The contribution of myelin to magnetic susceptibility-weighted contrasts in high-field MRI of the brain

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Introduction

Tissue myelin has been suggested as a primary contributor to magnetic susceptibility contrasts (GRE magnitude and frequency (phase) contrasts) (1-4). Recently, studies on dys- or no myelination have suggested significant changes in frequency contrast (5-7). Despite these findings, no study has shown a direct connection between brain myelin content and T_2^*. Here we used a mouse model of cuprizone-induced demyelination to investigate the contribution of myelin to T_2^* and frequency contrasts *in vivo* and in fixed tissue.

Methods

Fourteen 8-week-old male C57BL/6 mice were used for this IACUC approved study. Eight mice were fed with 0.2% cuprizone for 6 weeks and the other 6 mice were fed a control diet. Animals were scanned at 7T to measure T_2^* and frequency contrasts both *in vivo* and after fixation for 1 week in 10% formalin. Four coronal slices were scanned with in-plane resolution of 50 x 50 μm^2, slice thickness = 0.75 mm, TR = 1.5 s, and TE = 6/13/20 ms. Data were processed to generate T_2^* and frequency images. All animal images were aligned together to generate averaged images and ROIs (cortex and corpus callosum) were drawn on these images for quantification (*in vivo* and fixed separately). This averaging step was necessary because the GM-WM boundary was not clear in the cuprizone-fed mice. Averaged frequency and T_2^* images for cuprizone (CPZ) and control (CTL) groups were generated (Fig 1) and Student's t-tests were performed on each pair of conditions (i.e. CPZ vs. CTL, *in-vivo* vs. *in-vitro*, and GM vs. WM) with a significant threshold of p = 0.05. Because the average body weight was different between CPZ and CTL (16.1 ± 2.3 g and 28.3 ± 4.3 g resp.), its potential effect was investigated by regression analysis on T_2^* in GM, T_2^* in WM, and on the T_2^* GM-WM contrast across animals both *in vivo* and *in vitro* ($p \leq 0.05/8$ after Bonferroni). Brain areas were also measured. For one cuprizone-fed mouse and one control mouse, brains were stained for myelin (8).

Results

Averaged brain images are shown in Fig. 1 and the ROI results are summarized in Table 1 (last column shows statistical significance). Both *in-vivo* and fixed brains showed similar patterns of contrast changes. CPZ showed a significant reduction in GM-WM frequency contrast compared to CTL (Figs. 1B and B' vs. C and C'; Table 1, red). The GM-WM T_2^* contrast was significant only in CTL (Figs. 1D and D'; Table 1, blue) whereas CPZ showed almost no GM-WM T_2^* difference (Figs. 1E and E'; Table 1, light blue). The mean T_2^* GM showed a marginally significant increase in CPZ relative to CTL (Table 1, green). The mean WM T_2^* values were significantly increased in CPZ compared to CTL (Table 1, green). Tissue fixation had no significant effect on frequency contrast (Table 1, orange). On the other hand, the T_2^* values were significantly reduced in the fixed tissues (Table 1, light green). Despite overall decreases in T_2* values, the relative contrasts were sustained after fixation, giving a clear GM-WM differentiation in CTL (Fig. 1D'). The reduced T_2^* values in fixed brains may not have originated solely from the formalin fixation since there was a temperature difference (37 °C *in vivo* vs. room temperature fixed). When the body weight of individual animals was regressed out of each group for each contrast, none of them showed a significant correlation suggesting no influence of body weight. The brain size was not different between the two groups. The myelin stained images (Figs. 1F and F') show a pattern of changes similar to those observed in T_2^* and frequency images.

