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Magnetic susceptibility increases as diamagnetic molecules break down: myelin digestion during multiple sclerosis lesion formation contributes to increase on QSM

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Abstract

BACKGROUND—The pathological processes in the first weeks of multiple sclerosis (MS) lesion formation include myelin digestion that breaks chemical bonds in myelin lipid layers. This can increase lesion magnetic susceptibility, which is a potentially useful biomarker in MS patient management, but not yet investigated.

PURPOSE—To understand and quantify the effects of myelin digestion on quantitative susceptibility mapping (QSM) of MS lesions.

STUDY TYPE—Histological and quantitative susceptibility mapping (QSM) analyses on *in vitro* models of myelin breakdown and MS lesion formation *in vivo*.

POPULATION/SPECIMENS—Acute demyelinating white matter lesions from MS autopsy tissue were stained with the lipid dye oil red O (ORO). Myelin basic protein (MBP), a major membrane protein of myelin, was digested with trypsin. Purified human myelin was denatured with sodium dodecyl sulfate (SDS). QSM was performed on phantoms containing digestion products and untreated controls. *In vivo* QSM was performed on five multiple sclerosis patients with newly enhancing lesions, and then repeated within four weeks.

FIELD STRENGTH/SEQUENCE—Three-dimensional (3D) T2*-weighted spoiled multi-echo gradient echo scans performed at 3 Tesla

ASSESSMENT—Region of interest (ROI) analyses were performed by a biochemist and a neuroradiologist to determine susceptibility changes on *in vitro* and *in vivo* QSM images.

STATISTICAL TESTS—Not applicable.

RESULTS—MBP degradation by trypsin increased the QSM measurement by an average of 112 ± 37 ppb, in excellent agreement with a theoretical estimate of 111 ppb. Degradation of human myelin by SDS increased the QSM measurement by 23 ppb. As MS lesions changed from gadolinium enhancing to non-enhancing over an average of 30.6 ± 3.0 days, their susceptibility increased by an average of 7.5 ± 6.3 ppb.

DATA CONCLUSIONS—Myelin digestion in early stages of MS lesion formation contributes to an increase in tissue susceptibility, detectable by QSM, as a lesion evolves from gadolinium enhancing to non-enhancing.

Keywords

myelin; diamagnetism; multiple sclerosis; QSM

INTRODUCTION

The formation of multiple sclerosis (MS) lesions involves migration of peripheral, activated leukocytes into the central nervous system (1). This inflammatory infiltration is associated with breakdown of the blood brain barrier (BBB), caused by changes in endothelial tight junctions and damage to the basement membrane and astrocyte end-feet (2) and imaged routinely in clinical practice using T1 weighted imaging post gadolinium (Gd) injection (3). Macrophages are the principal cells that mediate demyelination by phagocytosing and degrading myelin, and the presence of foamy macrophages within an MS lesion indicates that it has formed within days or weeks (4,5). Breakdown of myelin reduces electron orbit sizes of valence bonds in myelin and consequently decreases diamagnetism (6–8). This myelin digestion causes an increase in the MS lesion susceptibility that may be measurable on quantitative susceptibility mapping (QSM) without Gd to improve MS patient management (9).

The diamagnetic change of myelin digestion has not been demonstrated in MRI literature, to the best of our knowledge. Accordingly, one purpose of this study is to identify the chemical bonds in myelin that may be broken in macrophage myelin digestion and measure their corresponding contributions to susceptibility increase using QSM. For this purpose, we will perform controlled in vitro experiments.

The main purpose of this study is to demonstrate the effect of active myelin digestion on tissue magnetic susceptibility in MS lesions. Because both myelin and iron are the major contributors to lesion tissue magnetic susceptibility, we need to differentiate myelin digestion effect from myelin debris removal and iron accumulation. The myelin debris removal occurs over several months (5), and the iron accumulation occurs a few months after the BBB closure (10). In contrast, myelin digestion is expected to occur immediately after phagocytosis. Therefore, susceptibility increase in the first few weeks following the detection of a Gd enhancing lesion probably comes from myelin digestion.

