INTRODUCTION: The cuprizone mouse model is a well characterized animal model of demyelination. Reproducible CNS demyelination will result within weeks after young adult mice are fed with the copper chelator cuprizone (bis-cyclohexanone oxalidihydrazone). After removal of the toxin, spontaneous remyelination occurs. In this model, demyelination is predominantly found in the corpus callosum (CC), with oligodendrocyte damage followed by demyelination associated with a microglial response. Recently, cortical demyelination has also been observed. Previous diffusion MRI studies have demonstrated the pathology of the CC in cuprizone mouse model. However, no cortical diffusion MRI changes have been reported. Diffusional Kurtosis Imaging (DKI) is a diffusion MRI technique that extends DTI and quantifies the non-Gaussian behavior of water diffusion, contributing additional information beyond that provided by DTI. Since non-Gaussian diffusion is believed to arise from the presence of diffusion barriers (cell membranes, organelles) and water compartments (extracellular and intracellular), the measures of DKI can be considered as natural indicators of tissue microstructural complexity in the grey matter in addition to white matter structures. Indeed, several animal studies have shown that the diffusional kurtosis metrics provides better differentiation of brain tissue type, and are sensitive to changes in brain microstructural association with brain development and in different diseases processes. The main goal of this study was to quantitatively characterize the diffusional kurtosis changes for grey and white matter during the demyelination process in the cuprizone mouse model.

METHODS: A total of 22 (8–10 weeks old) C57BL/6 mice were used in this study. In the cuprizone (CPZ) treated group (n=12) mice were fed a diet of ground mouse chow containing cuprizone (0.2%). (Bis-cyclohexanone oxalidihydrazone, Sigma-Aldrich) for a period of 10 weeks to induce CNS demyelination. The control (NC) group (n=10) was maintained on a normal diet for 10 weeks. All in vivo MRI experiments were performed on a 7T Agilent MR system. A respiratory-gated 4-shot SE-EPI sequence was used for DKI acquisition. The sequence parameters were: TR:TE=3000/30 ms, ΔΔ=517 ms, slice thickness=1 mm, data matrix=128×128, image resolution=234×234 μm², 4 averages, 30 gradient directions and five b-values for each gradient direction (0.5, 1, 1.5, 2 and 2.5 ms/mm²). Fractional anisotropy (FA), mean (MD), axial (λ₀) and radial (λ₁) diffusivity, as well as, mean kurtosis (MK), axial (K₀) and radial (K₁) kurtosis were derived from the DKI data set using an in-house software programmed in Matlab (The MathWorks, Inc., Natick, MA) called Diffusional Kurtosis Estimator (DKE). All parametric maps were masked (MD> 1.5 μm²/ ms) to reduce partial volume effects. Brain regions of interest (ROIs) at the level of corpus callosum (rostral (aCC), middle (bCC), and caudal (pCC)), cortex (CT), hippocampus and ventral (vHP) were manually drawn using ImageJ (http://rsb.info.nih.gov/ij). Two-tailed t-test was performed to assess differences in the ROI measurements between CPZ and NC mice, P < 0.05 was considered as statistically significant.

RESULTS & DISCUSSION: Illustrated in Fig.1 are representative b0 images. MK maps and the histological stain (Solochrome) for NC and CPZ mice to confirm demyelination. Fig.2 shows the ROI measurement of different brain regions. Consistent with previous reports, CPZ mice showed significantly reduced FA and increased MD and λ₁ throughout the entire CC. Also, the non-Gaussian diffusion metrics showed significantly reduced MK and λ₀ throughout the entire CC in the CPZ mice. These diffusion changes are qualitatively consistent with the degree of demyelination seen at this phase (10 weeks of cuprizone treatment). The axial diffusivity λ₀ was increased in the bCC, but decreased in the pCC when compared to that of NC, and the K₁ showed a trend for decrease, but it was significantly reduced only in the bCC. Since axonal damage in this model is variable and more prominent in early stages of pathology (before 4 weeks of cuprizone treatment), the exact interpretation of these axial diffusion changes (at 10 weeks of cuprizone treatment) can only be done with future histological correlation. A striking result was that only the non-Gaussian diffusion metrics MK, K₀, K₁ showed significant changes (decrease) in the cortex of the CPZ mice, which likely reflects cortical demyelination and reactive glial cells accumulation, confirming that kurtosis metrics are sensitive indicators of changes in structural complexity not only in white matter, but also in grey matter. In summary we observed, for the first time, non-Gaussian diffusion changes in the cortex of mice with demyelination induced by cuprizone, demonstrating the significant advantage of microstructural characterization using DKI, especially for abnormalities in grey matter.


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