MR Data Acquisition

All experiments were done in a 3 T MR scanner (Trio, Siemens AG, Erlangen Germany) with a TEM3000 (MR Instruments, Minneapolis, MN) circularly-polarized transmit-receive head coil. After placing each subject head first supine into the magnet, localizers were taken in 3 planes to ensure correct placement. Sagittal 3D Magnetization Prepared RApid Gradient Echo (MP-RAGE) images: *TE*/*TI*/*TR*=2.6/800/1360 ms, 256×256×160 mm^3 field-of-view (FOV) and 256×256×160 matrix were then acquired for MRSI VOI guidance and for tissue segmentation. They were reformatted into axial, sagittal and coronal slices (1 mm^3 isotropic pixels) at an angle rendering the genu and splenium of the corpus callosum in the same horizontal plane at the level of the longitudinal fissure, as shown in figure 1A. Next, we acquired thirty 3.7 mm thick axial T_2-weighted Fluid Attenuated Inversion Recovery (FLAIR) images, *TE*/*TI*/*TR*=88/2500/9000 ms FOV=256×256 mm^2, 512×512 matrix along the same planes for lesion volumetry.

Our chemical-shift imaging (CSI) based automatic shim procedure then adjusted the scanner's first and second order currents in under 5 minutes.[1] A 10×8×4.5=360 cm^3 ^{1}H-MRSI VOI was then image-guided over the corpus callosum, as shown in figure 1A-B. It was excited with *TE*/*TR*=35/1800 ms PRESS in three 1.5 cm thick, second-order Hadamard-encoded slabs (6 slices) interleaved every *TR* along the IS direction for optimal SNR and spatial coverage.[2] The axial slabs were encoded with 2D 16×16 CSI over a 16×16 cm^2 FOV and a 8×10 cm VOI defined in their planes with two 11.2 ms long numerically optimized 180° RF pulses (4.5 kHz bandwidths) under 1.34 and 1.1 mT/m gradients, yielding 480 voxels 0.75 cm^3 each in the VOI. The MR signal was acquired for 256 ms at ±1 kHz bandwidth. At two averages, the ^{1}H-MRSI took 34 minutes and the entire protocol less than an hour.

Voxel tissue Segmentation

The MP-RAGE images were segmented using SPM2 (Wellcome Department of Cognitive Neurology, Institute of Neurology, Queen Square, London, UK).[3] The resultant CSF, GM and WM masks were co-registered with the ^{1}H-MRSI grid using in-house software (The Mathworks Inc., Natick, MA), as shown in figure 2A-D, yielding their volume in every *j*-th voxel in each subject: V_j^{GM}, V_j^{WM}, V_j^{CSF}. Their VOI fractions: GM_f, WM_f, CSF_f was their respective sums over all 480 voxels divided by the 360 cm^3 VOI volume.

Global GM and WM concentrations

The ^{1}H-MRSI data was voxel-shifted to align the NAA grid with the VOI, Fourier and Hadamard reconstructed the spectral and spatial directions, then each spectrum was frequency-aligned and phased relative to its NAA peak, all with in-house IDL software (Research Systems Inc. Boulder CO). The relative levels of the *i*=NAA, Cr, Cho, *m*I metabolite in the *j*=1…480 voxel were obtained from their peak area, S_{ij}, using the SITools-FITT spectral modeling IDL software package.[4] They were scaled into absolute amounts, Q_{ij}, relative to a 2 L sphere of C_i^{vitro}=12.5, 10.0, 3.0 and 7.5 mM NAA, Cr, Cho and *m*I in water:

$$Q_{ij} = \frac{C_i^{vitro}}{V} \cdot \frac{S_{ij}}{S_{ijR}} \cdot \left(\frac{P_j^{180^\circ}}{P_R^{180^\circ}} \right)^{1/2} \text{millimoles}, \qquad [1]$$

where V is the voxel volume, the S_{ijR} is the sphere's voxels' metabolites' signal, $P_j^{180^\circ}$ and $P_R^{180^\circ}$ the RF power for a non-selective 1 ms 180° inversion pulse on the subject and reference.

Since the CSF does not contribute to the ^{1}H-MRSI signals, the *i*-th metabolite amount in the *j*-th voxel of each subject can be modeled as a sum of two compartments (GM, WM):

$$Q_{ij} = Q_{ij}^{GM} + Q_{ij}^{WM} = C_i^{GM} \cdot V_j^{GM} \cdot f_i^{GM} + C_i^{WM} \cdot V_j^{WM} \cdot f_i^{WM}, \qquad [2]$$

where C_i^{WM}, C_i^{GM} are that subject's *i*-th metabolite (unknown) global WM and GM concentrations and f_i^{GM}, f_i^{WM} account for the relaxation times differences between each metabolite, *i*, *in vivo* (T_1^{vivo}, T_2^{vivo}) and in the phantom (T_1^{vitro}, T_2^{vitro}) and are given by:

$$f_i = \frac{\exp\left(-TE/T_2^{vitro}\right)}{\exp\left(-TE/T_2^{vivo}\right)} \cdot \frac{1-\exp\left(-TR/T_1^{vitro}\right)}{1-\exp\left(-TR/T_1^{vivo}\right)} \quad [3]$$

