Comparison of multi echo T₂ relaxation and steady state approaches for myelin imaging in the central nervous system

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The traditional approach for measuring myelin-associated water with quantitative magnetic resonance imaging (MRI) uses multi-echo T₂ relaxation data to calculate the myelin water fraction (MWF). A fundamentally different approach, abbreviated “mcDESPOT”, uses a more efficient steady-state acquisition to generate an equivalent metric (f_m). Although previous studies have demonstrated inherent instability and bias in the complex mcDESPOT analysis procedure, f_m has often been used as a surrogate for MWF. We produced and compared multivariate atlases of MWF and f_m in healthy human brain and cervical spinal cord (available online) and compared their ability to detect multiple sclerosis pathology. A significant bias was found in all regions (p < 10⁻⁵), albeit reversed for spinal cord (f_m-MWF = −3.4%) compared to brain (+6.2%). MWF and f_m followed an approximately linear relationship for regions with MWF < ~10%. For MWF > ~10%, the relationship broke down and f_m no longer increased in tandem with MWF. For multiple sclerosis patients, MWF and f_m Z score maps showed overlapping areas of low Z score and similar trends between patients and brain regions, although those of f_m generally had greater spatial extent and magnitude of severity. These results will guide future choice of myelin-sensitive quantitative MRI and improve interpretation of studies using either myelin imaging approach.

Quantitative magnetic resonance imaging (MRI) techniques are developed with the aim of being specific to certain features of tissue microstructure. In the case of myelin water imaging, the water trapped between myelin bilayers can be used as a biomarker for myelin content.

The traditional approach for measuring myelin-associated water with quantitative magnetic resonance imaging (MRI) uses multi-echo T₂ relaxation data to calculate the myelin water fraction (MWF). A fundamentally different approach, abbreviated “mcDESPOT”, uses a more efficient steady-state acquisition to generate an equivalent metric (f_m). Although previous studies have demonstrated inherent instability and bias in the complex mcDESPOT analysis procedure, f_m has often been used as a surrogate for MWF. We produced and compared multivariate atlases of MWF and f_m in healthy human brain and cervical spinal cord (available online) and compared their ability to detect multiple sclerosis pathology. A significant bias was found in all regions (p < 10⁻⁵), albeit reversed for spinal cord (f_m-MWF = −3.4%) compared to brain (+6.2%). MWF and f_m followed an approximately linear relationship for regions with MWF < ~10%. For MWF > ~10%, the relationship broke down and f_m no longer increased in tandem with MWF. For multiple sclerosis patients, MWF and f_m Z score maps showed overlapping areas of low Z score and similar trends between patients and brain regions, although those of f_m generally had greater spatial extent and magnitude of severity. These results will guide future choice of myelin-sensitive quantitative MRI and improve interpretation of studies using either myelin imaging approach.

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including separate, exchanging $T_1$ and $T_2$ components for myelin-associated and intra-/extra-cellular water, which facilitates calculation of the mcDESPOT myelin water fraction ($f_M$). Compared to myelin imaging with multi-echo $T_2$ relaxation, mcDESPOT is appealing for its intrinsic acquisition efficiency, which can be leveraged to improve coverage, resolution, or acquisition time, and for its additional quantitative metrics, such as $T_1$ and exchange rate constants, which can provide supplemental information.

Potential inaccuracies or biases of the GRASE and mcDESPOT analysis procedures have previously been explored. Multi-component $T_2$ analysis of GRASE, or any multi-echo $T_2$ relaxation data, relies on suitable analysis parameters and sufficient data signal-to-noise ratio for MWF values to stabilize. mcDESPOT analysis requires accurate parameter fitting boundaries to be defined, but even then has been criticized for non-unique solutions and inherent, unpredictable bias in the fitting procedure.

Similarly, both acquisitions have a set of MR-signal related potential confounds for myelin imaging, which have been reasonably well studied. Previous studies have found differences between MWF and $f_M$ in brain tissue, with mcDESPOT providing consistently higher myelin estimates, especially in gray matter (GM), and a much more uniform distribution of values, especially in white matter (WM). However, MWF and $f_M$ values reported in cervical spinal cord are relatively similar, which is surprising given their disparity in the brain. This difference between brain and spinal cord could be related to the influence of MR physics related confounding factors, bias in analysis procedures, differences in cyto-architecture between brain and spinal cord, or likely a combination of the above.

Despite these differences, both GRASE and mcDESPOT have individually demonstrated sensitivity to pathology and developmental changes in the central nervous system, often with conclusions that corroborate one another. Previous work has reported a significant, moderately strong correlation between MWF and $f_M$ in brain tissue, further suggesting that steady-state approaches can serve as effective alternatives to multi-echo $T_2$ relaxation myelin imaging.

However, as previously reported values suggest that this relationship might not hold in the spinal cord, a direct comparison with spinal cord data would be of great value as it could strengthen or contradict the purported correspondence between MWF and $f_M$.

