# Histological Basis of Diffusely Abnormal White Matter in Multiple Sclerosis: Evidence for a Primary Lipid Abnormality

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## Background

The characteristically focal lesions of multiple sclerosis (MS) have been studied extensively using magnetic resonance imaging (MRI) and histology. However, very little is known about diffusely abnormal white matter (DAWM). DAWM is mildly hyperintense, typically periventricular with a poorly defined boundary[1] and is present in ~20% of MS cases. Because of DAWM's nebulous appearance which makes it difficult to precisely define and delineate, it is not typically included in quantitative measurements of MRI disease burden in clinical trials and research studies. However, as people with DAWM have a shorter disease duration and progress faster on disability measures[2], DAWM may contribute to disease symptoms and progression. Previous DAWM research studies have observed reduced magnetization transfer ratio, reflecting change in water and tissue content[3]; increased $T_{1,free}$, related to increased water content[4]; reduced fractional anisotropy, suggesting lower fibre ordering[5]; and reduced myelin water fraction (MWF), indicating less myelin[6,7]. Histological studies have reported reduced myelin and axons and gliosis[5-7]. In particular, decreased staining of myelin phospholipids by Luxol Fast Blue (LFB) and relatively preserved staining for myelin basic protein (MBP) and 2',3'-cyclic nucleotide 3'-phosphohydrolase (CNP) has been demonstrated. **Given the potential prognostic importance of non-lesional white matter abnormalities in disability and clinical progression, further histological studies are warranted to fully elucidate the underlying pathology giving rise to the MRI-defined phenomena of DAWM**.

## Methods

**MR Experiments & Analysis:** Fourteen slices of formalin-fixed brain from 9 MS cases (mean age = 65 yrs (35-88 yrs); 5F/ 4M; mean disease duration = 26yrs (13-38 yrs) were examined with a 32 echo T2 relaxation measurement at either 1.5T (n=9, GE, TR head coil, TR/TE=3000/10ms, 8 averages, matrix = 256x256, 3mm thick, in plane resolution = 586µm x 586µm, scan time = 102 minutes) or 7T (n=5, Bruker, TR/TE=1500/6.673ms, 6 averages, matrix = 256x256, field of view=6 cm, 1mm thick, in plane resolution = 234µm x 234µm, scan time = 38 minutes). Voxel-wise $T_2$ distributions were calculated with a non-negative least squares algorithm[8,9] and MWF was defined as the fractional area of the short $T_2$ component (<30ms at 1.5T, <20ms at 7T). Regions of interest (ROIs) outlined in normal appearing white matter (NAWM), DAWM and lesion and mapped to the voxel-wise MWF images. Mean MWF was determined for each ROI.

**Histological Staining & Analysis:** Brain slices were paraffin-embedded, sectioned at 10 µm-thickness, and stained with LFB and Weil's stains for myelin phospholipids, Alcian Blue for sialic acid residues on myelin phosopholipids, immunohistochemically for MBP, CNP, myelin-associated glycoprotein (MAG), myelin-oligodendrocyte glycoprotein (MOG), proteolipid protein (PLP), for astrocytes using glial fibrillary acidic protein (GFAP) and with modified Bielschowsky for axons. Histology images were registered to MRI and mean optical density (OD) for ROIs were determined using Image Pro Plus 5.1.

**Statistical Analysis:** DAWM and lesion were compared to NAWM with a t-test for each sample ($p<0.05$). Results were expressed as a % change relative to NAWM.

## Results

Figure 1 demonstrates DAWM (arrows) on MRI (proton density and myelin water map) and the corresponding histological features. Quantitative analysis is summarized in Table 1 and Figure 2. The most dramatic reductions were observed in the myelin phospholipids (demonstrated by Luxol Fast Blue, Weil's, Alcian Blue, $p<0.05$ for 14, 14, 8 cases respectively), while there was lesser involvement of the myelin proteins (MAG, MBP, MOG, PLP, CNP, $p<0.05$ for 3, 3, 1, 2, 3 cases respectively). Axonal involvement was intermediate ($p<0.05$ for 7 cases).

