Serial proton MR spectroscopy of gray and white matter in relapsing-remitting MS

ABSTRACT

Objective: To characterize and follow the diffuse gray and white matter (GM/WM) metabolic abnormalities in early relapsing-remitting multiple sclerosis using proton magnetic resonance spectroscopic imaging (1H-MRSI).

Methods: Eighteen recently diagnosed, mildly disabled patients (mean baseline time from diagnosis 32 months, mean Expanded Disability Status Scale [EDSS] score 1.3), all on immunomodulatory medication, were scanned semiannually for 3 years with T1-weighted and T2-weighted MRI and 3D 1H-MRSI at 3 T. Ten sex- and age-matched controls were followed annually. Global absolute concentrations of N-acetylaspartate (NAA), choline (Cho), creatine (Cr), and myo-inositol (mI) were obtained for all GM and WM in the 360 cm³ 1H-MRSI volume of interest.

Results: Patients’ average WM Cr, Cho, and mI concentrations (over all time points), 5.3 ± 0.4, 1.6 ± 0.1, and 5.1 ± 0.7 mM, were 8%, 12%, and 11% higher than controls’ (p < 0.01), while their WM NAA, 7.4 ± 0.7 mM, was 6% lower (p = 0.07). There were increases with time of patients’ WM Cr: 0.1 mM/year, Cho: 0.02 mM/year, and NAA: 0.1 mM/year (all p < 0.05). None of the patients’ metabolic concentrations correlated with their EDSS score, relapse rate, GM/WM/CSF fractions, or lesion volume.

Conclusions: Diffuse WM glial abnormalities were larger in magnitude than the axonal abnormalities and increased over time independently of conventional clinical or imaging metrics and despite immunomodulatory treatment. In contrast, the axonal abnormalities showed partial recovery, suggesting that patients’ lower WM NAA levels represented a dysfunction, which may abate with treatment. Absence of detectable diffuse changes in GM suggests that injury there is minimal, focal, or heterogeneous between cortex and deep GM nuclei.

GLOSSARY

Cho = choline; Cr = creatine; EDSS = Expanded Disability Status Scale; FLAIR = fluid-attenuated inversion recovery; GM = gray matter; 1H-MRS = proton magnetic resonance spectroscopy; 1H-MRSI = proton magnetic resonance spectroscopic imaging; mI = myo-inositol; MPRAGE = magnetization-prepared rapid gradient echo; MS = multiple sclerosis; NAA = N-acetylaspartate; RRMS = relapsing-remitting multiple sclerosis; VOI = volume of interest; WM = white matter.

In multiple sclerosis (MS), conventional MRI is sensitive to gray and white matter (GM/WM) atrophy and can distinguish active from chronic lesions, but lacks specificity to gliosis, inflammation, demyelination, and neuronal loss. These can be examined with proton magnetic resonance spectroscopy (1H-MRS) quantification of N-acetylaspartate (NAA) for neuronal health, creatine (Cr) for glial cell density, choline (Cho) for membrane turnover, and myo-inositol (mI) for astrocyte status. However, the utility of 1H-MRS in understanding MS evolution is limited by technological and cost constraints to mostly cross-sectional investigations. Although conclusions on disease course are drawn from such studies, only follow-up allows direct observation of disease progress. Therefore, there is a reiterated need for longitudinal 1H-MRS, in particular of diffuse pathology in normal-appearing tissue.

We report findings from a 3-year semiannual 1H-MRS follow-up of recently diagnosed patients with relapsing-remitting MS (RRMS). We also address the common 1H-MRS pitfalls: low sensitivity, partial volume, and quantification with metabolite ratios. Specifically, for maximum sensitivity
to diffuse changes, we estimated the global metabolic concentrations in the GM and WM of a ∼0.4 L volume of interest (VOI), larger than in any previous serial study. Partial volume effects were removed by using multivoxel, high spatial resolution (0.75 cm³) 3D proton magnetic resonance spectroscopic imaging (1H-MRSI), correcting each voxel for tissue fractions from coregistered segmented MRI. Our previous cross-sectional, non-tissue-specific study showing diffuse glial abnormalities, without neuronal dysfunction, motivated us to define metabolism by tissue type and establish its temporal course in the same cohort.