THEORY

According to the Langevin formula, the diamagnetic susceptibility, χ , of an atom is proportional to its mean square radius $\langle r^2 \rangle$:

$$\chi = -\frac{\mu_0 N e^2}{6m} \langle r^2 \rangle \quad (1)$$

where μ_0 is the vacuum permeability, N is the number of electrons in the atom, e and m are the electron charge and mass respectively. When atoms or monomers bond into molecules or polymers, the spread of weakly held π electrons over one or more units leads to an increase in the magnitude of diamagnetism (11,12). The increase in the magnitude of diamagnetism can be estimated using Pascal's rule, which states that the total magnetic susceptibility of a compound is the sum of the magnetic susceptibilities of the subunits and bonds (13).

Proteins consist of amino acids held together by peptide bonds (-CO-NH-), which are hybrid molecular orbitals consisting of delocalized electrons spread over the N, C and O atoms. Using Pascal's rule, the magnetic susceptibility of myelin basic protein (MBP) a biochemical marker of demyelination in MS patients (14), can be estimated as the sum of the diamagnetic susceptibilities of the constituent amino acids and peptide bonds. When a peptide bond is lost from proteolysis, the molar susceptibility of MBP will decrease by the molar susceptibility of a peptide bond. A peptide bond has the structure of glycine and molar susceptibility of $\chi_{molar}^{CGS} = -40.3 \cdot 10^{-6} \text{ cm}^3/\text{mol}$ in CGS units (15,16) or a volume susceptibility change in SI units of

$$\Delta\chi_{volume}(ppm) = 4 \times \pi \times \chi_{molar}^{CGS} \left(\frac{\text{cm}^3}{\text{mol}} \right) \times \rho(\text{g}/\text{cm}^3) / M(\text{g}/\text{mol}) \quad (2)$$

where $\rho = 1.35 \text{ g}/\text{cm}^3$ and $M = 18500 \text{ g}/\text{mol}$ for the density and molar mass, respectively of MBP (17). Inserting these values into Eq. 2 gives the expected volume susceptibility change of 37.1 ppb when one peptide bond is lost in MBP. According to the known MBP amino acid sequence and trypsin cleavage sites, human trypsin cleaves MBP at 3 sites (18). Therefore, digesting MBP with trypsin is expected to result in a volume susceptibility change of about 111 ppb.

Wiedemann's Law states that the total susceptibility of a mixture of non-interacting components is the sum of the susceptibilities of the components weighted by their volume fractions. Bond formation or breaking between mixture components results in difference between the experimentally measured susceptibility and the susceptibility predicted by Wiedemann's Law (19). When electrostatic bonds between proteins and lipids are broken, as occurs during myelin denaturation with a surfactant such as SDS, the change in the volume susceptibility of the mixture is proportional to its associated free energy change ($-G$) (20–23). The removal of a protein from the myelin phospholipid surface is estimated to increase the free energy of the surface by at least 4.9 kJ/mol (24), which corresponds to a molar

susceptibility increase of $15.5 \times 10^{-6} \text{ cm}^3/\text{mol}$ (22) or a volume susceptibility increase of 14 ppb.

MATERIALS AND METHODS

To identify the sources of magnetic susceptibility increase in demyelinating MS lesions, we performed QSM analysis of the following cases: 1) trypsin digestion of myelin basic protein *in vitro*, 2) disruption of the myelin lipid bilayer with SDS *in vitro*, and 3) acute lesion formation in MS patients. We used MEDI+0 implementation of QSM that enforces susceptibility homogeneity in a region known to consist of water for the zero reference (25).