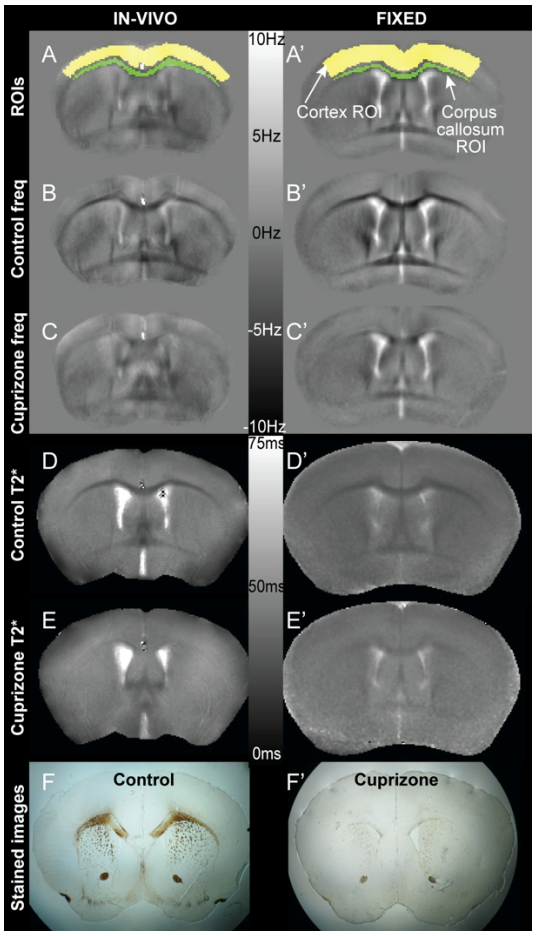

Figure 1. Images of control and cuprizone mice

	Comparison (measurement value)		p-value	
FREQ	In-vivo CTL GM-WM (3.58 ± 0.91 Hz)	In-vivo CPZ GM-WM (1.45 ± 0.43 Hz)	6.5×10^{-4}	*
	Fixed CTL GM-WM (3.91 ± 0.43 Hz)	Fixed CPZ GM-WM (1.10 ± 0.31 Hz)	1.4×10^{-7}	*
	In-vivo CTL GM-WM	Fixed CTL GM-WM	0.22	
	In-vivo CPZ GM-WM	Fixed CPZ GM-WM	0.066	
T_2^*	In-vivo CTL GM (34.0 ± 3.3 ms)	In-vivo CTL WM (28.4 ± 3.4 ms)	0.007	*
	Fixed CTL GM (30.3 ± 2.4 ms)	Fixed CTL WM (25.2 ± 1.8 ms)	0.0013	*
	In-vivo CPZ GM (37.2 ± 1.2 ms)	In-vivo CPZ WM (39.4 ± 2.8 ms)	0.055	
	Fixed CPZ GM (33.3 ± 2.2 ms)	Fixed CPZ WM (32.6 ± 2.2 ms)	0.29	
	In-vivo CTL GM	In-vivo CPZ GM	0.033	*
	Fixed CTL GM	Fixed CPZ GM	0.02	*
	In-vivo CTL WM	In-vivo CPZ WM	5.3×10^{-5}	*
	Fixed CTL WM	Fixed CPZ WM	1.9×10^{-5}	*
	In-vivo CTL GM	Fixed CTL GM	0.025	*
	In-vivo CTL WM	Fixed CTL WM	0.044	*
	In-vivo CPZ GM	Fixed CPZ GM	0.0014	*
	In-vivo CPZ WM	Fixed CPZ WM	1.5×10^{-4}	*

Table 1. CPZ: cuprizone group, CTL: control group

Discussion

The results suggest a causal relationship between myelin depletion and magnetic susceptibility contrast, confirming the notion that myelin is an important contributor to the magnetic susceptibility of white matter. It is plausible that the cuprizone diet may have affected tissue constituents not specific to myelin, such as proteins, iron and copper, as may have occurred in previous studies (5-7). In mice, the concentrations of iron and copper in cortex and corpus callosum are similar and significantly lower than in humans (9, 10), and proteins are not specific to white matter. Therefore, these confounds are likely to have minor contribution. Assuming the susceptibility changes are dominated by the induced demyelination, a rough estimate of the magnetic susceptibility of myelin is possible. Δf and $|\Delta R_2'|$ from our data are -2.13 Hz and 10.2 Hz respectively. From (11) and assuming corpus callosum fibers are perpendicular to B_0 and volume fraction of myelin in WM = 16% (12), we arrive at the following estimates for the susceptibility of myelin: -0.089 ppm (from Δf) and -0.068 ppm (from R_2^*).

References

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