In this study, we perform the first direct comparison of multi-echo $T_2$ relaxation (GRASE) and steady-state (mcDESPOT) myelin imaging in both the brain and cervical spinal cord in a cohort of healthy participants. Our comparison is supplemented with data in the context of demyelinating disease pathology, analyzed on an individual basis for people with multiple sclerosis. The goals of this study are to:

- create atlases of normative reference MWF and $f_M$ values for both brain and cervical spinal cord;
- use atlas-based methods to compare myelin imaging approaches with minimal influence from biological variation and measurement noise;
- improve characterisation of the relationship between MWF and $f_M$, by comparing values across a diverse range of tissue, including healthy and diseased brain and spinal cord.

**Results**

Five of the 28 participants had to be excluded from the MWF and $f_M$ spinal cord atlases, due to imaging artefacts that rendered their GRASE or mcDESPOT data unusable.

**Mean atlas ROI values.** Table 1 provides the mean and standard deviation (SD) of voxel values within each brain and spinal cord region of interest (ROI), calculated for the mean MWF and $f_M$ atlases. Mean MWF and $f_M$ ROI values across all healthy control participants differed significantly ($p < 10^{-5}$) for all ROIs. MWF values were higher than MWF in all brain ROIs by a factor of $\sim 2$, except for the posterior internal capsule where values were more similar ($f_M = 19.5 \pm 1.2\%$, MWF = $17.6 \pm 4.0\%$) and the caudate where $f_M$ was larger by a factor of $\sim 3$ ($f_M = 10.0 \pm 3.3\%$, MWF = $3.0 \pm 1.8\%$). In spinal cord, $f_M$ was lower than MWF in all ROIs except GM and the absolute difference between $f_M$ and MWF values was smaller than in brain.

**Healthy participant ROI values.** In Fig. 1 mean MWF and $f_M$ values within each ROI, for each healthy participant, are displayed. Figure 1A shows $f_M$ plotted against MWF, for each ROI value. The Bland–Altman plot in Fig. 1B shows the difference between each MWF and $f_M$ ROI value plotted against their mean.

There was an approximately linear relationship between MWF and $f_M$ for brain regions (circle data points in Fig. 1A) with MWF < $\sim 10\%$. The linear MWF-$f_M$ relationship did not hold in the spinal cord, or in brain regions with MWF > $\sim 10\%$. The non-linearity between $f_M$ and MWF appears qualitatively consistent across participants, with a tight spread of data points following the same distinct pattern. The intra-subject, intra-ROI variability of each metric, indicated by standard deviation uncertainty bars in Fig. 1A, is small compared to the range of $f_M$ and MWF values and could not account for the non-linear relationship.

In the spinal cord, MWF spanned a large range of values (12–30%) whereas $f_M$ had little variation (16–23%). A systematic bias exists between MWF and $f_M$ values, but with opposite sign for brain (mean WM&GM $f_M$-MWF = $6.2\%$) compared to spinal cord (mean whole cord $f_M$-MWF = $-3.4\%$).

**Templates and atlases.** Representative slices of the anatomical templates, $T_1$-weighted ($T_1w$) for brain and $T_2$-weighted ($T_2w$) for spinal cord, voxel-wise mean MWF and $f_M$ atlases, and voxel-wise SD MWF and $f_M$ atlases are displayed in Fig. 2A for brain and Fig. 2B for spinal cord. As observed in Fig. 1A, there is a larger dynamic range in the myelin estimates with MWF compared to $f_M$. In the mean MWF atlas of the spinal cord,
structures such as gray matter and dorsal column can be identified clearly. The templates, mean and SD atlases, and ROIs analyzed for MWF and fM in brain and spinal cord are openly available online33.

Representative slices of Z score maps comparing relapsing–remitting multiple sclerosis (RRMS) and primary progressive multiple sclerosis (PPMS) MWF and fM to the healthy participant atlases are shown in Fig. 3 for brain and for spinal cord. The RRMS Z score maps, Fig. 3A, show evidence of some small, focal areas of deficient MWF and fM compared to controls, likely corresponding to the location of lesions. The PPMS Z score maps, Fig. 3B, show widespread regions of low Z score for both MWF and fM in both brain and spinal cord.

In Fig. 3, histograms for brain and cord of each patient show similar distributions of Z score values for MWF and fM, with the largest differences visible in the extremes of the Z score range for PPMS. For RRMS, a slight shift towards lower values is apparent for the MWF Z score histogram, compared to fM. For PPMS, the MWF Z score maps are generally less extreme in terms of both spatial extent and statistical magnitude compared to those of fM. While PPMS Z score maps suggest that fM has greater sensitivity to the presence of pathological tissue changes, RRMS Z score histograms suggest that MWF may better identify subtle changes outside regions of more-extreme pathology. Regions of deficient MWF Z score are generally highlighted on the corresponding fM Z score map, although this congruity appears to be more consistent in brain.