QSM following *in vitro* myelin basic protein digestion by trypsin

1 mg of bovine myelin basic protein (MBP, Sigma-Aldrich, U.S.) was dissolved in phosphate buffered saline (PBS) to form a stock solution, which was then stored at -20°C . 2.5% (wt/vol) trypsin was added to 30 μL of 1 mg/mL MBP and PBS to form a 3 ml solution. Solutions of pure MPB in PBS and pure trypsin in PBS were also prepared. The final concentration of each solution was 0.01 mg/mL. All solutions were vortexed and incubated in a 37°C water bath for 2 hours. Following digestion, aliquots were removed, and a western blot was completed (Santa Cruz Biotechnology). The remaining samples were pipetted into latex balloons (Imperial Kaos, Tie-Not, US), which were securely tied and embedded within approximately 1000 cm^3 of 1% agarose gel in a large plastic bowl. A latex balloon of water was also embedded for use as a reference for QSM measurements. Multi-echo gradient echo MRI imaging of the plastic bowl was performed on a GE Discovery MR750 3T with the following parameters: voxel size = $0.35 \times 0.35 \times 0.4 \text{ mm}^3$, TE1/ TE = 6.3/6.8 ms, number of echoes = 9, TR = 65.7 ms, flip angle = 20° , 0.75 partial Fourier. QSM was reconstructed in MATLAB (R2012b) using MEDI+0 QSM reconstruction with the 1% agarose gel region as the homogenous region (25). The volume susceptibility of the contents of each balloon was measured by taking the mean voxel value of a region of interest (ROI) segmented using a region-growing algorithm with segmentation intensity thresholds specified so that the entire contents of the balloon were captured within the ROI mask (26). The mean susceptibility value of the water balloon was subtracted from that of the other balloons to correct for any diamagnetic effects of the balloon material. To estimate the change in volume susceptibility due to the digestion of MBP by trypsin, we can assume the digestion does not change the trypsin concentration and that MBP is completely digested. Then, any difference in volume susceptibility between the digested and undigested MBP solutions, that remains after subtracting the volume susceptibility measured for the trypsin solution must be due entirely to the digestion of MBP. More concretely, denoting the molar susceptibilities of digested and undigested MBP by χ_d and χ_u respectively, and that of PBS by χ_s , and the concentrations of MBP and PBS by $[m]$ and $[s]$ respectively, we can write the volume susceptibility change from MBP digestion as

$$\Delta\chi = [\chi_d - \chi_u][m] = (\chi_d[m] + \chi_s[s]) - \chi_s[s] - \chi_u[m] \quad (3)$$

This experiment was repeated using four more phantoms, and the average and standard deviation of all volume susceptibility changes were recorded.

QSM of in vitro myelin breakdown by SDS

Myelin was obtained from post mortem autopsy brain tissue of a patient without neurological disease. The post mortem interval was 8 hours. Briefly, 3 grams of white matter tissue from the frontal cortex was homogenized in 0.32 M sucrose using a Dounce homogenizer. Myelin was recovered as a pellet by three successive sucrose gradient centrifugation steps and washed extensively in Tris-Cl buffer, pH 7.4. The pellet was resuspended in 0.32 M sucrose, layered over 0.85 M sucrose and subjected to centrifugation at 75,000 g for 30 min. Myelin was recovered and suspended in PBS, pH 7.4. Protein concentration of the preparation was analyzed with a BCA-Kit (Pierce™). Purified human myelin was digested with 13% sodium dodecyl sulfate (SDS) for 16 hours at 37°C. To verify myelin digestion by SDS, myelin debris solution was labeled with fluoromyelin, a fluorescent stain that specifically stains the lipids in myelin because of the composition and density of lipids found in myelin, and not in the lipid bi-layer of other cell membranes. Using a fluorescence plater reader, fluorescence was quantified for purified myelin before and after incubation with SDS. Additionally, the purified myelin was treated with the Bodipy lipid stain after incubation with SDS, to confirm breakdown.

To measure magnetic susceptibility using QSM, one sample containing 13% SDS, one sample containing purified homogenized myelin, and one sample containing the myelin incubated with 13% SDS as described above were each placed on a bed of 1% agarose gel in 50-mL falcon tubes, which were then completely filled with 1% agarose gel, closed, and imaged in a wrist coil on a GE Discovery MR750 3T scanner using a 3D multi-echo gradient echo (GRE) pulse sequence with the following parameters: voxel size = $0.2 \times 0.2 \times 0.4$ mm³, TE1/ TE = 5.3/5.2 ms, number of echoes = 9, TR = 66.8 ms, flip angle = 20°, 0.75 partial Fourier. Multi-coil complex DICOM images were exported from the scanner and processed off-line into QSM maps in MATLAB (R2012b) using MEDI+0, enforcing homogeneity in the 1% agarose gel region (25). ROIs were created with a region-growing algorithm so that the entire region containing the digested or undigested within the agarose gel was captured within the ROI mask. Volumetric ROI analysis was used to measure the relative susceptibility of the sample in each of the three tubes, and the measured values referenced by subtracting the susceptibility measured in a ROI placed in the surrounding 1% agarose gel in each falcon tube. The volume susceptibility change due to myelin digestion was estimated using Eq. 3.

