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Multi-slice brain myelin water fractions at 3T in multiple sclerosis

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Abstract

Purpose: To evaluate a multi-slice nonlinearly-spaced 12-echo imaging sequence at 3T covering the supratentorial brain for the quantification of myelin water fraction (MWF) in MS patients.

Methods: Eighty-nine patients with, or at risk of, MS (69 RRMS, 7 SPMS, 13 CIS) and 28 controls were studied. 12-echo datasets were acquired using a multi-slice T2 prep spiral imaging sequence and were fitted using a nonnegative least squares algorithm. The mean MWF within NAWM, contrast enhancing (CE) and non-enhancing T2 lesions were calculated.

Results: Mean MWF in white matter for controls was 11.3%. Mean MWF was significantly reduced in NAWM of MS patients (10.6%, $P = 0.004$) relative to controls. SPMS/RRMS patients with disease duration > 5 years (10.3%) had lower MWF compared to CIS/RRMS with disease duration ≤ 5 years (10.8%, $P = 0.03$). Mean MWF was reduced by 26 % and 29 % within both CE ($P < 0.0001$) and non-enhancing T2 lesions ($P < 0.0001$) relative to controls.

Conclusion: Using a multi-component T2 sequence at 3T, a significant decrease in the supratentorial MWF was observed in MS NAWM and lesions relative to controls. The method was sensitive to detect white matter changes early in the disease process.

Key Words: Multi-component T2 relaxation times, myelin water fraction, multi-slice multi-echo T2 prep, high field MRI, multiple sclerosis

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INTRODUCTION

Multiple sclerosis (MS) is immune-mediated disease that affects the central nervous system and causes demyelinated plaques with glial scar formations in multiple focal areas as well as in macroscopically normal tissue ¹⁻⁵. Therefore, accurate measurement and quantification of the myelin water content would be useful for patients with MS. Although conventional magnetic resonance (MR) images play an important clinical role because of excellent contrast between normal and pathologic tissue, there is a need for more sensitive and specific markers of the biological effects of disease for analysis of patients with MS to monitor disease progression and response to therapy.

Previous studies have shown the existence of multi-component T2 relaxation decay curves in biological tissue as an indicator of compartmentation ^{6,7}. The individual T2 compartments have been interpreted in terms of different water compartments within heterogeneous tissue. It has also been shown that the short T2 component ($T_2 < 50$ msec) was observed mainly in the myelinated tissue ⁸ and reduction of the short T2 component has been observed in degenerated peripheral nerves ⁹. Moore et al. have also shown an absence of short T2 component in the chronic MS plaque using a formalin-fixed MS brain ¹⁰.

Previous *in vivo* studies have shown that the short T2 component is mainly observed within white matter (i.e. myelin water) for normal controls ¹¹, and that there is decreased myelin water in the lesions as well as normal appearing white matter for patients with MS relative to normal controls ¹². These latter findings relied on a single-slice 32-echo spin echo imaging sequence. T2 decay has traditionally been measured using multi-echo data sets acquired during a spin echo

readout train. Previous studies have shown that the accuracy of T2 quantification was improved by using non-slice selective rectangular composite refocusing pulses which reduced errors from B1 inhomogeneity^{13,14}. But these hard pulses prevent the acquisition of multiple slices within a single TR, and thus, imaging time increases linearly with the number of prescribed slices.

Therefore, to keep scan time within reasonable limits, coverage is often limited to a single slice¹⁵⁻¹⁷. Maier et al. have implemented a multi-echo spin echo sequence that uses slice selective refocusing pulses, which enables more time efficient multi-slice imaging. However, it was shown that slice selective refocusing pulses resulted in imperfect refocusing leading to stimulated echoes that introduced mixed T1 and T2 contrasts. It was also shown that off-resonance effects created by slice selective refocusing pulses introduced magnetization transfer contrast¹⁸. These effects could confound attempts to accurately quantify T2. Accurate quantification of the T2 relaxation times also requires a large number of echoes for the same location, typically ≥ 32 , with high signal to noise ratio (SNR) and results in a long acquisition time. Because of these limitations, multi-echo spin echo MR images have not been routinely acquired in a clinical environment. Vidarsson et al. have used a three spin echo sequence and a linear combination filter optimized for myelin imaging with an acquisition time of 5 min (6 slices)¹⁹. However this sequence is limited to myelin water content only, because signals from other white matter components are sufficiently suppressed.