Table 1. Mean and standard deviation (SD) of voxels within each region of interest (ROI) for the mean myelin water fraction atlases from multi-echo T2 relaxation (MWF) and steady-state (fM) approaches. ROI volumes are also provided. A paired, two-tailed t test found significantly different MWF and fM ROI values (p < 10-5) across healthy control participants, for all ROIs. Brain ROIs include all white matter (WM), all gray matter (GM) and combined WM&GM from T1w image segmentations, along with 9 additional WM ROIs (all JHU white matter labels combined, genu of corpus callosum, splenium of corpus callosum, whole corpus callosum (CC), posterior internal capsule, and frontal, occipital, parietal, and temporal lobes masked to WM), and 4 additional GM ROIs (cortical GM, caudate, thalamus, and putamen). Spinal cord ROIs include the whole cord, WM, GM, dorsal column, and lateral funiculi.

| ROI                          | MWF (%) | fM (%) | ROI volume   |
|------------------------------|---------|--------|--------------|
|                              | Mean ± SD | Mean ± SD | Number of voxels |
| All WM&GM                    | 5.7 ± 5.4 | 11.9 ± 5.5 | 1,239,957     |
| Brain white matter           |         |        |              |
| All WM                       | 8.0 ± 4.9 | 16.8 ± 4.1 | 538,746       |
| All JHU                      | 11.5 ± 4.5 | 19.9 ± 2.7 | 78,903        |
| Genu                         | 9.1 ± 2.3 | 20.0 ± 3.0 | 5260          |
| Splenium                     | 13.4 ± 2.8 | 20.4 ± 3.1 | 7527          |
| CC                           | 11.1 ± 3.0 | 19.6 ± 3.0 | 21,435        |
| Posterior internal capsule   | 17.6 ± 4.0 | 19.5 ± 1.2 | 5437          |
| Brain lobe white matter      |         |        |              |
| Frontal                      | 7.1 ± 3.5 | 17.6 ± 3.4 | 115,698       |
| Occipital                    | 7.3 ± 3.2 | 16.0 ± 3.9 | 72,205        |
| Parietal                     | 8.3 ± 3.7 | 17.8 ± 3.6 | 100,925       |
| Temporal                     | 6.8 ± 2.9 | 16.4 ± 3.7 | 45,173        |
| Brain gray matter            |         |        |              |
| All GM                       | 4.1 ± 5.2 | 8.1 ± 2.7 | 781,211       |
| Cortical                     | 3.7 ± 4.1 | 8.7 ± 2.8 | 445,356       |
| Caudate                      | 3.0 ± 1.8 | 10.0 ± 3.3 | 8130          |
| Thalamus                     | 7.4 ± 5.2 | 13.2 ± 3.7 | 14,105        |
| Putamen                      | 5.0 ± 3.8 | 12.0 ± 2.6 | 13,748        |
| Spinal cord                  |         |        |              |
| Whole                        | 22.1 ± 5.7 | 18.7 ± 2.9 | 18,673        |
| All WM                       | 23.8 ± 4.4 | 18.6 ± 3.2 | 14,614        |
| All GM                       | 15.7 ± 5.1 | 19.0 ± 1.0 | 4059          |
| Dorsal column                | 25.6 ± 4.6 | 19.2 ± 2.5 | 5541          |
| Lateral funiculi             | 22.8 ± 3.9 | 18.3 ± 3.3 | 7235          |

Z scores. Representative slices of Z score maps comparing relapsing–remitting multiple sclerosis (RRMS) and primary progressive multiple sclerosis (PPMS) MWF and fM to the healthy participant atlases are shown in Fig. 3 for brain and for spinal cord. The RRMS Z score maps, Fig. 3A, show evidence of some small, focal areas of deficient MWF and fM compared to controls, likely corresponding to the location of lesions. The PPMS Z score maps, Fig. 3B, show widespread regions of low Z score for both MWF and fM in both brain and spinal cord.

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Discussion
The atlas-based methods employed here provided an effective means of reducing biological variations and measurement noise present within and between individual participant quantitative metric maps. In addition to facilitating the creation of Z score maps, atlas-based methods provide increased statistical power for characterizing discrepancies between the quantitative myelin fraction metrics obtained with GRASE and mcDESPOT. The smooth, “high SNR” appearance of the atlases in Fig. 2 provide clear depictions of representative values expected
from each imaging modality. They can also serve as a reference for how MWF and fM values are expected to differ throughout commonly investigated regions in the central nervous system. The MWF and fM atlases are openly available and provide normative reference data to increase statistical power for future studies with limited ability to recruit and collect data from healthy controls.