The following T_2^{vivo} values for NAA, Cr, Cho and *m*I at 3 T were used in patients: GM: 221, 143, 205, 200 ms, WM: 298, 162, 222, 200 ms; and controls: GM: 275, 157, 241, 200, WM: 401, 185, 258, 200 ms.[5, 6] T_1^{vivo} = 1360, 1300, 1145, 1170 ms were used for both GM and WM in patients and controls.[7] The corresponding values in the phantom were T_2^{vitro}=483, 288, 200, 233 ms and T_1^{vitro}=605, 336, 235, 319 ms. The resulting over-determined 480 equation system of Eq. [2], was solved for the optimal C_{ik}^{WM} and C_{ik}^{GM} using linear regression, as described previously.[8]

T_2 Lesion Load

Lesion volumes were estimated from the FLAIR images using the MIDAS software package.[9] The process starts by automatic detection of the WM signal intensity, I_{WM}, in a periventricular "seed" region. Following selection of all pixels at or above 120, but below 500% of I_{WM}, a lesion-mask is constructed per slice in three steps: morphological erosion, recursive region growth retaining pixels connected to the "seed", and morphological inflation to reverse the effect of erosion. Each slice was then manually inspected and the mask was modified if needed. The lesion volume was the product of the number of "lesion" pixels" × their volume.

Statistical Analysis

Cross-sectional data: Two-way analysis of variance was used to compare patients to controls in terms each volume measure (GM_f, WM_f, CSF_f) and each metabolite (Cr, Cho, *m*I, NAA) within each region (GM, WM). Since there were no significant rates of change for the control group (see

Results), to improve statistical power the data for each control was represented as an average over all four time points. These average values were compared, in separate analyses, to the values of patients at each individual time point and, in an additional analysis, to the patient data represented for each subject as average over all seven time points. In each analysis, the indicator variable identifying subjects matched to each other in terms of age and gender was included as a blocking factor and the error variance was allowed to differ across comparison groups in order to remove the unnecessary assumption of variance homogeneity.

Longitudinal data: Random coefficients regression (RCR) was used to model changes in each study endpoint. Using RCR, a linear model is fit to the data from each subject. Specifically, the slope and intercept of the model for each subject are treated as random variables whose means equal the true slope and intercept of an aggregate model for the average change among subjects in the group. A separate RCR analysis was conducted for GM and WM concentrations of NAA, Cr, Cho and *m*I, CSF_f, GM_f, WM_f in patients and controls and lesion volume in patients. In each case, the dependent variable consisted of the values observed for each subject at all available times and the model included time from baseline as a numeric factor, subject group (patient, control) as a classification factor and the term representing the group-by-time interaction; a significant interaction implies a group difference in the rate of change of the relevant measure. Quadratic terms in elapsed time were tested to verify that changes could be reasonably approximated as linear. The correlation structure was modeled by assuming measures to be correlated only when acquired from the same subject with the strength of correlation inversely dependent on the time between observations, *i.e.*, measures are more strongly correlated when closer in time. Linear regression was used to test for correlations between changes in metabolites and in CSF_f, GM_f, WM_f, lesion volume, Expanded Disability Status Scale (EDSS) scores and relapses by estimating

the rate of change for each patient in each measure over the entire follow-up period. Pearson correlations were used to characterize the association between subject-level rates of change in each metabolite with the subject-level rates of change in CSF_f, GM_f, WM_f, lesion volume, EDSS and the total number of relapses. Statistical significance was defined as a two-sided *p* value less than 0.05. SAS 9.3 (SAS Institute, Cary, NC) was used for all computations.

e-References

1. Hu J, Javaid T, Arias-Mendoza F, Liu Z, McNamara R, Brown TR. A fast, reliable, automatic shimming procedure using 1H chemical-shift-imaging spectroscopy. J Magn Reson B 1995;108:213-219.
2. Goelman G, Liu S, Hess D, Gonen O. Optimizing the efficiency of high-field multivoxel spectroscopic imaging by multiplexing in space and time. Magn Reson Med 2006;56:34-40.
3. Ashburner J, Friston K. Multimodal image coregistration and partitioning--a unified framework. Neuroimage 1997;6:209-217.
4. Soher BJ, Young K, Govindaraju V, Maudsley AA. Automated spectral analysis III: application to in vivo proton MR spectroscopy and spectroscopic imaging. Magn Reson Med 1998;40:822-831.
5. Kirov II, Liu S, Fleysher R, et al. Brain metabolite proton T2 mapping at 3.0 T in relapsing-remitting multiple sclerosis. Radiology 2010;254:858-866.
6. Posse S, Otazo R, Caprihan A, et al. Proton echo-planar spectroscopic imaging of J-coupled resonances in human brain at 3 and 4 Tesla. Magn Reson Med 2007;58:236-244.
7. Traber F, Block W, Lamerichs R, Gieseke J, Schild HH. 1H metabolite relaxation times at 3.0 tesla: Measurements of T1 and T2 values in normal brain and determination of regional differences in transverse relaxation. J Magn Reson Imaging 2004;19:537-545.
8. Tal A, Kirov II, Grossman RI, Gonen O. The role of gray and white matter segmentation in quantitative proton MR spectroscopic imaging. NMR Biomed 2012; doi:10.1002/nbm.2812

9. Mikheev A, Nevsky G, Govindan S, Grossman R, Rusinek H. Fully automatic segmentation of the brain from T1-weighted MRI using Bridge Burner algorithm. J Magn Reson Imaging 2008;27:1235-1241.