Immunohistochemistry and histochemistry

Human central nervous system (CNS) tissue was obtained at autopsy following an Institutional Review Board-approved protocol. White matter CNS tissue was obtained from 4 subjects with relapsing-remitting MS. Patient 1 was a 42-year-old man, 12-year disease duration; Patient 2 was a 41-year-old man, 8-year disease duration; Patient 3 was a 32-year-old woman, unknown disease duration, and Patient 4 was a 36-year-old woman, 8-year disease duration. Brain tissue was fixed by immersing the brain in 10 to 20% formalin for 2

to 4 weeks. Some samples were embedded in paraffin; other samples were stored in formalin.

In formalin-fixed, non-embedded sections, in which lipids were not extracted through deparaffinization, myelin lipids were detected with Sudan Red 5B (Oil Red O), which stains neutral lipids. ORO powder was dissolved in isopropanol at a concentration of 0.5% and further diluted at a 3:5 dilution in dH₂O. Sections were incubated with ORO for 10 minutes, washed with tap water and cover-slipped with aqueous mounting medium without dehydration.

Formalin-fixed, paraffin-embedded (FFPE) sections were cut at 5- μ m thickness. After quenching with 0.03% hydrogen peroxide and blocking with normal serum, sections were incubated with primary fluorescent- labelled secondary antibodies and subsequently treated with 0.7% Sudan Black in ethanol and CuSO₄ to quench autofluorescence. Sections were counterstained with DAPI and mounted with VectaShield mounting medium (VectaShield Kit, Vector Laboratories). Adequate controls using isotype control antibodies were performed with each primary antibody (27). Fluorescent images were taken with an UltraVIEW VoX (Perkin Elmer) spinning disc confocal Nikon Ti-E Eclipse microscope. Image acquisition, visualization and quantification were performed using the Volocity 6.3 software (Improvision). Images were processed with the ImageJ software (28), by a biochemist (K.M.G. with 10 years of experience).

Susceptibility change during acute MS lesion formation

Five relapsing-remitting MS patients with Gadolinium (Gd) enhancing lesions were recruited for an MRI follow-up scan within four weeks of the previous scan as part of an ongoing longitudinal human MRI study. The mean age of the patients (2 men and 3 women) was 40.8 ± 8.8 years, with ages ranging from 28 to 51 years. This study was approved by the local IRB committee and written informed consent was obtained from all subjects prior to the start of the study. MRI was performed on a 3T Siemens Skyra system and the scanning protocol consisted of standard T1 and T2 weighted imaging for anatomy, multi-echo GRE imaging for QSM, and gadolinium-enhanced imaging to detect blood-brain barrier disruption. The 3D multi-echo GRE imaging parameters were as follows: TE1/ TE = 6.7/4.1 ms, number of echoes = 10, TR = 49 ms, axial FOV = 24 cm, phase FOV factor = 0.8, acquisition matrix = 320 $\times$ 256, slice thickness = 3 mm, flip angle = 15°, readout bandwidth = 260 Hz/pixel, parallel imaging GRAPPA factor = 2, scan time = 4.5 min.

Brain QSM maps were reconstructed from multi-echo GRE data (CSF) with MEDI+0, which enforces homogeneity within the CSF in the lateral ventricles(25).. Gd enhancing lesions were identified and traced by a neuroradiologist (S.Z. with 6 years of experience) using ITK-SNAP software (26). Lesion ROIs were manually drawn on co-registered T2w images and overlaid on QSM. When necessary, these ROIs were further edited to better match lesion geometry on QSM if lesions were visible on QSM. ROIs with similar sizes in contralateral NAWM not containing any potential white matter lesions were also traced for quantification of local susceptibility contrast. Veins or artifacts within the ROIs were removed manually.

RESULTS

ORO lipid staining of acute MS lesions obtained from Patient 1 demonstrated punctuate staining within macrophages, indicative of myelin breakdown and digestion (Fig. 1a–c). In surrounding normal appearing white matter, ORO staining showed intact myelin (Fig. 1d). Double labeling with fluoromyelin and the macrophage marker CD68 confirmed the presence of myelin fragments within macrophages (Fig. 1c). Similar results were obtained for the other 3 patients.