Although previous studies have compared MWF and fM in the brain, this is the first comparison of MWF and fM values in both the brain and spinal cord. Our combined analysis yielded some consequential results, especially when interpreting spinal cord results in concert with those from brain. First, the absolute difference in magnitude between myelin-related metric values produced by multi-echo T2 relaxation and steady-state approaches is much smaller in spinal cord than in brain. Despite having more-similar magnitudes than in brain, MWF and fM in the spinal cord bear almost no correspondence. Secondly, the average bias between GRASE and mcDESPOT is reversed in spinal cord (mean whole cord fM-MWF = −3.4%) compared to brain (mean WM&GM fM-MWF = 6.2%). The inversion of the MWF versus fM bias between brain and spinal cord is clearly depicted by the individual participant mean ROI values plotted in Fig. 1A, where most brain and spinal cord data points lie on opposite sides of the line of unity.

Although comparisons in human brain have shown significant, moderately strong correlations between MWF and fM, the same linear relationship does not hold in the spinal cord. Figure 1A suggests that this relationship...
actually begins to break down in brain regions as well. Specifically, MWF and $f_M$ do not appear to be correlated in regions with MWF $> \sim 10\%$, where $f_M$ no longer increases in tandem with MWF. In hindsight, this is visible in some brain regions examined by previous studies$^{26,34}$, but the magnitude and extent of the incongruity between the two methods is made clear by our unified, direct comparison with both brain and spinal cord data.

Our atlas ROI results (Table 1) show that MWF is lower than $f_M$ throughout all investigated brain ROIs, in agreement with previous studies that compared values in the brain$^{24–26}$. Lack of contrast in $f_M$ between WM ROIs, as demonstrated by Table 1 values and qualitatively by mean $f_M$ atlases in Fig. 2, also matches expectations based on previous brain studies with mcDESPOT$^{24–26}$.

Previous work has provided evidence that myelin content within human brain WM is heterogeneous. Aboitiz et al. studied the corpus callosum in post-mortem human brain using the Loyez histological stain for myelin and electron microscopy$^{35}$. They found that the genu had a smaller median axon diameter (0.6 $\mu$m) compared to the splenium (1.0 $\mu$m), which is expected to correspond with lower oligodendrocyte proliferation and myelin content$^{35}$. They also found a high percentage of unmyelinated fibers in the genu (16%) compared to the rest of the corpus callosum (< 5%)$^{35}$. Both of these findings support the expectation of lower myelin content in the genu, compared to the splenium, which we found reflected in our results for MWF but not for $f_M$. In more recent studies using histological staining markers for myelin, the optical density of Luxol fast blue showed regional variation by factors $>2$ within non-lesional WM, albeit in MS brain tissue where pathological changes could increase stain contrast$^{5,6}$. Bürgel et al. demonstrated strong regional contrast in WM myelination when they used a modified Heidenhain–Woelcke stain$^{36}$ to distinguish major tracts (including the corticospinal tract, optic and acoustic radiations, fornix, cingulum, corpus callosum, superior longitudinal, superior and inferior occipito-frontal and uncinate fascicles) in 10 post-mortem brains by their relative degrees of myelination$^{37}$. Therefore, the low contrast of $f_M$ within WM is likely not representative of myelin content but rather could relate to a confounding factor for

Figure 2. Representative slices of the anatomical templates as well as quantitative atlases for the myelin water fraction from multi-echo T2 relaxation (MWF) and steady-state ($f_M$) approaches, for the (A) brain and (B) C2 and C3 level of the cervical spinal cord. From left to right, columns display the: anatomical template, mean atlases, and the standard deviation atlases. Mean and standard deviation atlases are calculated voxel-wise across 28 participants for brain and 23 participants for spinal cord.
mcDESPOT, such as the increased influence of magnetization transfer. Similarly to fM, magnetization transfer ratio maps show relatively little contrast between WM regions compared to MWF, but studies investigating the relationship between magnetization transfer and mcDESPOT have not been able to confirm that magnetization transfer has a prominent influence on fM values.

Our GRASE and mcDESPOT values agree with mean whole cord MWF and fM values reported previously by our group in healthy cervical spinal cord: 22.1% versus 22.9% reported by Ljungberg et al. and 18.7% versus 20.5% reported by Kolind et al. In our study, WM accounted for 78% of the spinal cord volume, compared to 43% of brain tissue volume. As a result, higher mean MWF and fM can generally be expected across the whole spinal cord. Although this does not explain higher MWF in both WM and GM individually, previous studies have reported approximately 2.5 times higher myelin content in the cord, compared to the cerebrum, of rat nervous system, providing a precedent for a similar pattern to exist in human tissue.

There are many possible explanations for the low fM in the spinal cord, compared to expectations from the brain. Like all quantitative MRI techniques, GRASE and mcDESPOT each have their own set of confounds, which could affect the results more or less in the spinal cord, causing the relationship between MWF and fM to change.

