# ASSESSMENT OF WHITE MATTER TRACT INTEGRITY METRICS IN CUPRIZONE-INDUCED WHITE MATTER DEGENERATION WITH DIFFUSION MRI

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**TARGET AUDIENCE**: Researchers, clinicians interested in quantifying neurodegeneration with diffusion MRI.

**PURPOSE**: Myelin damage and axonal loss are major hallmarks of white matter (WM) degeneration. Unlike axonal loss, myelin breakdown can potentially be restored under pharmaceutical intervention. We have recently proposed compartment specific diffusion white matter tract integrity (WMTI) metrics as derived from clinically feasible diffusion MRI methods such as diffusion kurtosis imaging (DKI).[1] In particular, these include axonal water fraction (AWF), intra-axonal diffusivity ($D_a$), extra-axonal axial and radial diffusivity ($D_{e\parallel}$ and $D_{e\perp}$), and tortuosity α of extra-axonal space. The aim of this study was to assess the proposed WMTI imaging biomarkers against conventional MRI myelin indicators (transverse relaxation time (T2), magnetization transfer ratio (MTR), radial diffusivity ($D\perp$))[2,3] and histopathology in an experimental model of cuprizone-induced WM degeneration.

**METHODS**: Female C57 mice were fed a cuprizone-supplemented chow (0.2% Sigma Aldrich USA, n=7), and control animals (n=3) were fed a standard pallet diet *ad libitum*. MR imaging was performed at 7 Tesla (Bruker BioSpec) *ex vivo*, 12 weeks after the treatment. For brain extraction, animals were anesthetized using pentobarbital (250 ml/kg), and perfused intracardially with 50 ml of 0.1 M saline followed by 50 ml of 4% paraformaldehyde. Mid-sagittal images were acquired with 0.5 mm slice thickness, 2.2x2.2 $cm^2$ FOV, and 256x256 matrix size, resulting in the in-plane resolution of 86x86$\mu m^2$. A series of diffusion-weighted images were acquired using a 2D-GRASE sequence[4] (TR/TE=1000/53ms, echo train length=4, Δ/δ=18/3 ms) with 30 diffusion gradient directions and 4 b-values (950, 2100, 2900, 4200 s/$mm^2$), in addition to 6 b=0 s/$mm^2$ images. T2 measurements were performed with a multi-spin echo sequence (TR/TE=2000/7ms, number of echoes=32, echo spacing=7, NA=15). For magnetization transfer ratio (MTR), two FLASH images (TR/TE=47/5.5ms, FA=8°, NA=60) were acquired with and without an MT saturation pulse (Gaussian-shaped, length=12ms, RF peak power=4.7μT, frequency offset=1500Hz). DKI analysis was performed, followed by derivation of the WMTI parametric maps.[1] In addition to the metrics derived from the diffusion tensor (fractional anisotropy (FA), mean (MD), radial ($D\perp$), axial ($D_\parallel$) diffusivity) and kurtosis tensor (not shown), WMTI metrics ($D_a$, $D_{e\perp}$, $D_{e\parallel}$, AWF, and $\alpha=D_{e\parallel}/D_{e\perp}$) were calculated in three regions of interest (ROI): the rostral (rCC), and caudal (cCC) region of the corpus callosum (CC), as well as anterior commissure (ac). The same ROIs were chosen for T2 and MTR assessments. For T2 calculation the data were fitted to a monoexponential decay. Histopathological evaluation was performed using electron microscopy (EM). A two-tailed unpaired t-test was applied for statistical analysis between the groups of animals. Cohen's d (difference between two group means normalized by a standard deviation) was employed to find the quantitative group separation indicators.