Multiple echo data can also be acquired using T2 prep²⁰. Images with different T2-weighting are generated by changing the number of refocusing pulses in T2 prep. Wright et al. have demonstrated that an RF cycling scheme can mitigate the effects of T1 recovery between T2 prep and multi-slice readouts²¹. Therefore, with T2 prep, multiple slices can be acquired within a TR,

enabling time efficient multi-slice imaging with all the advantages afforded by hard composite refocusing pulses. A previous study from our laboratory has shown that short T2 components were mainly observed within the white matter and the short T2 water component fraction was 10 – 12 % of total water for normal volunteers using a multi-slice nonselective T2 prep spiral MR imaging sequence with unequal echo sampling at 3T equipped with an 8-channel phased array coil ²².

This current study was designed to implement a clinically relevant 16-slice 12-echo T2 prep spiral MR imaging sequence using a commercially available receive only 8-channel phased array coil at 3T for quantification of the T2 relaxation times in patients with MS.

MATERIAL AND METHODS

Study Population

Eighty-nine MS patients were included in this study from a cohort of patients who were followed at the University of California, San Francisco Multiple Sclerosis Center. Sixty-nine patients had RRMS and seven patients had clinically definite SPMS as defined by Poser criteria ²³. Thirteen patients with clinically isolated syndrome (CIS) were also included (all with abnormal brain MRI defined by ≥ 2 white matter lesions). To test the dependence of MWF on disease duration, the patients were divided into two groups; one for CIS and RRMS patients with disease duration (DD) of less than or equal to 5 years (N = 48) and the other for SPMS and RRMS patients with DD greater than 5 years (N = 41). Neurological evaluations included the expanded disability status scale (EDSS) ²⁴. Twenty-eight control subjects were examined using the same MR

protocol. All subjects gave written and informed consent for participating in the study. The mean (range) age, disease duration, and EDSS for all sub-groups are listed in Table 1.

Conventional MR Imaging

MR images were acquired with a 3T Signa scanner (GE Healthcare, Waukesha, WI, USA) equipped with an 8-channel phased array coil. The conventional MR imaging examination included a dual-echo PD-/T2-weighted sequence (TR = 2000 msec, TE = 20/80 msec, 512×512 matrix, 240×240 mm field of view, 44 3-mm interleaved slices), a T1-weighted three dimensional (3D) inversion recovery spoiled gradient echo (IR-SPGR) sequence (TR/TE/TI = 7/2/400 msec, 15° flip angle, $256 \times 256 \times 180$ matrix, $240 \times 240 \times 180$ mm field of view, 180 1-mm slices), and a post-gad T1-weighted spin echo sequence (TR/TE = 467/8 msec, 256×256 matrix, 240×240 mm field of view, 44 3-mm interleaved slices, covering the same locations as the pre-contrast dual-echo images). Single dose (0.1 mM/kg) gadolinium diethylenetriamine-pentaacetic acid (Gd-DTPA) was used as a contrast agent. The contrast enhancing T1-weighted spine echo sequence was acquired 5 minutes after injection of the contrast agent.

Multi-slice multi-echo T2 prep Spiral MR Imaging

A 16-slice 12-echo T2 prep spiral sequence was used in this study to acquire multi-echo data^{20, 22}. In brief, the T2 prep portion of the sequence consisted of a nonselective 90° tip-down pulse, a train of equally spaced (6 msec) composite 180° hard refocusing pulses (90_x - 180_y - 90_x) and a hard -90° tip-up pulse²⁵. The length of the refocusing trains determines the amount of T2 contrast in the resultant image. Large gradient spoilers following the tip-up pulse de-phase any remaining transverse magnetization. Image acquisition follows the T2 prep and includes a spectral-spatial

pulse and spiral readout for each prescribed slice. An RF cycling scheme was used to mitigate the effects of T1 recovery between T2 prep and the multi-slice spiral readouts²¹. Any residual longitudinal magnetization was then nulled using chemical shift selective (CHESS) pulses²⁶ consisted of three sets of 90° excitations and spoiler gradients applied sequentially. The period of longitudinal recovery was preserved for different echo times by shifting the delay between spiral readout and the CHESS pulses while TR is held constant. The echo time of this sequence was the duration of the refocusing train corrected by the period of T1-weighted signal decay during each composite pulse as shown in Foltz et al¹³.

The 12-echo MR imaging parameters were TR = 2000 msec, $TE_n = 7, 17, 28, 38, 49, 60, 70, 92, 124, 177, 220$ and 294 msec, 128×128 matrix, 240×240 mm field of view, 4096 points with 4 spiral interleaves resulting in an effective resolution of 2×2 mm, 16 5-mm slices, and NEX = 6 (scan time of 10 minutes). The 12-echo MR imaging was acquired before the injection of the contrast agent.