GRASE is expected to have some magnetization transfer effects due to repeated refocusing pulses throughout the echo train. For mcDESPOT, the short, high-amplitude excitation pulses used to acquire SPGR and bSSFP

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**Figure 3.** Representative slices of Z score maps for the myelin water fraction from multi-echo T2 relaxation (MWF) and steady-state (fM) approaches. Deficient Z scores, with Z ≤ −1.96, are superimposed on the anatomical templates for (A) one participant living with relapsing–remitting multiple sclerosis (female, age 26 years, expanded disability status scale (EDSS) 1.5, disease duration 3 years) and (B) one with primary progressive multiple sclerosis (female, age 64 years, EDSS 8.5, disease duration 29 years). From left to right, columns display: axial brain slices, axial cervical spinal cord slices, sagittal cervical cord views, and the corresponding vertebral levels of the cord. For brain and cord of each patient, histograms are plotted to show the distribution of Z score values for MWF (dashed blue line) and fM (solid red line). Z scores were calculated as the difference between multiple sclerosis metric values and the mean atlas, divided by the standard deviation atlas.
data minimize undesirable finite radio-frequency pulse effects but increase the effects of magnetization transfer. By sensitizing the signal to macromolecular protons present in biological tissue, magnetization transfer can have a profound effect on estimates of $T_1$ and $T_2$, relaxation values. Modified versions of mcDESPOT have been introduced with additional model parameters in an attempt to quantify and correct for magnetization transfer.

Upon investigation, Zhang et al. confirmed that removal of magnetization transfer has a strong effect on bSSFP signal curves. However, values for $f_M$ remained much higher than MWF, suggesting that magnetization transfer does not drive the MWF-$f_M$ difference in brain. The fact that signal changes did not propagate into the expected parameter changes helped motivate subsequent investigation into the mcDESPOT analysis procedure.

The GRASE MWF is generally underestimated in regions with very low signal contribution from myelin water, such as cortical GM brain regions, where there is insufficient signal-to-noise for accurate myelin water quantification. The multi-component $T_2$ analysis used with GRASE ignores the effects of $T_1$ relaxation. Using a short repetition time (TR) with GRASE could therefore artificially increase MWF values, due to additional $T_1$ weighting. However, our TR of 1073 ms is not expected to have a large influence on MWF and does not explain the discrepancy with $f_M$ cord values. Recent studies have shown that the MWF will decrease if the inter-compartmental exchange rate between myelin water and intra/extra-cellular water increases.

Therefore, GRASE MWF values may be underestimated, especially in regions with small axons and thin myelin sheaths, where an increase in inter-compartmental exchange can be expected. In comparison, mcDESPOT should be immune to this effect, assuming the analysis includes and accurately estimates the myelin water residence time parameter that is intended to account for exchange. However, omitting exchange from the mcDESPOT model so it is not accounted for, as with GRASE, does not remove the discrepancy between MWF and $f_M$ values.

The caveat that the residence time must be accurately estimated becomes problematic in the context of recent studies evaluating the mcDESPOT analysis. Degeneracy of parameter solutions in mcDESPOT, a fundamental problem afflicting many MRI microstructure mapping techniques, was first shown by Lankford and Does studying mcDESPOT precision with Cramér–Rao lower bounds. Imprecision of mcDESPOT solutions has been further demonstrated by Zhang et al. and Bouhrara et al., who both showed flat residual plots of mcDESPOT parameter fits. This can result in substantial instability in metric values, especially for moderate or low signal-to-noise data. They also showed that the instability could be reduced by removing fit parameters, specifically by ignoring the effects of exchange.

A recent investigation by West et al. further showed that mcDESPOT solutions are inaccurate and imprecise when exchange is included in the model. As in previous work, they found that ignoring exchange improved the stability of the analysis, but at the cost of significantly biased results. West et al. also found that changes in mcDESPOT analysis results were non-linear with respect to changes in their true values, and that changing a single parameter could induce marked changes in other, static parameters. For example, a change in the $T_1$ or $T_2$ value of intra-/extra-cellular water can result in a large change in $f_M$ results, even when the true value of $f_M$ has not changed. The notable sensitivity of $f_M$ to pathological tissue differences could be in part explained by certain pathology-related changes to $T_1$ or $T_2$ inducing a large, non-linear response in the $f_M$ results.

West et al. suggested that unpredictable bias in mcDESPOT results can be at least partially mitigated by the use of consistent acquisition and analysis parameters. However, our results suggest that even with consistent acquisition and analysis conditions, changes in signal characteristics due to the presence of pathology (multiple sclerosis) or unique tissue structures (spinal cord) propagate into $f_M$ values in a way that does not consistently reflect MWF values.

The anatomical structure of the spinal cord likely plays an important role in our observed breakdown of the MWF-$f_M$ relationship as well as the lack of contrast observed in our $f_M$ spinal cord results. The non-linear behaviour of the mcDESPOT analysis procedure could lead to biased results that do not necessarily reflect the true underlying values. Spinal cord WM is similar to that of corpus callosum in the brain, and cord GM is similar to putamen. Therefore, based on contrast between brain values, we can expect that MWF will have more contrast between these regions than $f_M$. Another consideration is the presence of a low $f_M$ boundary around the cord, larger than that visible for MWF, which is likely related to partial volume effects with cerebrospinal fluid.