ROI-based estimates from QSM maps showed that digestion of myelin by trypsin caused the myelin volume susceptibility to increase by an average of 112 ± 37 ppb (Fig. 2a&b), in close agreement with the theoretical estimate of 111 ppb, which assumes cleavage of 3 peptide bonds in MBP. Digestion of MBP by trypsin was confirmed using Western blotting (Fig. 2c).

Degradation of purified myelin with SDS resulted in a substantial reduction of fluoromyelin intensity (Fig. 3c&d). Bodipy staining of myelin before and after SDS treatment confirmed lipid breakdown into smaller liposomes (Fig. 3e&f). Representative QSM images from the region of the falcon tube with purified myelin, and from the region with myelin incubated with SDS, are shown in Fig. 3a&b. Although, the volume susceptibility difference between purified and denatured myelin looks substantial, after correcting for the volume susceptibility due to SDS, the change due to denaturation of myelin was found to be just 23 ppb.

DISCUSSION

Our data from digestion of human MBP with trypsin and dissolution of human myelin with SDS demonstrated that the susceptibility of digested MBP increased by 112 ± 37 ppb, consistent with theoretical predictions, while the susceptibility of denatured myelin increased by 23 ppb. We also observed similar susceptibility increase in MS patients, where susceptibility of white matter lesions rose by 7.5 ± 6.3 ppb within 4 weeks after detection of enhancement. These in-vitro QSM results can be interpreted using Pascal's rule of additivity of magnetic susceptibility that the magnetic susceptibility of a system is the sum of the magnetic susceptibilities of its subunits and bonds. Loss of bonds in a biomolecule results in a decrease in the magnitude of its diamagnetic susceptibility or a susceptibility increase.

MBP is a polymer of 170 amino acids and is known to be cleaved by the enzyme trypsin at 3 sites (18). Trypsin digestion of MPB *in vitro* is expected to result in a volume susceptibility change that is three times the molar susceptibility of the peptide bond divided by the molar volume of MBP. This estimate of the volume susceptibility change evaluates to 111 ppb, which is close to the experimental value of 112 ± 37 ppb obtained in this study. Since MBP provides support to the myelin lipid bilayer, its digestion in the initial stage of MS lesion development results in additional loss of bonds within the bilayer, with a concomitant increase in susceptibility. Although proteolysis is a minor component in the overall process of myelin digestion, it can make a significant contribution to susceptibility increase. From (29), 100 grams of normal white matter contains 28 grams of dry white matter, of which 14 grams is myelin, i.e., 14% of white matter is myelin. In addition, 30% of myelin, or 4% of

white matter, is made up of proteins (30). Applying Wiedemann's Law to our *in vitro* results, the susceptibility change due to protein digestion and the dissolution of the myelin sheath is $(4\% \times 112 \text{ ppb}) + (14\% \times 23 \text{ ppb})$, which is approximately 7.7 ppb. This is close to the average value of $7.5 \pm 6.3 \text{ ppb}$ measured *in vivo*. The value measured for a specific lesion *in vivo* will be determined by the extent of demyelination.

In MS patients, tissue susceptibility increased rapidly in active lesions that transitioned from Gd-enhancing to non-enhancing. This is consistent with the recently reported MRI phase changes during acute lesion formation (31). We hypothesize that early myelin breakdown may be the main contributor to positive lesion susceptibility. Restitution of the BBB occurs within several weeks of lesion formation, and although the events accompanying this process are incompletely known, research suggests substantial myelin digestion by macrophages during this period (32,33). A possible additional contributor to susceptibility increase is the exit of myelin-laden macrophages from lesions, which represents a reduction of diamagnetic materials in lesions. However, the macrophage exit from lesions is a slow process, as demonstrated in histological evidence that elevated lipid signals can persist for up to 8 months after the initial lesion appearance (5,34,35). This is illustrated by lesions that are positive for the non-specific debris stain PAS (Periodic acid-Schiff) (5), and the presence of ORO in acutely demyelinating lesions that shows accumulation of lipids (denatured myelin) in macrophages, while the overall lipid content is still comparable between the lesion and surrounding normal appearing white matter. Another possible additional contributor to susceptibility increase in MS lesions is iron (36–38). However, iron accumulates several months later than this early lesion stage (1,10). The susceptibility change is further heightened and ultimately maintained for several years due to myelin debris removal and iron accumulation (36,38). QSM can, in principle, detect susceptibility shifts of 1 ppb (39), and is very sensitive at detecting susceptibility change in MS lesions at all their stages including early stages (36,38,40). The increases in QSM in newly formed lesions may therefore be indicative of a post-enhancing state and, in conjunction with T2 weighted imaging, may be used to substitute the use of gadolinium in routine follow-up MRI of MS patients (9).