METHODS Human subjects. Twenty-nine patients (9 men, 20 women) with clinically definite RRMS for less than 6 years were recruited prospectively to be scanned semiannually for 3 years (7 scans each). Exclusion criteria at enrollment were the

Figure 1 Positioning of the 1H-MRSI volume of interest and example spectra

Sagittal T1-weighted magnetization-prepared rapid gradient echo (A) and axial T2-weighted fluid-attenuated inversion recovery (B) of patient 8 in the table, superimposed with the volume of interest (VOI) and field of view (solid and dashed white frames). The location of (B) is indicated on (A) by an arrowhead. (C) Real part of the 8 × 10 (LR × AP) 1H spectra matrix from the VOI on (B), on common 1.4-3.8 ppm and intensity scales. Note the consistent spectral quality in these 0.75 cm³ voxels acquired in ∼30 minutes of acquisition. (D) Expanded and magnified spectra from the 6 spectroscopic voxels, delineated in (B) and (C), superimposed with their fitted model functions (gray lines). Note the spectral resolution and the fidelity of the fitting procedure. Cho = choline; Cr = creatine; 1H-MRSI = proton magnetic resonance spectroscopic imaging; ml = myo-inositol; NAA = N-acetylaspartate.
Table

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Average: 33 32 1.3 1.3 1.3 1.4

Abbreviations: EDSS = Expanded Disability Status Scale; GA = glatiramer acetate; IFN = interferon; IVIg = IV immunoglobulin; N-mab = natalizumab.

Data acquisition. All measurements were done in a 3T magnetic resonance scanner (appendix e-1 on the Neurology® Web site at www.neurology.org). Sagittal 3D magnetization-prepared rapid gradient echo (MPRAGE) images were acquired for MRSI VOI guidance and for tissue segmentation and axial T2-weighted fluid-attenuated inversion recovery (FLAIR) images for lesion volumetry (appendix e-1). A 10 × 8 × 4.5 = 360 cm³ 1H-MRSI VOI was then image-guided over the corpus callosum, as shown in figure 1. A and B (appendix e-1). At 2 averages, the 1H-MRSI took 54 minutes and the entire protocol less than an hour.

Segmentation. After segmentation of the MPRAGE images (appendix e-1), the resultant CSF, GM, and WM masks were coregistered with the 1H-MRSI grid using in-house software, as shown in figure 2, A–D, yielding their volume in every voxel in each subject. Their VOI fractions GM, WM, CSF were their respective sums over all 480 voxels divided by the 360 cm³ VOI volume. Lesion volumes were estimated from the FLAIR images using an automated approach and manual inspection (appendix e-1).

1H-MRSI postprocessing and analyses. Absolute metabolite amounts were obtained using phantom replacement with correction for T1 and T2 relaxation time differences. Global GM and WM concentrations were calculated using linear regression as previously described (appendix e-1). Two-way analysis of variance was used to compare patients to controls in cross-sectional comparisons and random coefficients regression was used to model longitudinal changes (appendix e-1). Linear regression was used to test for correlations between changes in metabolites and in CSF, GM, WM, lesion volume, Expanded Disability Status Scale (EDSS) scores, and relapses (appendix e-1). Statistical significance was defined as a 2-sided p value less than 0.05.

RESULTS Eighteen of the 29 patients met all enrollment criteria. Their demographics at baseline, yearly EDSS scores, and disease-modifying medications are compiled in the table. Their EDSS scores increased by an average of 0.2 (range −2.5 to 2.5), i.e., a rate of 0.05/ year. Clinical relapses occurred in 11 patients over the 3 years. Seven patients were scanned at all 7 time points, 9 at 6, and 2 at 5. The 10 controls (mean age 30, range 24–43) were all scanned 4 times each. Mean scanning intervals were 6.4 months in patients and 12.2 months in controls.

Our shim procedure yielded a VOI water line width of 22 ± 4 Hz (mean ± SD). An example of VOI placement and spectra is shown in figure 1. The individual voxels’ signal-to-noise ratios, from all 73,440 voxels (153 datasets × 480 spectra each), were NAA = 31 ± 6, Cr = 15 ± 2, Cho = 13 ± 2, and mI = 8 ± 1, with 6.7 ± 1.1 Hz linewidth. Coregistration of the 1H-MRSI matrix to the GM, WM, and CSF masks segmented from the MPRAGE images (figure 2, A–D) revealed that in controls, 1% of voxels comprised >90% GM, 15% had >90% WM, and 86% had minimal, <10%, CSF fraction.