Partial volume effects could indicate imperfections in the motion correction alignment of each image volume in mcDESPOT data sets. Misalignment from motion relates to the fact that mcDESPOT acquires central k-space data separately for each image volume, spread across the entire acquisition time, whereas GRASE acquires central k-space data only once. Imperfect alignment of mcDESPOT image volumes would lead to blurred metric maps and reduced contrast between ROIs inside the cord.

MWF and $f_M$ can be used to identify pathology on an individual basis in the brain or spinal cord. Here, we demonstrated this using Z score maps, shown in Fig. 3, which showed greater spatial extent and magnitude of severity for mcDESPOT $f_M$ Z scores, compared to those of GRASE MWF.

The high sensitivity of mcDESPOT to pathological differences has been previously demonstrated in the brain by O'Muircheartaigh et al. Although there is no definitive explanation, the high sensitivity of mcDESPOT is likely due to multiple confounding factors. Relatively low inter-participant $f_M$ variability, as evidenced by the low values of $f_M$, standard deviation between participants (Fig. 2), will tend to amplify Z score values. Another influence may be reduced specificity to myelin, which would grant $f_M$ sensitivity to a variety of pathological changes compared to MWF. Strong correlation (median $\rho = 0.8$) between $f_M$ and quantitative $T_1$ results from mcDESPOT provides evidence suggesting that $f_M$ is closely related to, if not driven by, relaxation values. Considering this, $f_M$ may not provide enough additional independent, myelin-specific information, compared to the quantitative $T_1$ metric from DESPOT1-HIFI, to justify the increased acquisition time to acquire a full mcDESPOT data set.

Despite their different acquisitions, analyses, and confounding factors, MWF and $f_M$ Z score histogram plots in Fig. 3 show similarity between participants and anatomical structures. Similarity between Z scores and the higher sensitivity of $f_M$ to pathological tissue changes support the interpretation that MWF and $f_M$ are both sensitive to myelin content but that $f_M$ has reduced specificity due to the unpredictable, non-linear analysis response to signal.
changes. For specificity to myelin, recent technical developments allow acquisition of multi-echo \(T_2\) relaxation data with compressed sensing acceleration, which can provide improved spatial and parametric resolution in less acquisition time, compared to the GRASE sequence presented here\(^8\). When possible within time constraints, studies should ideally include multiple myelin-sensitive metrics. Additional metrics incorporated in a multimodal imaging approach can provide supplemental information as well as increase confidence in the findings.

In conclusion, we have produced and provided normative reference MWF and \(f_M\) atlases for both brain and cervical spinal cord. In comparing these atlases, we found that MWF and \(f_M\) follow an approximately linear relationship for regions with MWF < ~10%, above which the relationship breaks down and \(f_M\) no longer increases in tandem with MWF. In the context of demyelinating disease pathology, Z score maps from \(f_M\) showed greater spatial extent and magnitude of severity. The high sensitivity of \(f_M\) is a benefit for detecting pathological changes but is likely related to the mcDESPOT analysis procedure propagating MR signal changes into the \(f_M\) results non-linearly and greatly limits the ability to attribute those differences solely to changes in myelin. Despite the fundamental differences in these two techniques, MWF and \(f_M\) Z score maps showed overlapping areas of low Z score and similar trends between patients and brain and spinal cord regions. Ultimately, these results will guide future choice of myelin-sensitive quantitative MRI and help improve retrospective interpretation of clinical studies using multi-echo \(T_2\) relaxation or steady-state myelin imaging measures in the context of healthy or diseased central nervous system.

**Methods**

**Ethics.** The study operated under ethics approval from the University of British Columbia Clinical Research Ethics Board. All participants provided written informed consent. All methods were performed in accordance with the relevant guidelines and regulations.

**Participants.** Data were collected from 28 healthy participants without a history of brain or spinal cord disease or injury (10 male and 18 female, mean age 46 years, range 22–65 years). To facilitate comparison of MWF and \(f_M\) in the context of pathology, data were also collected from two participants with clinically definite multiple sclerosis fulfilling the 2017 revised MacDonald criteria for diagnosis\(^55\): one participant with RRMS (female, age 26 years, expanded disability status scale (EDSS)\(^56\) 1.5, disease duration 3 years) and one with PPMS (female, age 64 years, EDSS 8.5, disease duration 29 years).

**MRI.** Imaging was performed at the UBC MRI Research Centre on a 3.0T MRI scanner (Achieva, Philips Medical Systems, Best, The Netherlands).