Our histological data demonstrates an MS lesion with active myelin digestion but no iron accumulation and no substantial myelin removal but with susceptibility increase (Fig.1). This lesion may correspond to the lesion observed in patients using very short time interval (~2 to 4 weeks) immediately following the identification of an enhancing lesion. The consistency among the lesion susceptibility values measured in MS brain specimens and in MS patients and the theoretical predication discussed in the above supports the myelin digestion interpretation. The previous longer time intervals used in prior phase studies (11,44) may contain effects of both myelin digestion and myelin debris removal, the latter caused by myelin laden macrophages leaving the lesion. The 4-week interval used in this study that was designed to minimize contamination from myelin debris removal seems to allow almost complete myelin digestion, as indicated by the agreement of the observed susceptibility increase with the theoretical estimate as discussed in the above.

An obvious limitation of this study is the limited sample sizes. While it is difficult to find in autopsied MS brains a lesion with active myelin digestion, we hope to recruit with greater

efforts more MS patients in *in vivo* MRI study. Another limitation is the myelin digestion interpretation of in vivo data. We assume that myelin digestion is the dominant contributor to magnetic susceptibility increase during the four weeks immediately after the identification of an enhancing lesion. While myelin debris removal may be marginal, there may be myelin disruption and phagocytosing. The myelin orientation change from order to disorder seems to have little change on QSM, as suggested by the lack of susceptibility contrast of enhancing MS lesions (14,48). The generalized Lorentz model for the myelin change from linear order to point order may (52) predominantly reflects diamagnetic effects of myelin breaking down from line to points, but this theoretically confusing issue requires further investigation. QSM (16), by deconvolving the non-local phase used in prior studies (11,12,44), reveals local tissue susceptibility property. The QSM method here assumes no anisotropy and may not be sensitive to residual intact or reconstituted myelin. Myelin may be quantified using additional measurement based on relaxation (53,54), which belongs to another interesting and important study.

In conclusion, our data suggests that continuous breakdown of myelin contributes to an increase in magnetic susceptibility as MS lesions evolve from enhancing to early post-enhancing. (14,48)

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References

1. Kutzelnigg A, Lassmann H. Pathology of multiple sclerosis and related inflammatory demyelinating diseases. *Handb Clin Neurol*. 2014; 122:15–58. [PubMed: 24507512]
2. Sofroniew MV. Astrocyte barriers to neurotoxic inflammation. *Nat Rev Neurosci*. 2015; 16(5):249–263. [PubMed: 25891508]
3. Absinta M, Sati P, Reich DS. Advanced MRI and staging of multiple sclerosis lesions. *Nat Rev Neurol*. 2016; 12(6):358–368. [PubMed: 27125632]
4. Greenfield JG, Love S, Louis DN, Ellison D. *Greenfield's neuropathology*. London: Hodder Arnold; 2008.
5. Kuhlmann T, Ludwin S, Prat A, Antel J, Brück W, Lassmann H. An updated histological classification system for multiple sclerosis lesions. *Acta Neuropathol*. 2017; 133(1):13–24. [PubMed: 27988845]
6. Nolting W, Ramakanth A. *Quantum theory of magnetism*. Heidelberg; New York: Springer; 2009. xiii752
7. Wang Y. *Principles of Magnetic Resonance Imaging: Physics Concepts, Pulse Sequences, & Biomedical Applications: CreateSpace Independent Publishing Platform*. 2012
8. Kahn O. *Molecular magnetism*. New York, NY: VCH; 1993. xvi380
9. Zhang Y, Gauthier SA, Gupta A, et al. Magnetic Susceptibility from Quantitative Susceptibility Mapping Can Differentiate New Enhancing from Nonenhancing Multiple Sclerosis Lesions without Gadolinium Injection. *AJNR American journal of neuroradiology*. 2016
10. Zhang Y, Gauthier SA, Gupta A, et al. Quantitative Susceptibility Mapping and R2* Measured Changes during White Matter Lesion Development in Multiple Sclerosis: Myelin Breakdown, Myelin Debris Degradation and Removal, and Iron Accumulation. *AJNR American journal of neuroradiology*. 2016; 37(9):1629–1635. [PubMed: 27256856]
11. Anantakrishnan S V, Varadachari P S. The hydrogen bond and diamagnetism: 1944. :128–137.

