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 (1H-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 1H-MRS techniques, however, can examine only very small volumes (few tenths of cm³), thus rendering the status of most normal-appearing tissue invisible not only to MRI, but to 1H-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 cm³ 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 (32±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 10x8x4.5 LRx4.5 IS = 360 cm³ VOI centered on the corpus callosum excited with TR/TE = 35/1800 ms PRESS in 3 sequentially-acquired slabs each with 2nd order Hadamard-encoding in the IS direction. A 16x16x16 LRx4.5 IS cm³ field of view containing the VOI was partitioned into 1.0x1.0x0.75 IS = 0.75 cm³ voxels with 16x16x16 LR 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 480x22 fold increase in the signal-to-noise-ratio (SNR). Alignment of spectra before summation exploited better homogeneity across small voxels and thus increased their narrow linewidths in the sum, yielding better spectral resolution compared to acquiring signal from a single 360 cm³ voxel. Improved SNR and resolution allowed for accurate fitting (STTools 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, ml 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 ml 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 ml 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 ml 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 1H-MRS surrogate markers. Consequently, ml 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.