Fitting of Multi-echo Data to Quantify T2 Relaxation Times

The 12-echo data was fit to a distribution of T2 values using a nonnegative least square (NNLS) algorithm²⁷. The NNLS algorithm was implemented using Matlab (The MathWorks, Natick, MA, USA). The solution of the NNLS algorithm was iteratively regularized such that the ratio of chi-square misfit between the regularized and unregularized solution was less than 1%. The objective here was to have smoothly varying T2 distributions^{28,29}. The T2 axis was partitioned into 80 logarithmically spaced compartments between 15 and 2000 msec¹¹. T2 value, peak area and pool fraction for each peak were estimated from the T2 distributions. Estimated T2

components below a specific threshold (peak area for $T2_j < 3\%$ of total water) were ignored to eliminate the dependence of the fit to the noise. A median filter with kernel size of 3 was applied to the original images before fitting. The pixel-by-pixel myelin water fraction (MWF) (defined as ratio between peak area for T2 component < 50 msec and total water) map (%) was created. The fitting algorithm was implemented using a parallel processing computing grid (processing time of approximately 12 minutes).

Post-Processing

T2 hyperintensity lesions were drawn based on a semi-automated threshold with manual editing on the PD and T2-weighted images by an experienced neurologist (DP). Contrast enhancing (CE) lesions were defined manually based on the contrast enhancing T1-weighted spin echo images. The CE lesions were segmented out from the T2 hyperintensity lesion to create non-enhancing T2 hyperintensity (T2) lesions. Masks for both T2 and CE lesions were generated.

The scalp and skull were removed from the T1-weighted 3D IR-SPGR volume images using an automated brain extraction tool in order to improve the robustness of the subsequent image segmentation³⁰. The T1-weighted 3D IR-SPGR volume images were resampled to correspond to the PD-/T2-weighted images using nearest neighbor interpolation and then used to segment white matter, gray matter and cerebral spinal fluid structures using a hidden Markov random field model with an expectation maximization algorithm³¹. Normal appearing white matter (NAWM) masks was created for patients by excluding both CE and non-enhancing T2 lesions from the white matter (WM) masks.

The NAWM, non-enhancing T2 and CE masks were then regridded to the lower resolution MWF map from the 12-echo data yielding the percent content within each pixel. The mean MWF values were calculated in the NAWM, non-enhancing T2 and CE lesions for all subjects. Pixels contain within $\geq 90\%$ NAWM or non-enhancing T2 lesion masks were included in the calculation of their respective mean MWF. To include as many CE lesions possible, pixels contain within $\geq 50\%$ CE lesion mask were included for the MWF calculation.

Statistical Analysis

Statistical analyses were performed using standard least square means (LSM) tests with age adjustment to consider relatively younger mean age of controls with respect to the MS patients. The results were reported as LSM (standard error) unless otherwise noted. The non-parametric Spearman method was used for correlation tests. In this study, $p < 0.05$ is regarded as significant.

RESULTS

Volume of the Contrast Enhancing and Non-enhancing T2 Lesions

13/89 patients had contrast enhancing lesions and the average volume of CE lesion load was 1.2 ml with a range of 0.1 – 4.9 ml. The average volume of non-enhancing T2 lesion load for all patients was 5.5 ml with a range of 0.0 – 36.9 ml.

Myelin Water Fraction Values within Regions of Interests

A. Supratentorial NAWM Regions

The age adjusted mean MWF values within the WM/NAWM, non-enhancing T2 and CE lesions for all sub-groups are listed in Table 2. Figure 1 shows representative 8-slice of the MWF map

from a control. As shown in Figure 1, short T2 components were mainly detected within the white matter. The mean MWF value within the WM region was 11.3 (0.2) % for the controls. It was observed that the mean MWF value was significantly decreased in the NAWM for all patients ($P = 0.004$) relative to the controls. Differences were observed for both patients with CIS/RRMS with $DD \leq 5$ years ($P = 0.05$) and for patients with SPMS/RRMS with $DD > 5$ years ($P = 0.0004$) relative to the controls. The mean MWF value within the NAWM for patients with SPMS/RRMS with $DD > 5$ years was also significantly lower than that for patients with CIS/RRMS with $DD \leq 5$ years ($P = 0.03$).