12. Tomiyasu K, Sato T, Orimo S. Estimation of bonding nature using diamagnetic susceptibility. *Chem Commun (Camb)*. 2015; 51(41):8691–8694. [PubMed: 25908250]
13. Pascal P. Magnetochemical Researches. *Annales de Chimie et de Physique*. 1910; 9(19):5–70.
14. Ohta M, Ohta K. Detection of myelin basic protein in cerebrospinal fluid. *Expert Rev Mol Diagn*. 2002; 2(6):627–633. [PubMed: 12465458]
15. Babaei M, Jones IC, Dayal K, Mauter MS. Computing the Diamagnetic Susceptibility and Diamagnetic Anisotropy of Membrane Proteins from Structural Subunits. *J Chem Theory Comput*. 2017; 13(6):2945–2953. [PubMed: 28418668]
16. Kumar M, R G. *Diamagnetic Susceptibility and Anisotropy of Organic Compounds*. Springer; Berlin Heidelberg: 2008.
17. Smith R. Self-association of myelin basic protein: enhancement by detergents and lipids. *Biochemistry*. 1982; 21(11):2697–2701. [PubMed: 6178428]
18. Medveczky P, Antal J, Patthy A, et al. Myelin basic protein, an autoantigen in multiple sclerosis, is selectively processed by human trypsin 4. *FEBS Lett*. 2006; 580(2):545–552. [PubMed: 16412431]
19. Gopalakrishna R. Diamagnetic susceptibility of binary liquid mixtures. *Proceedings of the Indian Academy of Sciences - Section A*. 1963; 58(4):229–232.
20. Hu Y, Doudevski I, Wood D, et al. Synergistic interactions of lipids and myelin basic protein. *Proc Natl Acad Sci U S A*. 2004; 101(37):13466–13471. [PubMed: 15353595]
21. Min Y, Kristiansen K, Boggs JM, Husted C, Zasadzinski JA, Israelachvili J. Interaction forces and adhesion of supported myelin lipid bilayers modulated by myelin basic protein. *Proc Natl Acad Sci U S A*. 2009; 106(9):3154–3159. [PubMed: 19218452]
22. Takahashi F, Sakai Y, Yoshida T. Diamagnetic Susceptibilities of Various Inorganic Phosphates and Organic Phosphate Esters. *Bulletin of the Chemical Society of Japan*. 1993; 66(2):371–373.
23. Takahashi F, Sakai Y, Nakazawa Y, Mizutani Y. The Change of Diamagnetic Susceptibility and the Thermodynamic Data due to Hydrogen Bonding between 2-Propanol and Various Hydrogen Acceptors. *Bulletin of the Chemical Society of Japan*. 1994; 67(11):2967–2971.
24. Martenson RE. *Myelin*: Taylor & Francis; 1992.
25. Liu Z, Spincemaille P, Yao Y, Zhang Y, Wang Y. MEDI+0: Morphology enabled dipole inversion with automatic uniform cerebrospinal fluid zero reference for quantitative susceptibility mapping. *Magn Reson Med*. 2017
26. Yushkevich PA, Piven J, Hazlett HC, et al. User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. *Neuroimage*. 2006; 31(3):1116–1128. [PubMed: 16545965]
27. Ponath G, Ramanan S, Mubarak M, et al. Myelin phagocytosis by astrocytes after myelin damage promotes lesion pathology. *Brain*. 2017; 140(2):399–413. [PubMed: 28007993]
28. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods*. 2012; 9(7):671–675. [PubMed: 22930834]
29. Laule C, Vavasour IM, Moore GR, et al. Water content and myelin water fraction in multiple sclerosis. A T2 relaxation study. *J Neurol*. 2004; 251(3):284–293. [PubMed: 15015007]
30. Quarles RH, Macklin WB, Morell P. Myelin formation, structure and biochemistry. *Basic neurochemistry: molecular, cellular and medical aspects*. 2006; 7:51–71.
31. Wiggermann V, Hernandez Torres E, Vavasour IM, et al. Magnetic resonance frequency shifts during acute MS lesion formation. *Neurology*. 2013; 81(3):211–218. [PubMed: 23761621]
32. Wiggermann V, Hernández Torres E, Vavasour IM, et al. Magnetic resonance frequency shifts during acute MS lesion formation. *Neurology*. 2013; 81(3):211–218. [PubMed: 23761621]
33. Ortiz GG, Pacheco-Moisés FP, Macías-Islas M, et al. Role of the blood-brain barrier in multiple sclerosis. *Arch Med Res*. 2014; 45(8):687–697. [PubMed: 25431839]
34. Prineas J. Pathology of the early lesion in multiple sclerosis. *Hum Pathol*. 1975; 6(5):531–554. [PubMed: 170186]
35. Prineas JW, Raine CS. Electron microscopy and immunoperoxidase studies of early multiple sclerosis lesions. *Neurology*. 1976; 26(6 PT 2):29–32. [PubMed: 944890]