- Brain MRI were acquired using an 8-channel head coil.
- A sagittal 3D \(T_1\)-weighted magnetization-prepared rapid gradient-echo image (\(T_1w\)) was acquired with TR = 8.1 ms, echo time (TE) = 3.5 ms, inversion time (TI) = 1052 ms, shot interval = 3000 ms, FA = 8°, resolution = 1.0 × 1.0 × 1.0 mm\(^3\), field of view (FOV) = 256 × 256 × 165 mm\(^3\), acquisition time = 6 min 26 s.
- Multi-echo \(T_2\) relaxation data were acquired using a 3D multi-echo GRASE sequence with TR = 1073 ms, TE = 8 ms, echo spacing (\(\Delta TE\)) = 8 ms, 48 echoes, acquired resolution = 1.0 × 2.0 × 5.0 mm\(^3\), reconstructed resolution = 1.0 × 1.0 × 5.0 mm\(^3\), FOV = 230 × 190 × 100 mm\(^3\), slice oversampling factor = 1.3, sensitivity encoding (SENSE)\(^57\) acceleration factor = 2, and acquisition time = 7 min 31 s.
- Steady-state mcDESPOT brain acquisitions consisted of multiple SPGR, a single IRSPGR, and multiple bSSFP images, all acquired with a common resolution = 1.7 × 1.7 × 1.7 mm\(^3\) and FOV = 217 × 217 × 156 mm\(^3\). SPGR were acquired with TR = 6.5 ms, TE = 3.6 ms, and FA = 2, 3, 4, 6, 9, 13, and 18°; IRSPGR with TR = 6.5 ms, TE = 3.2 ms, TI = 450 ms, and FA = 5°; and bSSFP with TR = 5.8 ms, TE = 2.9 ms, FA = 7, 11, 15, 19, 24, 30, and 47° and phase cycling patterns of 0° and 180° for each FA. Total mcDESPOT acquisition time, for all FA and phase cycling patterns, was 9 min 26 s.
- Cervical spinal cord imaging used a 6-channel spine coil, with all imaging FOV centred at the C2/C3 level of the cervical spinal cord and angulation aligned with the cord.
- An axial 2D multi-slice \(T_2^*\)-weighted multi-echo gradient echo image (\(T_2^*\)w) was acquired with TR = 815 ms, TE = 6.5 ms, \(\Delta TE\) = 8.2 ms, 5 echoes (cumulated on the scanner), FA = 28°, acquired resolution = 0.8 × 0.8 × 2.5 mm\(^3\), number of slices = 16, slice gap = 0.25 mm, FOV = 150 × 150 × 44 mm\(^3\), and acquisition time = 5 min 7 s.
- Multi-echo \(T_2\) relaxation MWI data were acquired as adapted by Ljungberg et al.\(^22\) using a 3D multi-echo GRASE sequence with TR = 1501 ms, TE = 10 ms, \(\Delta TE\) = 10 ms, 32 echoes, acquired resolution = 0.75 × 0.75 × 5.0 mm\(^3\), reconstructed resolution = 0.63 × 0.63 × 2.5 mm\(^3\), FOV = 180 × 150×40 mm\(^3\), slice oversampling factor = 1.2, SENSE acceleration factor = 2, and acquisition time = 8 min 36 s.
- Steady-state mcDESPOT cord acquisitions had a common acquired resolution = 0.75 × 0.75 × 4.0 mm\(^3\), reconstructed resolution = 0.70 × 0.70 × 4.0 mm\(^3\), and FOV = 180 × 201 × 56 mm\(^3\). SPGR were acquired with TR = 5.5 ms, TE = 2.7 ms, and FA = 2, 3, 4, 6, 8, 10, 13, and 18°; IRSPGR with TR = 5.5 ms, TE = 2.7 ms, TI = 350 ms, and FA = 5°; and bSSFP with TR = 10.0 ms, TE = 5.0 ms, FA = 7, 14, 21, 28, 35, 42, and 49° and phase cycling patterns of 0° and 90° for each FA. Total mcDESPOT acquisition time, for all FA and phase cycling patterns, was 6 min 7 s.

The \(T_1w\) brain and \(T_2^*\)w cord images were acquired for their strong white-gray matter contrast, to facilitate alignment of anatomical structures between participants. All acquisitions were performed without cardiac or respiratory triggering. Brain and cord acquisitions for GRASE and mcDESPOT differed slightly due to differences in optimisation of parameters for each application\(^22\).
Quantitative MRI analysis. GRASE data was analyzed using a temporally-regularized non-negative least squares algorithm with stimulated echo correction (https://mriresearch.med.ubc.ca/news-projects/myelin-water-fracti...), 40 T2 relaxation delta functions logarithmically spaced from 15 to 2000 ms, 8 refocusing flip angles between 90 and 180°, and a \( \chi^2 \) regularization factor of 1.02. The MWF was calculated as the fraction of total signal with 15 < T2 < 40 ms.

mcDESPOT data underwent motion correction to align the imaging volumes before the DESPOT1-HIFI code was used to obtain an estimated FA correction map from the SPGR and IRSPGR data. Using the FA correction map, mcDESPOT analysis was performed to obtain the fM. The analysis was matched to that of a previous study on mcDESPOT stability, using a 3-pool model and the same stochastic-region-contraction parameter boundaries: fM [0.00000001, 0.35], myelin water T1 [300, 650] ms, myelin water T2 [1, 30] ms, free water T1 [700, T1,max] ms, free water T2 [50, 165] ms, and myelin water residence time \( \tau \) [25–600] ms.