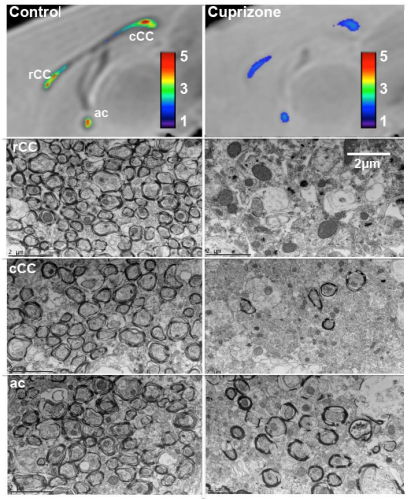


**Figure 1 Tortuosity maps in rCC, cCC, and ac for a control and cuprizone mouse with corresponding EM images.**

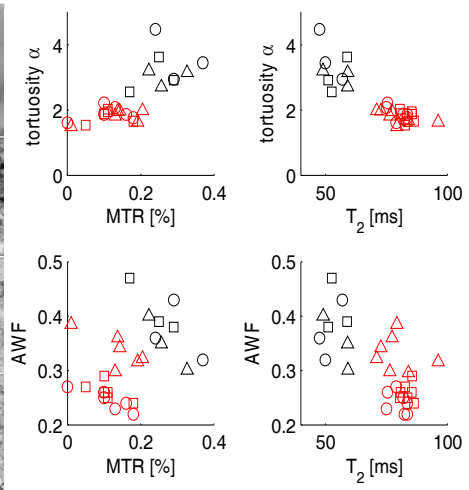


**Figure 2 Scatter plots of (tortuosity, AWF) vs (MTR, T2) for control (black) and cuprizone (red) mice in rCC (circle), cCC (square), ac (triangle).**

**RESULTS**: EM revealed severe demyelination in rCC, and cCC, and moderate demyelination in ac in cuprizone mice (**Figure 1**). The effect of cuprizone could also be observed in all MR imaging modalities. The regional DTI parameter analysis yielded results consistent with previously reported data[2,3] in all investigated regions (i.e. significant FA decrease and MD, $D\perp$, and $D_\parallel$ increase in the cuprizone group compared to healthy mice). WMTI metrics also revealed the following significant changes: reduced AWF (rostral cc: 39%, p=0.02, caudal cc: 46%, p<0.04, ac: 6%, ns), increased α (rostral cc: 40%, p=0.05, caudal cc: 48%, p<0.05, ac: 40%, p<0.01), increased $D_{e\perp}$ (rostral cc: 38%, p=0.04, caudal cc: 55%, p<0.0001, ac: 36%, p=0.02), and increased $D_{e\parallel}$ (rCC: 19%, p=0.001), in cuprizone mice. A non-significant increase was found in $D_a$ for all regions, and in $D_{e\parallel}$ for cCC, and ac. The conventional myelin measurements showed a significant T2 increase (rCC: 34%, p=0.02, cCC: 36%, p=0.002, ac:30%, p=0.02), and MTR contrast drop in cuprizone mice (rCC: 54%, p=0.04, cCC:62%, p=0.01, ac: 49% p=0.02). Correlations graphs (**Figure 2**) showed relations of α vs MTR and α vs T2. Also, there was also relation between AWF and T2, however there was no relation between AWF and MTR. The greatest separation using Cohen's d was achieved for T2, AWF, α (in rCC); α, T2, AWF (cCC), and α, FA, T2 (ac).

**DISCUSSION AND CONCLUSION**: In this study, conventional myelin imaging markers were compared with novel WMTI metrics in the cuprizone model of WM-degeneration. Both conventional and WMTI metrics were sensitive to the effect of the cuprizone treatment, however the overall comparison between DTI, WMTI, T2 and MTR showed that α, T2, and AWF were the most sensitive measures for cuprizone-induced changes. Notably, MTR and $D\perp$, were approximately 2-3 times less sensitive to distinguish between the groups. It is commonly known that the T2 relaxation time is a very sensitive MR probe of microstructure, however its specificity to different degenerative processes such as demyelination and axonal loss is of concern. Conversely, it was suggested that the tortuosity might be a specific marker of demyelination.[5] Correlation graphs showed that tortuosity and conventional myelin metrics (T2, MTR) were related, indicating α to be associated with the demyelination process. Overall, our preliminary results indicate that WMTI imaging markers can be valuable in assessing WM degeneration processes. Further assessment using EM is underway to investigate the association of myelin thickness with WMTI metrics.

**REFERENCES**: **1**. E. Fieremans et al. Neuroimage 2011;58:177-188 **2.** SW Sun et al Magn Reson Med 2006;55(2):302-8. **3**. Thiessen et al. NMR Biomed 2013;26(11):1562-81 **4**. D. Wu et al. Neuroimage 2013;83:18-26. **5**. E. Fieremans et al. In Proc. 20th ISMRM, Melbourne, Australia, #0465.
**ACKNOWLEDGEMENTS**: Grant NIH R21 NS081230, Microscopy Core at New York University Langone Medical Center.