

## Reversible NAA decreases in active MS lesions are not due solely to water content changes

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**Introduction:** Multiple sclerosis (MS) is pathologically characterised by inflammation, demyelination and axonal loss. Previous proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS) studies have shown a decrease in N-acetyl-aspartate (NAA) signal when a new MS lesion appears followed by a rise in NAA signal after some months [1-4]. Although NAA concentration has been linked with axonal integrity, an initial loss of axons followed by axonal re-growth seems unlikely. One of the most plausible explanations proposed is that dilution from increased water content (WC) due to tissue edema that accompanies active inflammation causes NAA to decrease while resolution of edema leads to a subsequent NAA increase. Therefore, we examined both water content and NAA concentrations ([NAA]) in new MS lesions over 6 months to determine how shifts in WC affect [NAA].

**Methods: Subjects** Twenty subjects with relapsing remitting MS (15 female / 5 male; median EDSS = 2.5; mean age = 40yrs; mean disease duration = 8.5yrs) were volunteers for this study. Each subject had a corresponding age and gender matched control.

**MR Examinations** MRI and <sup>1</sup>H-MRS examinations were performed on a Philips 3T Achieva MR Scanner. T<sub>1</sub> images were obtained from an inversion recovery prepared sequence (5 T<sub>1</sub>'s from 150 to 3500ms, TR/TE=6.4/3.1ms, matrix size=256x256). T<sub>2</sub> relaxation was measured from a 3D 32 echo CPMG sequence (TR/TE=1200/10ms, matrix size=256x128) [5]. The multi-voxel <sup>1</sup>H-MRS experiment consisted of a PRESS sequence (TR/TE=1000/35ms, 5 slices, 132 voxels per slice, voxel size = 10 x 10 x 5mm). Additional scans included an axial FLAIR for lesion identification (28 slices, TR/TE=10000/125ms, TI=2800 ms, matrix=256 x 203) and post Gadolinium-DTPA enhanced T<sub>1</sub>-weighted spin echo scan (28 slices, TR/TE=800/10ms, matrix=256x163) for identification of newly active lesions. All slice thicknesses were 5 mm. The MS subjects were scanned at month 0, 1, 2, 3, 4, 5, 6. The controls were scanned at month 0 and month 6.

**Analysis** T<sub>1</sub> relaxation maps were created by fitting the inversion recovery curve to a single exponential at each voxel in the image. T<sub>2</sub> data was fit to a multi-exponential using a regularized non-negative least-squares algorithm [6]. Analysis of <sup>1</sup>H-MRS data was performed using LCModel [7]. Water-scaling was used, referencing the metabolite signal to the water signal for NAA. Spectra were included in subsequent analysis based on criteria determined from LCModel output parameters: standard deviation < 20%, full width at half-maximum ≤ 0.10 ppm and a signal to noise ratio ≥ 5. Regions of interest (ROIs) corresponding to the placement of voxels from the <sup>1</sup>H-MRS study were created for enhancing and chronic (stable over the 6 months) lesions, normal appearing white matter (NAWM) and normal white matter (NWM). NAA metabolite concentration for each ROI was corrected for water content and relaxation ([NAA<sub>corrected</sub>]) using equation 1, where [NAA<sub>LCModel</sub>] is the NAA concentration output from LCModel, T<sub>1</sub>water is the T<sub>1</sub> for water in the ROI, T<sub>2</sub>(35ms) and T<sub>2</sub>(0ms) are the T<sub>2</sub> signal intensities in the ROI as determined by interpolating and extrapolating the multi-exponential T<sub>2</sub> fit to 35 and 0ms respectively and T<sub>1</sub>NAA is the T<sub>1</sub> of NAA as found in the literature [8]. WC was determined from the fast-exchange relationship, 1/WC is linearly related to 1/T<sub>1</sub>, using WC and T<sub>1</sub> data from a previous study [9] to be WC=1/(0.8447+0.4765/T<sub>1</sub>water).

$$[NAA_{corrected}] = [NAA_{LCModel}] \left(1 - e^{-TR/T_{1,water}}\right) \left(\frac{T_2(35ms)}{T_2(0ms)}\right) \left(\frac{1}{1 - e^{-T_1/NAA}}\right) \times WC \quad (1)$$