Image analysis and template creation. Advanced Normalization Tools software (ANTS), Spinal Cord Toolbox (SCT), and some FMRIB Software Library (FSL) tools were used for image analysis. Non-linear registrations used symmetric diffeomorphic normalization (SyN) transformations. To prevent the introduction of circularity bias, no quantitative maps were involved in image registrations. Workflow for the multivariate template creation process is outlined in Fig. 4.

All T1w, GRASE echo 1, and IRSPGR brain images underwent N4 bias field correction for low frequency intensity nonuniformities. Brain segmentations were initialized with registration using the OASIS template and priors then refined using Atropos n-tissue segmentation. Each participant's GRASE echo 1 and IRSPGR images were masked to brain tissue before being rigidly aligned with their corresponding T1w image and masked again using the higher-resolution T1w brain segmentation.

For spinal cord images, cord segmentations were generated for each image modality using T2*w, GRASE echo 16, and IRSPGR images with an automated tool (sct_propseg) before quality control was performed manually. The cord segmentations were used to rigidly align GRASE echo 1 and IRSPGR with T2*w cord images. Finally, all images were masked using the higher-resolution T2*w cord segmentation.

Multivariate template creation was performed by iteratively co-registering the aligned and masked GRASE echo 1, IRSPGR and anatomical images (T1w for brain, T2*w for cord) using iterative rigid, affine, and SyN
transformation. Regressions were guided by mutual information (for linear stages) and neighborhood cross-correlation (for SyN) similarity metrics. The $T_1w$ and $T_1^*w$ similarity metrics were weighted twice as heavily as those of GRASE and IRSPGR, to account for their better resolution and tissue contrast.

**Region of interest definition.** A reference anatomical $T_1w$-weighted template was used to generate brain ROIs. This reference template was created in-house using data from 100 healthy volunteers, including those in the current study. After registration between our $T_1w$ template and the reference $T_1w$ template, JHU-ICBM-DTI-81 WM labels and MNI structural regions, optimized for the reference template using the probabilistic joint label fusion framework, were transformed to our multivariate template space. Atropos n-tissue segmentation was performed on our $T_1w$ template to provide WM and GM segmentations.

To generate spinal cord ROIs, registration was performed between our $T_2w$ spinal cord template and the $T_2^*w$-weighted SCT PAM50 template, centred at the C2/C3 level of the cord, and refined using a manually defined cord GM segmentation to improve alignment of intra-cord structure. This provided probabilistic cord ROIs, which were thresholded and binarized to only include voxels with probability $>0.5$, to reduce partial volume effects.

**Multiple sclerosis image analysis.** RRMS and PPMS images were processed the same as those of healthy participants, except they were excluded from template creation. Instead, their $T_1w$ and $T_1^*w$ images were registered to the $T_1w$ and $T_1^*w$ templates, and transformations were concatenated to align their MWF and $f_M$ maps in template space for comparison. Since the goal of the study was not to derive clinical conclusions, images from participants with multiple sclerosis were not segmented into lesion/non-lesion tissue.

**Statistical analysis.** From the healthy participant MWF maps, aligned in template space, atlases were created by calculating the voxel-wise mean and SD between participants, then cropped to exclude regions not covered by all individual participant MWF and fM FOV. For the mean MWF and fM atlases, the mean and SD within each ROI were calculated. Mean MWF and fM ROI values were compared across all healthy control participants using a paired, two-tailed t test. MWF and fM Z score maps were calculated by subtracting the mean atlas from the MS participant quantitative map, then dividing by the SD atlas. Z score maps were also masked to exclude highly variable voxels, where the coefficient of variation (calculated voxel-wise as the SD divided by the mean) was over 0.75.

**Data availability**

Ethics approval did not allow for making individual subjects’ imaging data available. However, the anatomical templates, quantitative atlases, and ROIs created and analysed in this study have been made openly available along with example code for the template creation process.

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Author contributions
A.V.D. was involved in study conception, design, data acquisition, analysis and interpretation, and created the figures, drafted, and substantially revised the work. E.L.J. was involved in study conception, data acquisition, interpretation, and helped draft and substantially revise the work. I.M.V., A.L.M. and S.H.K. contributed to study conception, design, interpretation, and substantial revision. L.E.L. and S.A. contributed to data acquisition and substantial revision. D.K.B.L. and A.T. contributed to study conception and interpretation.

Competing interests
The authors declare no competing interests.

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