B. Contrast Enhancing Lesions

The mean MWF value was significantly reduced within the CE lesions for 13 patients relative to controls ($P < 0.0001$). The same findings were observed for both patients with CIS/RRMS with $DD \leq 5$ years ($P = 0.0001$) and for patients with SPMS/RRMS with $DD > 5$ years ($P = 0.0003$) relative to the controls. Both patient groups did not differ from each other. Figure 2 (a) shows a PD image ($TE = 20$ msec) from a control (left), the corresponding WM mask overlaid on the PD image (middle), and the corresponding MWF map overlaid on the PD image (right). Figure 2 (b) shows a PD image with T2 lesions (top left) and NAWM (top right) masks, and post contrast T1-weighted spin echo image with CE lesion mask (bottom left) in a patient with RRMS. The MWF map was overlaid on the PD image (bottom right). Reduced MWF within the contrast enhancing MS lesion was observed compared to surrounding NAWM.

C. Non-enhancing T2 Lesions

A significant reduction of the mean MWF value was observed within the non-enhancing T2 lesions for all patients ($P < 0.0001$) relative to the controls. The same findings were observed for both patients with CIS/RRMS with $DD \leq 5$ years ($P < 0.0001$) and SPMS/RRMS with $DD > 5$ years ($P < 0.0001$) relative to controls. Both patient groups did not differ from each other.

Relationships between Lesion Load, Disease Duration, Disability and Myelin Water Fraction within the Regions of Interests

The volume of the non-enhancing T2 lesion for all patients was significantly correlated with the mean MWF values within the NAWM ($r = -0.3$, $P = 0.002$) and within the non-enhancing T2 lesions ($r = -0.4$, $P < 0.0001$). A weak correlation was observed between EDSS and the mean MWF within the non-enhancing T2 lesions ($r = -0.3$, $P = 0.02$) but not within the NAWM regions ($r = -0.1$, $P = 0.163$).

DISCUSSION

To authors' knowledge, this is the first study evaluating a clinically relevant multi-slice nonlinearly sampled 12-echo MR imaging technique measuring multi-component T2 relaxation times at 3T with the objective of estimating myelin water fractions derived from large supratentorial brain regions of interest in MS patients. We first observed that the mean myelin water fraction was significantly reduced in NAWM regions of MS patients and found that our method was sensitive enough to detect changes in patients within five years of disease onset. The second finding was that the mean myelin water fractions within both CE and non-enhancing T2 lesions were even more profoundly reduced for all patients relative to the controls. Lastly, only

modest relationships were observed between T2 lesion volume and myelin water fraction within NAWM regions, EDSS and myelin water fraction within T2 lesions.

Multi-component T2 relaxation imaging techniques are being investigated intensively since they have the potential to provide the most specific *in vivo* MRI marker of myelin integrity. Beaulieu et al. demonstrated three-component T2 relaxation times (i.e. myelin-, axonal- and interaxonal-water) *in vitro* for cranial nerves of the garfish at 2.35T⁸. In their study, the authors concluded that the short T2 components ($T_2 < 50$ msec) were assigned to myelin water because it was present in myelinated trigeminal and optic nerves, but absent in non-myelinated olfactory nerves. In our current study, we observed two- or three-component T2 relaxation times mainly in white matter with an estimated myelin water fraction for controls of 11.3 % as calculated from the multiple slices acquired. Laule et al. showed that mean myelin water fraction from several white matter structures was 11.2 % with a range of 7 – 16 % for controls using a single-slice 32-echo spin echo sequence¹². This myelin water fraction value is consistent with our results using the multi-slice nonlinearly spaced 12-echo T2 prep spiral sequence. Laule et al. also showed that the reduction of the myelin water fraction in NAWM for patients with MS was dominated by myelin loss of integrity rather than increased diffuse edema or inflammation¹².

Although a study involving the peripheral nervous system, Does et al. demonstrated three-component T2 relaxation times (mean T2 values of 19, 63 and 241 msec) in the sciatic nerve of the amphibian *Xenopus laevis* at 2.35T³² and also showed that after crush injury these three components were reduced remote from the crush due to Wallerian degeneration, reflecting collapse and loss of myelinated fibers⁹. In the current study, we observed a 6 % reduction of the

mean myelin water fraction within the NAWM for all patients relative to controls. The same metric within the NAWM was significantly lower for patients with SPMS/RRMS with DD > 5 years (9 % reduction) than that for patients with CIS and RRMS with DD ≤ 5 years (4 % reduction). This likely reflects more loss of myelin integrity with more advanced disease and/or more effective remyelination which may be prominent at the early stages of the disease³³. Moreover, we found a relationship, although modest, between T2 lesion volume and reduced MWF within NAWM regions representing damage that may be due to Wallerian degeneration caused by distant MS lesions.