The cross-sectional differences in metabolite levels between patients and controls are shown in figure 3. The average (over all time points) patients’ WM Cr, Cho, and mI concentrations, 5.3 ± 0.4, 1.6 ± 0.1, and 5.1 ± 0.7 mM, were higher than the controls’ 4.9 ± 0.2, 1.4 ± 0.1, and 4.6 ± 0.4 mM (all p < 0.01). Patients’ values were higher at all time points by a range of 8%–16% for Cr, 4%–13% for Cho, and 7%–17% for mI. The patients’ WM NAA was lower with a trend on average (7.4 ± 0.7 mM vs 7.9 ± 0.6 mM, p = 0.07), but lower significantly at 4 of the 7 time points (6 months, −8%, p = 0.05, 12 months, −7%, p = 0.02, 18 months, −9%, p = 0.05, and 36 months, −6%, p = 0.05). While the average concentrations, over all time points, of GM NAA, Cr, Cho, and mI in patients, 8.8 ± 0.4, 7.1 ± 0.4, 1.3 ± 0.1, and 5.5 ± 0.5 mM, were not different from the controls’ usual MRI contraindications, alcohol or drug abuse, inability to provide consent, HIV infection, or history of psychiatric or other neurologic disease. Ten age- and sex-matched (2 men, 8 women) healthy volunteers with identical exclusion criteria, but also including unremarkable MRI, were to be scanned annually (4 scans each). Post hoc exclusion criteria was less than 5 scans for patients and less than 4 for controls.

Standard protocol approvals, registrations, and patient consents. This study was approved by the Institutional Review Board of New York University School of Medicine and written informed consent was obtained from all participants.

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8.5 ± 0.6, 6.8 ± 0.6, 1.2 ± 0.1, and 5.4 ± 0.6 mM (all \( p > 0.16 \)), there were cross-sectional increases at one time point each of patients’ Cho (6 months, +10%, \( p = 0.01 \)), Cr (28 months, +7%, \( p = 0.03 \)), and NAA (28 months, +6%, \( p = 0.02 \)). However, since each of these was observed only once, we are uncertain whether they represent true differences or type I errors.

The serial changes in the distributions of all metabolites are also shown in figure 3. There were significant intracohort rates of change only for patients, who showed increasing WM Cr (0.1 mM/year, \( p < 0.001 \)), Cho (0.02 mM/year, \( p = 0.05 \)), and NAA (0.1 mM/year, \( p = 0.04 \)), as well as decreasing GM Cho (−0.03 mM/year, \( p < 0.01 \)) and mI (−0.1 mM/year, \( p = 0.01 \)). There were no significantly different intercohort (patients’ vs controls’) rates of change.

Cross-sectional and serial changes in all volumetric metrics are shown in figure 4. Only the patients’ average CSFf 9.9% ± 1.4%, was different from the controls’ 8.4% ± 1.9% (\( p = 0.03 \)). The average GMf and WMf were 38.3% ± 1.9% and 51.8% ± 1.9% for patients and 39.6% ± 2.4% and 52.0% ± 3.1% for controls. Patients’ average VOI T2 lesion load was 3.9 ± 6.1 cm\(^3\) (median 2.1 cm\(^3\)). Intracohort rates of change were significant only in patients, who had increasing CSFf (0.2%/year) and lesion volume (0.4 cm\(^3\)/year) and decreasing WMf (−0.3%/year) (all \( p \leq 0.01 \)). Although no intercohort rate differences were found, there was a trend for different CSFf rates (\( p = 0.06 \)).

Finally, the rates of change in GM or WM metabolite levels did not correlate with the rates of change of CSFf, GMf, WMf, lesion volume, EDSS, or relapses, with no trends observed.

DISCUSSION
While much is known about MS-associated 1H-MRS changes, their time progression is still unclear due to the limited utility of comparing cross-sectional studies using different patient cohorts and techniques. Despite the reiterated need for serial studies,\(^{1,3,4}\) practical constraints impede regular follow-up over considerable time periods. To our knowledge, the data here represent the most frequent 1H-MRS follow-up for the longest duration. In addition, most previous studies employed metabolite ratios which decrease the specificity of 1H-MRS, and used single voxels or region-of-interest analysis. In contrast to previous serial 1H-MRS, this study assesses metabolism of a large brain volume, accounts for partial volume effects, and investigates widespread diffuse involvement.

