessment. Alternatively, mild CO₂ mixtures (5% CO₂, 21% oxygen gas, and 74% nitrogen gas) are often used as a safe and reliable vasodilator for CVR measures. Increased CO₂ tension in blood (referred to as hypercapnia) is known to cause an increase in CBF.³⁰ As performed in our study, comparing CBF at normocapnia (breathing room air) and hypercapnia (breathing 5% CO₂) allows for a direct estimation of CVR. Compared with regular continuous or pulsed ASL, the recently developed pCASL sequence is optimized for a better signal to noise ratio, as well as high tagging efficiency within a reasonable short scan time.¹⁹ ³¹

| Table 2. Regions With Significantly Decreased CVR in Patients With MS* |
|-------------------|-----------------|-----------------|
| Subregion                      | Significant GM, % | Mean (SD) t Value |
| Temporal lobe                | 17.1            | 3.2 (0.6)        |
| Superior temporal gyrus       | 43.7            | 3.2 (0.5)        |
| Heschl gyrus                  | 30.4            | 3.0 (0.4)        |
| Middle temporal gyrus         | 21.2            | 3.3 (0.7)        |
| Temporal pole, superior temporal gyrus | 20.0   | 3.4 (0.7)        |
| Parietal lobe                 | 16.9            | 3.0 (0.4)        |
| Supramarginal gyrus           | 36.1            | 3.1 (0.5)        |
| Inferior parietal, excluding supramarginal and angular gyri | 20.6 | 2.9 (0.3)        |
| Precuneus                     | 16.4            | 2.9 (0.3)        |
| Postcentral gyrus             | 14.5            | 2.9 (0.3)        |
| Sublobar region               | 11.7            | 2.9 (0.4)        |
| Lenticular nucleus, pallidum  | 27.9            | 2.8 (0.2)        |
| Insula                        | 16.4            | 2.9 (0.4)        |
| Limbic lobe                   | 10.9            | 2.9 (0.3)        |
| Anterior cingulate and paracingulate gyri | 28.5 | 3.1 (0.4)        |
| Posterior cingulate gyrus     | 24.3            | 2.8 (0.3)        |
| Median cingulate and paracingulate gyri | 14.7 | 2.8 (0.3)        |
| Frontal lobe                  | 5.6             | 2.9 (0.4)        |
| Rolandic operculum            | 31.9            | 2.9 (0.4)        |
| Superior frontal gyrus, median orbital | 19.2 | 3.1 (0.5)        |
| Superior frontal gyrus, medial | 14.0            | 3.2 (0.5)        |
| Occipital lobe                | 4.3             | 16.9 (0.4)       |
| Cuneus cortex                 | 17.4            | 2.9 (0.3)        |

Abbreviations: CVR, cerebrovascular reactivity; GM, gray matter; MS, multiple sclerosis.

*Percentage of GM of each lobe and subregion that appears significant (corrected P < .05) on voxel-based analysis of CVR comparison between patients with MS and controls (only regions >10% included). The mean (SD) t values of voxels within each region are indicated.
A significant drawback of the ASL-type technique is its larger variations of CBF values in WM, which are due to the poor sensitivity and heterogeneous transit time, than in GM. Therefore, we do not consider WM CVR changes as reliable, but reduced CVR is expected because it is accepted that, in MS, inflammatory activities with lesion formation are more prominent in WM than in GM. Because nitric oxide is a highly lipophilic and diffusible gas, it permeates biological membranes, reaching distances far beyond where it was generated. In light of this, the chronic elevation of nitric oxide is expected to affect the vasculature reserve function found in the GM throughout the brain, which is shown by our finding of a diffuse decrease in CVR. Our results favor the notion that, in MS, atrophy is more predominantly seen in GM than in WM because GM has a higher vascular density; thus, the CVR compromise may be more pronounced in GM than in WM. However, with several recent improvements in ASL, including 3-dimensional gradient- and spin-echo readout schemes and background suppression techniques, a more accurate characterization of WM CVR may be possible in the near future.

In the present study, neither of the CBF conditions show significant differences in the patients with MS compared with controls; however, previous literature has reported both increased and decreased perfusion in patients. More regions are likely associated with underlying inflammatory activities, and regions with decreased perfusion may be due to either decreased neuronal activity (demand) or vasoreactivity. Our study only investigates global mean CBF values because regional CBF is not reliable with current ASL techniques owing to low signal to noise ratio. The CVR of the patients with MS, however, is significantly lower than the CVR of the controls. This is likely driven by the significantly smaller CBF increase in the MS group than in the control group when both groups were exposed to hypercapnia, as opposed to the ETCO₂ increase, which is similar in both groups. There is an imbalance in the number of male and female participants in the control group; however, in a study of 152 healthy controls, Lu et al. show that no sex effects were observed in CVR.

We show a globally diffuse, significant decrease in CVR in the MS group, which can indicate a widespread impairment in blood flow regulation or neurovascular coupling. We hypothesize that such impairment can lead to inefficient blood supply on demand and eventually neuronal dysfunction and death, which supports the increasingly recognized evidence of widespread neurodegeneration in MS. In our study, the GM CVR deficits found in certain cortical areas are consistent with structural changes shown in previous studies and are likely responsible for the common symptoms experienced by patients with MS. For example, decreased GM volume and thickness have been shown in the superior temporal gyrus of patients with MS and have been a suggested marker of cognitive impairment related to attention and information speed processing. We report that 43.7% of this region has significantly decreased CVR, which could be the underlying cause of GM thinning. Further studies are needed to explore the correlation between symptoms, structural changes, and CVR deficits; however, we provide evidence that CVR may play an important role in initiating these changes that lead to clinical symptoms.

Cerebrovascular reactivity is an important clinical measure because it assesses vascular health and therefore may indicate overall neuronal health. It is a strong predictor of tissue at risk and can guide medical treatment of many disorders, such as stroke. For the MS group, the global GM CVR and the fGM volume are significantly correlated. This is to be expected because a decreased fGM volume indicates neurodegeneration, which can be partly due to decreased CVR in patients with MS. This significant correlation is unlikely to be attributed to the partial volume effect of CSF because similar analysis for the control group does not show a significant correlation between CVR and fGM volume.

**Conclusions**

In conclusion, the direct measure of CVR with hypercapnia perfusion MRI reveals important characteristics of vascular hemodynamic function, and its impairment in patients with MS is usually only detectable in in vivo studies. This technique allows for a better understanding of the underlying mechanism of neurodegeneration and the worsening of symptoms in MS.
the integrity of the data and the accuracy of the data analysis.

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