The inflammatory infiltration within the active MS lesion (contrast enhancing lesions) is composed of active T cells, macrophages and microglial cells which are all involved in the pathogenesis of demyelination in MS^{1,34}. It was observed that there was a 26% reduction of mean myelin water fraction within the CE lesions for 13/89 patients in the current study relative to controls, which may indicate active demyelination within inflammatory lesions. Bruck et al. also showed that the highest number of macrophages was observed in actively demyelinating as well as early remyelinating lesions and the acute stage inflammatory macrophage markers were selectively expressed in early and late active lesions³⁵. In the current study, although significant reduction of the mean myelin water fraction was observed within the CE lesion, the variation of the same value was large (range of 4.0 – 13.0 %, N = 13). This may suggest that there is a mixture of active demyelination and early remyelination within the inflammatory lesions.

It was observed that there was a 29 % reduction, relative to controls, of the mean myelin water fraction within the non-enhancing T2 lesions for all patients. Wide heterogeneity of the myelin

water fraction within the non-enhancing T2 lesions (0.0 – 15.9 %) was also observed. This may be because non-enhancing T2 lesions were at different stages of demyelination or, possibly, remyelination.

In most previous studies the relationships between EDSS and metrics derived from several *in vivo* MRI techniques were found to be modest^{36,37}. A similar pattern was observed between EDSS and the myelin water fraction in this current cross sectional study ($r = -0.3$, $P = 0.02$). This is not surprising considering that MWF, although specific to myelin integrity, does not take into account axonal injury, which is more likely to contribute to disability in MS. Combining MRI metrics to assess the major CNS tissue elements such as N-acetyl-aspartate for neuro-axonal integrity and myo-inositol for glial content with WMF measurements may improve our ability to find relationship with clinical measures of disability. For the same reason the relationship with brain atrophy determinants will need to be evaluated with such MRI markers, such studies are now underway at our institution.

CONCLUSION

This study demonstrated that it is possible to use a multi-slice nonlinearly spaced 12-echo T2 prep spiral imaging sequence to quantify *in vivo* multi-component T2 relaxation times using the NNLS algorithm for patients with MS at 3T in clinically acceptable scan times. It was observed that the mean myelin water fraction was 11.3 (%) for controls and significantly reduced in NAWM for patients. Myelin water was even more profoundly reduced within both CE and non-enhancing T2 lesions for all patients relative to controls. Longitudinal studies following the

temporal evolution of myelin water fractions in newly developed lesions are needed, which may improve our ability to monitor lesion severity and/or myelin repair.

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For Peer Review

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Table 1

Clinical characteristics for individual sub-groups

	Number of subjects	Age (year)	Disease Duration (year)	EDSS
Controls	28	34.2 (9.7) [18.0 – 51.0]	N/A	N/A
All CIS/MS	89	43.4 (9.5) [24.0 – 66.0]	9.7 (9.7) [1.0 – 45.0]	1.7 (1.4) [0.0 – 6.0]
CIS/RRMS with DD ≤ 5y	41	40.0 (9.0) [24.0 – 60.0]	2.7 (2.0) [1.0 – 10.0]	1.3 (1.2) [0.0 – 6.0]
SPMS/RRMS with DD > 5y	48	46.4 (9.0) [31.0 – 66.0]	15.7 (10.0) [4.0 – 45.0]	2.0 (1.5) [0.0 – 6.0]

Note - Data are the mean (standard deviation) [range].

EDSS (Expanded disability status scale), CIS (Clinically isolated syndrome), MS (Multiple sclerosis), RRMS (Relapsing remitting MS), SPMS (Secondary progressive MS), DD (Disease duration).

Table 2

Age adjusted least square mean values of myelin water fraction for sub-groups

Sub-groups	Myelin water fraction (%)		
	WM/NAWM	Non-enhancing	CE lesions
	T2 lesions		
Controls	11.3 (0.2) [N = 28]	N/A	N/A
CIS/MS	10.6 (0.1) * [N = 89]	8.0 (0.3) * [N = 88]	8.4 (0.4) * [N = 13]
CIS/RRMS with DD ≤ 5y	10.8 (0.2) * [N = 41]	8.0 (0.4) * [N = 40]	8.5 (0.6) * [N = 7]
SPMS/RRMS with DD > 5y	10.3 (0.2) * [N = 48]	8.1 (0.4) * [N = 48]	8.4 (0.6) * [N = 6]

Note - Data are the age adjusted least square mean (standard error) [Number of subject].