High sensitivity without partial volume bias is achieved by 3D 1H-MRSI in a large (360 cm\(^3\)) VOI using each voxel’s signal and tissue composition to estimate each metabolite’s global concentration per tissue type,\(^{3}\) thereby boosting the sensitivity, i.e. the precision.\(^{8}\) It also renders changes in T2-visible lesions insignificant, since they comprise less than 1% of the VOI’s volume, as reflected by a lack of correlation between their load and metabolic levels. Therefore, abnormalities detected with this approach must be diffuse throughout the VOI.

Our specific goals were to assign the glial abnormalities previously found in these patients\(^{6}\) to tissue type and follow this cohort in order to establish the temporal dynamics of their GM and WM metabolism vs controls.

Previously, we showed diffusely elevated Cr, Cho, and mI in the entire VOI of these patients, but could not assign them to a specific tissue type. Given the VOI’s 3:2 WM:GM ratio, however, we conjectured that
they represent WM status. This hypothesis is supported by the results here indicating diffuse glial abnormalities in patients’ WM relatively early in their RRMS course. The Cr, Cho, and mI concentrations are consistently higher in patients’ WM than in controls (on average by 8%, 12%, and 11%, figure 3). Importantly, the increased sensitivity due to separating tissue types helped identify the patients’ smaller (6% on average) WM NAA deficits, indicating that axonal impairment accompanies the glial pathology.

Note that the differences in concentrations were on the order of the coefficients of variation, suggesting that they might not always be statistically identifiable due to biological and instrumental “noise.” Indeed, higher standard deviations in patients’ WM, despite larger sample size, may reflect heterogeneity of disease course or immune or medication responses, suggesting caution in drawing general conclusions from cross-sectional studies, where they can mask subtle pathologic effect. This point may be most pertinent for NAA, which showed the smallest differences, explaining the conflicting reports of both unchanged and decreased levels in cohorts of similar disease duration. This has important implications for timing and monitoring the neurodegenerative phase of RRMS. In this study, we conclusively show diffuse changes in Cho, Cr, mI, and NAA in the WM of patients with RRMS within ~3 to 6 years from diagnosis.

The single time point GM metabolic differences between patients and controls preclude us from concluding definite GM dysfunction. Previous 1H-MRSI in RRMS has ascribed GM changes to effects of axonal transection associated with WM lesions, or even only to their inflammation. Given the paucity of lesions and relapses in our cohort, it is plausible that GM injury is below the detection threshold since it may still be focal; is still confined to specific structures, e.g., basal ganglia but not the cortex; or is spatially heterogeneous. Such hypotheses, testable in the original 3D data, are beyond the scope here. We conclude, therefore, that at this stage, MS is not characterized by diffuse 1H-MRSI-detectable GM damage.

None of the intercohort rates were significantly different, but patients as a group had significant rates of change. In GM, these were decreases of Cho and mI, and although they may reflect normalizations from a previous elevation, they seem to be driven mainly by a sharp decrease at the last time point, a behavior mimicked by the controls, who have almost identical rates (Cho: -0.03 mM/year for patients and -0.02 mM/year for controls, mI: -0.1 mM/year for both), as seen in figure 3. We are therefore uncertain whether these changes are meaningful or resulted from a systematic bias, also present in controls, where rates were not significant due to their smaller sample size.

In WM, there were significant increases in patients’ Cr, Cho, and NAA with much larger intercohort rate differences: fivefold faster Cr and ~twofold Cho, as well as NAA increases in patients compared to controls. We infer the Cr and Cho as progression of glial pathology and the NAA as concurrent axonal recovery or sparing, possibly due to treatment. This is the first serial report of increasing WM Cr and Cho, and likely reflects progressing glial pathology. In contrast, stable or recovering WM NAA is reported in the majority (10 of 14) of serial studies (e.g., references 16-18), where most patients were
on interferons or glatiramer acetate, the main medications of our cohort and a possible reason for the observed partial NAA recovery. Indeed, WM NAA/Cr increased in interferon-treated and glatiramer acetate–treated patients and declined in nontreated ones. However, as studies with untreated patients are rare, extrapolating these effects to medication per se is speculative. In addition, since most serial studies employed NAA/Cr ratios, Cr elevation, as shown here, may confound concurrent NAA recovery.