36. Chen W, Gauthier SA, Gupta A, et al. Quantitative susceptibility mapping of multiple sclerosis lesions at various ages. *Radiology*. 2014; 271(1):183–192. [PubMed: 24475808]
37. Wisnieff C, Ramanan S, Olesik J, Gauthier S, Wang Y, Pitt D. Quantitative susceptibility mapping (QSM) of white matter multiple sclerosis lesions: Interpreting positive susceptibility and the presence of iron. *Magnetic resonance in medicine*. 2015; 74(2):564–570. [PubMed: 25137340]
38. Zhang Y, Gauthier SA, Gupta A, et al. Longitudinal change in magnetic susceptibility of new enhanced multiple sclerosis (MS) lesions measured on serial quantitative susceptibility mapping (QSM). *Journal of magnetic resonance imaging : JMRI*. 2016; 44(2):426–432. [PubMed: 26800367]
39. Duyn JH, Schenck J. Contributions to magnetic susceptibility of brain tissue. *NMR Biomed*. 2017; 30(4)
40. Li X, Harrison DM, Liu H, et al. Magnetic susceptibility contrast variations in multiple sclerosis lesions. *Journal of magnetic resonance imaging : JMRI*. 2016; 43(2):463–473. [PubMed: 26073973]

Figure 1.

Acutely demyelinating white matter lesion. (a) staining with the lipid dye, Oil Red O (ORO), demonstrates punctate staining within the lesion. (b) magnification of the rectangle 1 in A. ORO⁺ lipids are indicative of broken-down myelin. (c) Confocal image of the lesion from rectangle 1 stained with the macrophage marker CD68 (green), fluoromyelin (red) and nuclear stain Hoechst 33342 (blue) demonstrates the presence of myelin fragments within phagocytosing macrophages. (d) Magnification of rectangle 2 shows intact ORO⁺ myelin in the normal appearing white matter.

Figure 2.

QSM of undigested MBP (a) and MBP digested for 2 hours with trypsin (b) embedded in a gel phantom, showing increased susceptibility with MBP digestion. Western blotting of MBP (c; left lane) and MBP incubated for two hours with trypsin (c; right lane), confirm the digestion of MBP by trypsin. The left lane shows three isoforms of MBP (marked by asterisks)..

Figure 3.

QSM of homogenized purified human myelin (red arrow) (a) and myelin degraded by SDS (red arrow) (b) embedded in agarose gel. Corresponding lipid stains show that lipids in intact myelin are visible when stained with fluoromyelin (c) and Bodipy 493/503 (e), while lipids released from myelin treated with SDS are no longer visible with fluoromyelin (d) but still visible as smaller fluorescent dots with the Bodipy stain (f).

Figure 4.

Gd enhanced T1 weighted images (a and b) and corresponding QSM (c and d) of a new acute MS lesion acquired at the time of Gd enhancing (a and c) and 19 days later (b and d). The BBB is closed as evidenced by the non-enhancing appearance in (b). Notice the increase in susceptibility within the lesion (arrow), suggesting myelin digestion by macrophages.