* P < 0.05 relative to controls.

WM (White matter), NAWM (Normal appearing white matter).

FIGURE LEGEND

Figure 1 Representative 8-slice of MWF map from a control.

Figure 2 (a) PD image (TE = 20 msec, left) and overlaid WM mask on the same image (middle) for a control. The myelin water fraction map was overlaid on the same PD images (right).

Figure 2 (b) PD image with MS lesion (TE = 20 msec, left) for a patient with RRMS. The NAWM mask was overlaid on the post contrast T1-weighted spine echo image (middle). The myelin water fraction map was overlaid on the PD images (right). Note reduced myelin water fraction within the contrast enhancing MS lesion compared to surrounding NAWM.

Figure 1

Figure 2 (a)

Figure 2 (b)

Multi-slice brain myelin water fractions at 3T in multiple sclerosis

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Abstract

Purpose: To evaluate a multi-slice nonlinearly-spaced 12-echo imaging sequence at 3T covering the supratentorial brain for the quantification of myelin water fraction (MWF) in MS patients.

Methods: Eighty-nine patients with, or at risk of, MS (69 RRMS, 7 SPMS, 13 CIS) and 28 controls were studied. 12-echo datasets were acquired using a multi-slice T2 prep spiral imaging sequence and were fitted using a nonnegative least squares algorithm. The mean MWF within NAWM, contrast enhancing (CE) and non-enhancing T2 lesions were calculated.

Results: Mean MWF in white matter for controls was 11.3%. Mean MWF was significantly reduced in NAWM of MS patients (10.6%, $P = 0.004$) relative to controls. SPMS/RRMS patients with disease duration > 5 years (10.3%) had lower MWF compared to CIS/RRMS with disease duration ≤ 5 years (10.8%, $P = 0.03$). Mean MWF was reduced by 26 % and 29 % within both CE ($P < 0.0001$) and non-enhancing T2 lesions ($P < 0.0001$) relative to controls.

Conclusion: Using a multi-component T2 sequence at 3T, a significant decrease in the supratentorial MWF was observed in MS NAWM and lesions relative to controls. The method was sensitive to detect white matter changes early in the disease process.

Key Words: Multi-component T2 relaxation times, myelin water fraction, multi-slice multi-echo T2 prep, high field MRI, multiple sclerosis

INTRODUCTION

Multiple sclerosis (MS) is immune-mediated disease that affects the central nervous system and causes demyelinated plaques with glial scar formations in multiple focal areas as well as in macroscopically normal tissue¹⁻⁵. Therefore, accurate measurement and quantification of the myelin water content would be useful for patients with MS. Although conventional magnetic resonance (MR) images can generate high contrast differences between MS plaques and surrounding tissue, there is a need for more sensitive and specific MR markers of pathological processes for monitoring disease progression and response to therapy. Myelin water fraction derived from the quantification of the transverse relaxation time is a relatively new MR metric that has shown specificity for myelin content and/or integrity in neurological tissue⁶⁻¹⁰, it is utilized in this study to assess MS subjects with early and late disease duration.

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Previous studies have shown the existence of multi-component T2 relaxation decay curves in biological tissue as an indicator of compartmentation^{6,7}. The individual T2 compartments have been interpreted in terms of different water compartments within heterogeneous tissue. It has also been shown that the short T2 component (T2 < 50 msec) was observed mainly in the myelinated tissue⁸ and reduction of the short T2 component has been observed in degenerated peripheral nerves⁹. Moore et al. have also shown an absence of short T2 component in the chronic MS plaque using a formalin-fixed MS brain¹⁰.

Previous *in vivo* studies have shown that the short T2 component is mainly observed within white matter (i.e. myelin water) for normal controls¹¹, and that there is decreased myelin water in the lesions as well as normal appearing white matter for patients with MS relative to normal

