Investigating Axonal Damage in Multiple Sclerosis by Diffusion Tensor Spectroscopy at 7T

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Introduction: Diffusion tensor spectroscopy (DTS) *(1-5)* combines features of both diffusion tensor imaging (DTI) and MRS, allowing measurement of the diffusion properties of intracellular metabolites. As such, it may be sensitive to disruption of tissue microstructure within neurons and might consequently serve as a useful marker of axonal integrity and reversible damage in multiple sclerosis (MS). DTI provides information about microscopic structural features of anisotropic tissues such as white matter tracts. However, its pathological sensitivity is limited because the signal is derived from water protons, which are found in all tissue types (including inflammatory cells, myelin, and neurons). By contrast, MRS is neurochemically specific and can provide more detailed information about a measured tissue, but the spatial resolution of MRS is low due to low metabolite concentrations compared to water. In this study, we compare the diffusion properties of NAA and water in the CC between MS patients and healthy controls at 7 tesla.

Methods: 15 MS patients and 14 healthy controls (HC) were scanned on a 7T Philips Achieva scanner using quadrature volume transmit and 32-channel receive head coils (Nova Medical). For each volunteer, a T_1-weighted structural scan and DTI and DTS spectra from a 3.6 cm^3 volume of interest (VOI) in the normal appearing anterior CC (VOI = 3.0 (AP) x 1.5(RL) x 0.8(FH) cm^3) were collected. The T_1-w (3D MPRAGE) was used for voxel positioning, segmentation of CSF from tissue, and measurement of CC cross-sectional area on midline sagittal sections. The segmented spectroscopy VOI mask was applied to the DTIs to derive average water diffusion values. NAA diffusion measurements were obtained by incorporating bipolar diffusion gradients within a point-resolved spectroscopic sequence (PRESS; Fig 1: TR = 2000 ms, TE_1 = 64, TE_2 = 56, TE = TE_1+TE_2 = 120 ms, $\Delta = (TE_1+TE_2)/2$ = 60 ms, δ = 14 ms, t_{sep} = 47 ms). Diffusion measurements used two *b*-values in addition to *b*=0 (lower *b* = 440 s/mm^2, higher *b* = 2250 or 3600 s/mm^2) with diffusion weighting in 6 non-collinear directions. For each scan, 2048 points were collected with spectral bandwidth 3 kHz. NAA was characterized by averaging 32-40 spectra. Acquisition time was 14-17 minutes. The NAA spectra were acquired with frequency-selective excitation/dephasing water suppression. The water suppression radiofrequency pulse was optimized to allow reliable NAA quantification while retaining enough water signal for zero-order phase correction prior to spectral averaging. Individual spectra were phased and frequency-drift corrected using MATLAB scripts (Mathworks, Inc., Natick, MA). Spectra acquired at each diffusion direction and b-value combination were quantified with LCModel *(6)*. Due to low SNR at the highest b-values, NAA and N-acetylaspartylglutamate (NAAG) were not well separated. Therefore, LCModel-derived NAA+NAAG concentration values were fit to a diffusion tensor, which was diagonalized to yield mean diffusivity (MD), perpendicular diffusivity ($\lambda\perp$), parallel diffusivity ($\lambda\parallel$) and fractional anisotropy (FA) for NAA within the VOI. NAA concentration ([NAA]) was estimated from the LCModel ratio of NAA+NAAG to creatine+phosphocreatine from the b=0 spectrum.

Results: See table 1. NAA $\lambda\parallel$ was lower, whereas water $\lambda\parallel$ was higher, in patients compared to controls. In fact, NAA $\lambda\parallel$ and water $\lambda\parallel$ were negatively correlated (p=0.02). NAA MD and $\lambda\parallel$ were the only diffusion measures correlated with clinical status (EDSS, Fig 2). Additionally, lower [NAA] was associated with worse clinical scores for EDSS (p=0.035) and motor (9-Hole Peg Test; p<0.001) and cognitive (PASAT; p<0.001) tasks.

Figure 1

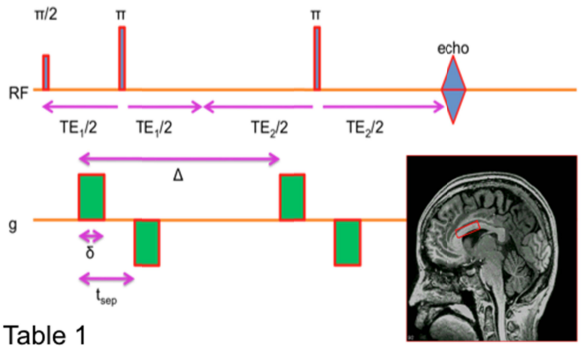
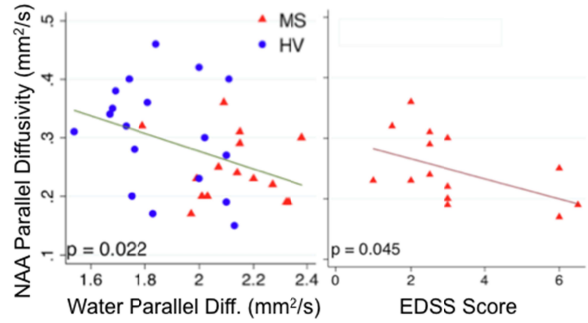


Table 1

Mean Values		Patients (n = 15)	Controls (n = 14)	p value
CC area (mm^2)		575	653	0.017
[NAA] (Cr Ratio)		1.47	1.84	< 0.001
FA	NAA	0.53	0.56	ns
	Water	0.43	0.49	0.016
MD (μm^2/ms)	NAA	0.15	0.18	ns
	Water	1.48	1.22	<0.001
$\lambda\parallel$ (μm^2/ms)	NAA	0.25	0.31	0.030
	Water	2.13	1.86	<0.001
$\lambda\perp$ (μm^2/ms)	NAA	0.11	0.12	ns
	Water	1.15	0.90	<0.001

Figure 2



Discussion: As NAA exists primarily within neurons, our measures of NAA diffusion anisotropy provide information about axonal structure without contributions from extra-axonal compartments. Axon transection and degeneration would restrict the diffusion of molecules along the length of an axon and would be associated with lower NAA $\lambda\parallel$ as seen here. Multiple studies of axonal transection and demyelination in mice have demonstrated a relationship between decreased water $\lambda\parallel$ and axonopathy, whereas water $\lambda\perp$ has been associated with demyelination *(7)* but is more likely nonspecific. On the other hand, most human studies have demonstrated increases in parallel and perpendicular diffusivity in MS patients in both lesions and normal appearing white matter *(8-10)*. As NAA is intra-axonal, decreased NAA $\lambda\parallel$ might confirm the presence of axonopathy in the face of overall increased water diffusion due to inflammation and edema in MS. This is highlighted by the correlation between clinical status and NAA $\lambda\parallel$ (but not water $\lambda\parallel$), since disability may be more closely related to neuro-axonal pathology than to inflammation *(11-13)*. Further studies will investigate NAA diffusion in animal models to address this possibility.

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