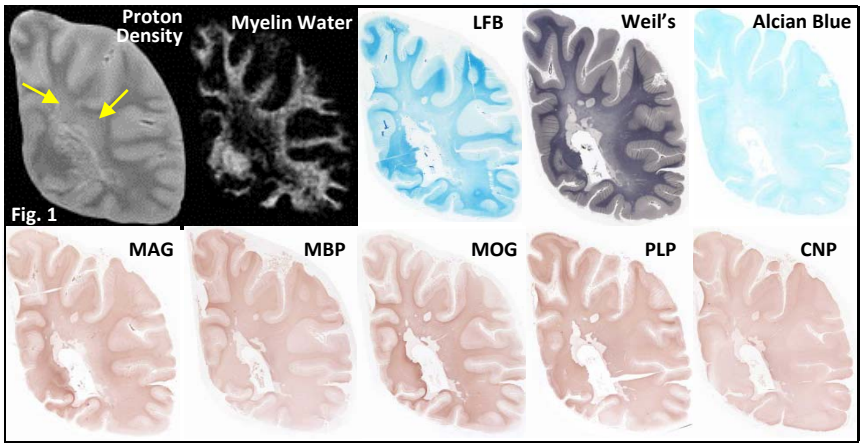


Fig. 2 – Boxplots of MWF and optical density mean % difference for all cases showing median, lower & upper quartiles, outliers, mean & standard error (SE)

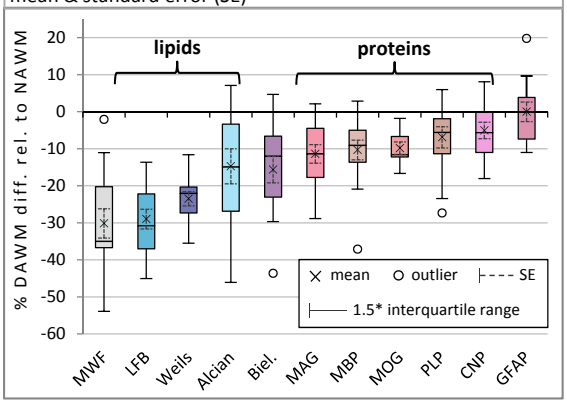


**Table 1 – Mean % difference (standard error) of MWF and optical density of myelin lipid, myelin protein and axon stains from all DAWM and lesion relative to NAWM**

| | Myelin Water (%) | LFB (%) | Weils (%) | Alcian (%) | Biel. (%) | MAG (%) | MBP (%) | MOG (%) | PLP (%) | CNP (%) | GFAP (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DAWM | -30 (4) | -29 (3) | -24 (2) | -15 (5) | -15 (4) | -11 (3) | -10 (3) | -10 (2) | -7 (3) | -5 (2) | 0 (3) |
| Lesion | -71 (6) | -57 (5) | -45 (6) | -37 (4) | -49 (6) | -42 (5) | -32 (5) | -48 (7) | -49 (5) | -27 (5) | -1 (6) |

## Discussion & Conclusion

DAWM demonstrated abnormalities in myelin water, myelin lipids, myelin proteins and axons. Relative to NAWM, DAWM showed substantial decreases in myelin water fraction (-30%) and the myelin phospholipid markers Luxol fast blue (-29%) and Weil's (-24%), with much less involvement of the myelin proteins MAG (-11%), MBP (-10%), MOG (-10%), PLP (-7%) and CNP (-5%). Lesions demonstrated more severe abnormalities than DAWM, showing substantial decreases in both myelin lipids and myelin proteins. Our findings are consistent with a primary lipid abnormality or perturbation that antedates myelin protein loss and axonal degeneration in DAWM. These phenomena may be important in the pathogenesis of MS and its clinical progression, which is more prominent in individuals with DAWM.

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