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controls ¹². These latter findings relied on a single-slice 32-echo spin echo imaging sequence. T2 decay has traditionally been measured using multi-echo data sets acquired during a spin echo readout train. Previous studies have shown that the accuracy of T2 quantification was improved by using non-slice selective rectangular composite refocusing pulses which reduced errors from B1 inhomogeneity ^{13, 14}. But these hard pulses prevent the acquisition of multiple slices within a single TR, and thus, imaging time increases linearly with the number of prescribed slices. Therefore, to keep scan time within reasonable limits, coverage is often limited to a single slice ¹⁵⁻¹⁷. Maier et al. have implemented a multi-echo spin echo sequence that uses slice selective refocusing pulses, which enables more time efficient multi-slice imaging. However, it was shown that slice selective refocusing pulses resulted in imperfect refocusing leading to stimulated echoes that introduced mixed T1 and T2 contrasts. It was also shown that off-resonance effects created by slice selective refocusing pulses introduced magnetization transfer contrast ¹⁸. These effects could confound attempts to accurately quantify T2. Accurate quantification of the T2 relaxation times also requires a large number of echoes for the same location, typically ≥ 32 , with high signal to noise ratio (SNR) and results in a long acquisition time. Because of these limitations, multi-echo spin echo MR images have not been routinely acquired in a clinical environment. Vidarsson et al. have used a three spin echo sequence and a linear combination filter optimized for myelin imaging with an acquisition time of 5 min (6 slices) ¹⁹. However this sequence is limited to myelin water content only, because signals from other white matter components are sufficiently suppressed.

Multiple echo data can also be acquired using T2 prep ²⁰. Images with different T2-weighting are generated by changing the number of refocusing pulses in T2 prep. Wright et al. have

demonstrated that an RF cycling scheme can mitigate the effects of T1 recovery between T2 prep and multi-slice readouts^{21,22}. Therefore, with T2 prep, multiple slices can be acquired within a TR, enabling time efficient multi-slice imaging with all the advantages afforded by hard composite refocusing pulses. A previous [3T](#) study from our laboratory has shown that short T2 components were mainly observed within the white matter and the short T2 water component fraction [from various white matter regions](#) was 10 – 12 % ([with scan-rescan intra-region coefficients of variation of 0.05 – 0.10](#)) of total water for normal volunteers using a multi-slice nonselective T2 prep spiral MR imaging sequence with unequal echo sampling²².

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This current study was designed to implement a clinically relevant 16-slice 12-echo T2 prep spiral MR imaging sequence [at 3T](#) using a commercially available receive only 8-channel phased array coil for quantification of the T2 relaxation times in patients with MS.

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MATERIAL AND METHODS

Study Population

Eighty-nine MS patients were included in this study from a cohort of patients who were followed at the University of California, San Francisco Multiple Sclerosis Center. Sixty-nine patients had RRMS and seven patients had clinically definite SPMS as defined by Poser criteria²³. Thirteen patients with clinically isolated syndrome (CIS) were also included (all with abnormal brain MRI defined by ≥ 2 white matter lesions). To test the dependence of MWF on disease duration, the patients were divided into two groups; one for CIS and RRMS patients with disease duration (DD) of less than or equal to 5 years (N = 48) and the other for SPMS and RRMS patients with DD greater than 5 years (N = 41). Neurological evaluations included the expanded disability

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status scale (EDSS)²⁴. Twenty-eight control subjects were examined using the same MR protocol. All subjects gave written and informed consent for participating in the study. The mean (range) age, disease duration, and EDSS for all sub-groups are listed in Table 1.

Conventional MR Imaging

MR images were acquired with a 3T Signa scanner (GE Healthcare, Waukesha, WI, USA) equipped with an 8-channel phased array coil. The conventional MR imaging examination included a dual-echo PD-/T2-weighted sequence (TR = 2000 msec, TE = 20/80 msec, 512 × 512 matrix, 240 × 240 mm field of view, 44 3-mm interleaved slices), a T1-weighted three dimensional (3D) inversion recovery spoiled gradient echo (IR-SPGR) sequence (TR/TE/TI = 7/2/400 msec, 15° flip angle, 256 × 256 × 180 matrix, 240 × 240 × 180 mm field of view, 180 1-mm slices), and a post-gad T1-weighted spin echo sequence (TR/TE = 467/8 msec, 256 × 256 matrix, 240 × 240 mm field of view, 44 3-mm interleaved slices, covering the same locations as the pre-contrast dual-echo images). Single dose (0.1 mM/kg) gadolinium diethylenetriamine-pentaacetic acid (Gd-DTPA) was used as a contrast agent. The contrast enhancing T1-weighted spin echo sequence was acquired 5 minutes after injection of the contrast agent.