**Results:** Eight chronic lesions and 12 new lesions were investigated in 12 subjects. Fifteen NAWM and 24 NWM regions were chosen to match most frequent locations of lesions. Plots of [NAA<sub>corrected</sub>] and WC over time are shown in Figures 1 and 2. Values were shifted relative to the time of first appearance of the lesion and averaged, with month 0 corresponding to the month when the lesion first appeared. New lesion results for months -6 to -3 and 5 to 6 had too few ROIs and therefore were not included. WC of new lesions showed an increase at the time of lesion formation followed by a steady decrease over subsequent months with a slight increase at month 4. [NAA<sub>corrected</sub>] from NAWM was significantly different than chronic lesions (p=0.02) and NWM (p=0.003). [NAA<sub>corrected</sub>] from new lesions were decreased at months 0-2 after lesion formation but rose to pre-lesion values at months 3-4 after lesion formation. Although this difference did not reach statistical significance due to the small number of lesions, a trend was seen where [NAA<sub>corrected</sub>] at month 0 compared to month 3 and 4 gave a p=0.09. Therefore, even when taking into account the change in water content of new lesions, NAA shows a reversible change. The plot of uncorrected [NAA] in new lesions showed a similar shape to [NAA<sub>corrected</sub>] but the concentrations were lower.

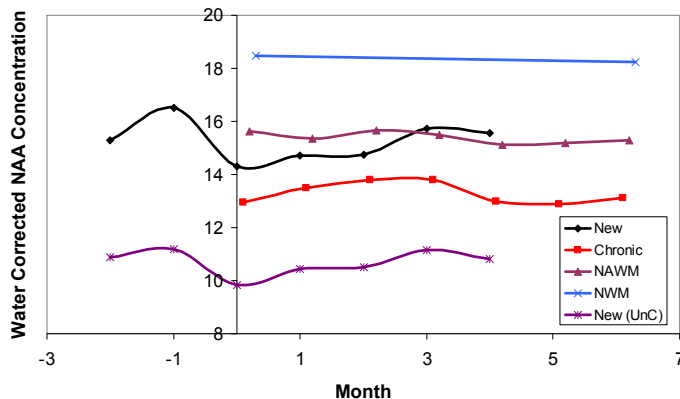


Figure 1: NAA concentration corrected for water content in new lesions, chronic lesions, NAWM and NWM over 6 months. The uncorrected values of NAA as directly output from LCModel are also included (UnC). New lesions have been shifted so that month 0 represents the time of first appearance.

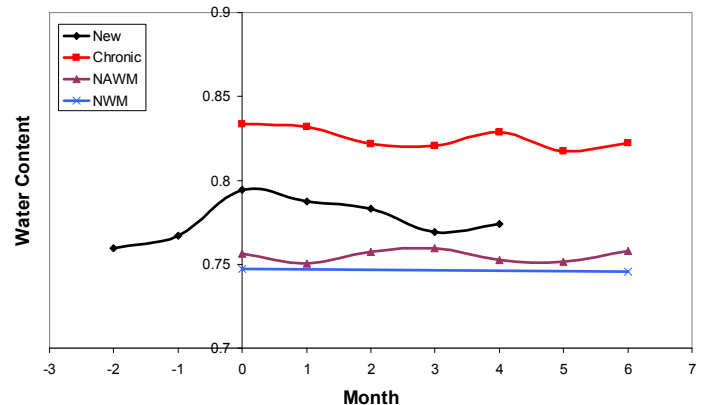


Figure 2: Water content in new lesions, chronic lesions, NAWM and NWM over 6 months. New lesions have been shifted so that month 0 represents the time of first appearance.

**Conclusion:** The change in NAA concentration even after correction for water content suggests that the reversible NAA changes in new lesions are not a simple dilution effect. Other hypotheses put forth to explain the reversible NAA concentration changes include altered chemistry causing a change in NAA relaxation time, damaged mitochondria making less NAA and oligodendrocyte progenitor cells (which contain NAA) proliferating locally in order to enhance remyelination [1].

**References:** [1]Davie. Brain. 1994;117:49. [2]Narayana. Ann Neurol. 1998;43:46. [3]Arnold. Ann Neurol. 1994;36:76. [4]DeStefano. Ann Neurol. 1995;38:901. [5]Mädler. ISMRM 2006 #958. [6]Whittall. JMRI 1989;84:134. [7]Provencher Magn Reson Med. 1993;30:672. [8]Srinivasan. Brain 2005;128, 1016. [9]Vavasour. ISMRM 2006 #3119.