The specificity of 1H-MRS can differentiate glial from neuronal injury. In vitro and ex vivo studies have established Cr, Cho, and mI as markers of the former and NAA of the latter. The total Cr resonance comprises free creatine and phosphocreatine, found in all cell types, but at higher concentrations in glia than neurons. In MS, high WM phosphocreatine levels have been associated with dysfunctional astrocytes, while recent absolute quantification showed that high Cr levels are due to both free and phosphocreatine and therefore represent gliosis rather than change in energy metabolism. Presence of gliosis in WM has been documented ex vivo and corroborated here by the elevated mI, an osmolyte involved in signal transduction in astrocytes and thought to represent astrogliosis. Further glial involvement is suggested by high levels of Cho, which indicate abnormal membrane turnover from demyelination and remyelination.

In contrast, NAA is almost exclusive to neurons and is therefore considered their marker. NAA levels decline not only with the number of neurons but also under metabolic stress, so by itself a decrease is nonspecific. In this study, however, we observe concurrent increasing NAA and decreasing WMf, suggesting that at this stage of RRMS, neuronal dysfunction dominates. Recently, energy failure due to mitochondrial dysfunction has been proposed as a mechanism for neuronal degeneration in MS. Since NAA is synthesized in the mitochondria, lower levels may reflect reduced mitochondrial activity. If that is driven by inflammation, it is possible that the increasing NAA reflects mitochondrial recovery in response to anti-inflammatory medication. The fact that in the progressive phase of MS neuronal death is independent of inflammation, however, may be related to the steady increase in glial abnormalities, which are more pronounced than NAA loss in early RRMS, and which, unlike NAA, do not seem to abate.

Patients’ CSFf was higher than controls’, and increased significantly, reflecting progressive enlargement of lateral and third ventricle and sulci around the medial part of the longitudinal fissure. It was the only rate that showed any evidence of intercohort difference (p = 0.06). This type of deep central atrophy characterizes the RRMS phenotype, as it starts early and progresses faster than global atrophy. Patients and controls had statistically indistinguishable WMf and GMf, but there was evidence of loss of both tissue types in patients. First, their WMf had a significant rate of decrease, suggesting accumulating WM atrophy. Second, they had consistently lower GMf medians and distributions (figure 4), suggesting greater GM contribution to atrophy. Indeed, GM loss may be apparent as early as clinical onset with involvement of the cortex, basal ganglia, and thalamus. It is possible that some

![Figure 4 Volumetry](image-url)
WM atrophy is masked by inflammatory changes, such as astrogliosis and remyelination, which are less pronounced in GM, as demonstrated by the high WM Cr, Cho, and ml but mostly normal GM metabolite levels.

The postprocessing choice of obtaining global tissue values was to investigate diffuse changes only. Since the original 3D data are available, exploring the contribution of specific structures or regions is feasible. The advantage of our approach, however, was in assessing the largest tissue volumes possible in order to increase the sensitivity, which might otherwise be insufficient to distinguish subtle changes in smaller regions of interest. Second, since our goal was to assess the overall disease load by tissue type, the reported WM changes represent the combined contribution of normal-appearing and "dirty" WM (figure 1D), with unknown contribution of each to the signal. Third, not accounting for lesions in segmentation can result in volume underestimation or overestimation depending on their intensity. Given their low loads in our patients (~4 cm³), in the most common scenario of misclassification as GM (figure 2C), volume errors would be under 0.7%. Finally, while the reported findings are from a relatively large volume (~40% of the brain’s WM and 20% of its GM), they are pertinent only to the regions inside the VOI (figure 1, A and B) and may not be representative of other domains, such as the rest of the cortex.

Glial abnormalities in WM, consistent with myelin breakdown and astrogliosis, are more pronounced than the axonal deficits attributed to dysfunction, such as mitochondrial impairment. In the context of increasing central atrophy and WM tissue loss, the glial changes progressed, while the axons showed partial recovery, presumably in response to treatment. Absolute quantification allowed us to assign metabolic profiles by cell class, which revealed a different time course of glial and neuronal metabolism. This has implications for understanding the natural history of MS as well as monitoring response to treatment paradigms.

AUTHOR CONTRIBUTIONS
Drafting/revising the manuscript for content: Drs. Kirov, Tal, Babb, Herbert, and Gonen. Study concept or design: Drs. Kirov and Gonen. Analysis or interpretation of data: Drs. Kirov and Gonen. Contribution of vital tools: Drs. Tal and Gonen. Acquisition of data: Dr. Kirov. Statistical analysis: Dr. Babb. Study supervision or coordination: Drs. Kirov and Gonen. Obtaining funding: Dr. Gonen.

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DISCLOSURE
The authors report no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.

REFERENCES


