

# Diffuse Subclinical Disease Activity in Normal-Appearing White Matter During Remission in Multiple Sclerosis: A Proton MR Spectroscopy Study

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**INTRODUCTION:** The macroscopic hallmarks of multiple sclerosis (MS) in the brain are white matter (WM) lesions and atrophy. Both are assessed with T1- and T2- weighted MRI, but findings correlate only moderately with clinical disability. This incongruity is due to the relative insensitivity of conventional MRI to *microscopic* pathology, as well as to its lack of specificity to distinguish inflammation from demyelination, axonal loss or gliosis (1). These processes can be identified, among other techniques, with proton MR spectroscopy (<sup>1</sup>H-MRS) through their surrogates: *N*-acetylaspartate (NAA) for neuronal integrity; choline (Cho) for membrane turnover; creatine (Cr) and *myo*-inositol (mI) for glial status. The widely used single voxel or 2D <sup>1</sup>H-MRS techniques, however, can examine only very small volumes (few tenths of cm<sup>3</sup>), thus rendering the status of most normal-appearing tissue invisible not only to MRI, but to <sup>1</sup>H-MRS as well. To comprehensively assess this occult pathology early on in the course of relapsing-remitting (RR) MS, we used 3D coverage to obtain metabolite concentrations from a 360 cm<sup>3</sup> volume of interest (VOI) of normal-appearing white matter around the corpus callosum.

**METHODS:** 21 recently diagnosed (mean disease duration 2.3 years), mildly disabled (mean Expanded Disability Status Score of 1.5) patients (33±6 years old, 15 women, 6 men) on immunomodulatory drugs and 15 matched controls (30±6 years old, 12 women, 3 men) were scanned at 3 Tesla. MRI guided a 10<sub>AP</sub>×8<sub>LR</sub>×4.5<sub>IS</sub> = 360 cm<sup>3</sup> VOI centered on the corpus callosum excited with *TE/TR* = 35/1800 ms PRESS in 3 sequentially-acquired slabs each with 2<sup>nd</sup> order Hadamard-encoding in the IS direction. A 16<sub>AP</sub>×16<sub>LR</sub>×4.5<sub>IS</sub> cm<sup>3</sup> field of view containing the VOI was partitioned into 1.0<sub>AP</sub>×1.0<sub>LR</sub>×0.75<sub>IS</sub> = 0.75 cm<sup>3</sup> voxels with 16<sub>AP</sub>×16<sub>LR</sub> 2D chemical-shift imaging matrix, yielding 480 nominal voxels. Their spectra were summed to obtain one global VOI spectrum per subject (Fig. 1), representing a 480<sup>1/2</sup>≈22 fold increase in the signal-to-noise-ratio (SNR). Alignment of spectra before summation exploited better *B*<sub>0</sub> homogeneity across small voxels and thus retained their narrow linewidths in the sum, yielding better spectral resolution compared to acquiring signal from a single 360 cm<sup>3</sup> voxel. Improved SNR and resolution allowed for accurate fitting (SITools software (3)) and peak-area quantification (phantom replacement method (4)). To correct for atrophy, metabolite concentrations were divided by the subject's tissue volume fraction (tissue-volume/VOI-volume) with smaller values indicating higher atrophy. Segmentation from sagittal MP-RAGE MRI was performed with MIDAS software (5). Analysis of variance based on ranks was used to compare patients and controls with respect to NAA, Cho, Cr, mI and tissue fraction adjusting for age and gender.

**RESULTS:** Patients' average VOI tissue volume fraction, 0.92, and NAA levels, 9.62 mM, were not significantly different (*p* > 0.2) from controls' 0.94 and 9.56 mM. In contrast, the Cr, Cho and mI concentrations: 7.69, 1.87, 4.07 mM were significantly, 9%, 14% and 20%, *higher* in the patients versus 7.09, 1.64 and 3.40 mM in the controls (*p* = 0.0097, 0.003 and 0.0023). All differences retained significance after Bonferroni correction.

**CONCLUSION:** Diffuse glial proliferation (elevated mI and Cr) and membrane turnover (elevated Cho), even in the absence of tissue loss or axonal dysfunction (normal NAA levels), are observed in RR MS patients. These suggest widespread ongoing disease activity: inflammation, de- or re-myelination processes, as well as astrogliosis (elevated Cho, Cr and mI levels) early on in the disease course, even during clinical remission and despite ongoing immunomodulatory treatment. The results suggest that these diffuse and widespread processes can be monitored non-invasively via their respective <sup>1</sup>H-MRS surrogate markers. Consequently, mI in combination with Cho and Cr elevation may serve as earlier MRS markers of disease activity than the more accepted NAA decline and atrophy, which may represent in order a later and the final stages of MS progression.

**REFERENCES:** 1. Bakshi R *et al.* Lancet Neurol 2008 2. Miller *et al.* J Neurol 2003. 3. Soher *et al.* MRM 1998. 4. Soher *et al.* MRM 1996. 5. De Santi *et al.* Neurobiol Aging 2001.



**Figure 1:** Real part of the <sup>1</sup>H spectra sums of all 480 voxels in the 360 cm<sup>3</sup> VOI (thin black lines) for all subjects: patients (1–20) and controls (21–36, circled). Each spectrum is superimposed with its fitted model functions (thick gray lines) (3) and all are on common scales. Note: (i) excellent 80 – 500 SNRs and 6.2±1.0 Hz linewidths; (ii) the quality of the fit; and (iii) the visual similarity in NAA levels between patients and controls vs. markedly elevated Cr, Cho and mI.