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Multi-slice multi-echo T2 prep Spiral MR Imaging

A 16-slice 12-echo T2 prep spiral sequence was used in this study to acquire multi-echo data^{20, 22}. In brief, the T2 prep portion of the sequence consisted of a nonselective 90° tip-down pulse, a train of equally spaced (6 msec) composite 180° hard refocusing pulses (90_x-180_y-90_x) and a hard -90° tip-up pulse²⁵. The length of the refocusing trains determines the amount of T2 contrast in the resultant image. Large gradient spoilers following the tip-up pulse de-phase any remaining

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transverse magnetization. Image acquisition follows the T2 prep and includes a spectral-spatial pulse and spiral readout for each prescribed slice. A RF cycling scheme was used to mitigate the effects of T1 recovery between T2 prep and the multi-slice spiral readouts^{21,22}. Any residual longitudinal magnetization was then nulled using chemical shift selective (CHESS) pulses²⁶ which consisted of three sets of 90° excitations and spoiler gradients applied sequentially. The period of longitudinal recovery was preserved for different echo times by shifting the delay between spiral readout and the CHESS pulses while TR is held constant. The echo time of this sequence was the duration of the refocusing train corrected by the period of T1-weighted signal decay during each composite pulse as shown in Foltz et al¹³.

The 12-echo MR imaging parameters were TR = 2000 msec, $TE_n = 7, 17, 28, 38, 49, 60, 70, 92, 124, 177, 220$ and 294 msec, 128×128 matrix, 240×240 mm field of view, 4096 points with 4 spiral interleaves resulting in an effective resolution of 2×2 mm, 16 5-mm thick slices, and NEX = 6 (scan time of 10 minutes). The 12-echo MR imaging was acquired before the injection of the contrast agent.

Fitting of Multi-echo Data to Quantify T2 Relaxation Times

The 12-echo data was fit to a distribution of T2 values using a nonnegative least square (NNLS) algorithm²⁷. The NNLS algorithm was implemented using Matlab (The MathWorks, Natick, MA, USA). The solution of the NNLS algorithm was iteratively regularized such that the ratio of chi-square misfit between the regularized and unregularized solution was less than 1%. The objective here was to have smoothly varying T2 distributions^{28,29}. The T2 axis was partitioned into 80 logarithmically spaced compartments between 15 and 2000 msec¹¹. T2 value, peak area

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and pool fraction for each peak were estimated from the T2 distributions. Estimated T2 components below a specific threshold (peak area for $T2_j < 3\%$ of total water) were ignored to eliminate the dependence of the fit to the noise. A median filter with kernel size of 3 was applied to the original images before fitting. The pixel-by-pixel myelin water fraction (MWF) (defined as ratio between peak area for T2 component < 50 msec and total water) map (%) was created. The fitting algorithm was implemented using a parallel processing computing grid (processing time of approximately 12 minutes).

Post-Processing

T2 hyperintensity lesions were drawn based on a semi-automated threshold with manual editing on the PD and T2-weighted images by an experienced neurologist (DP). Contrast enhancing (CE) lesions were defined manually based on the contrast enhancing T1-weighted spin echo images. The CE lesions were segmented out from the T2 hyperintensity lesion to create non-enhancing T2 hyperintensity (T2) lesions. Masks for both T2 and CE lesions were generated.

The scalp and skull were removed from the T1-weighted 3D IR-SPGR volume images using an automated brain extraction tool in order to improve the robustness of the subsequent image segmentation³⁰. The T1-weighted 3D IR-SPGR volume images were resampled to correspond to the PD-/T2-weighted images using nearest neighbor interpolation and then used to segment white matter, gray matter and cerebral spinal fluid structures using a hidden Markov random field model with an expectation maximization algorithm³¹. Normal appearing white matter (NAWM) masks was created for patients by excluding both CE and non-enhancing T2 lesions from the white matter (WM) masks.

The NAWM, non-enhancing T2 and CE masks were then regridded to the lower resolution MWF map from the 12-echo data yielding the percent content within each pixel. The mean MWF values were calculated in the NAWM, non-enhancing T2 and CE lesions for all subjects. Pixels contain within ≥ 90 % NAWM or non-enhancing T2 lesion masks were included in the calculation of their respective mean MWF. To include as many CE lesions possible, pixels contain within ≥ 50 % CE lesion mask were included for the MWF calculation.

Statistical Analysis

Statistical analyses were performed using standard least square means (LSM) tests with age adjustment to consider relatively younger mean age of controls with respect to the MS patients. The results were reported as LSM (standard error) unless otherwise noted. The non-parametric Spearman method was used for correlation tests. In this study, $p < 0.05$ is regarded as significant.

RESULTS

Volume of the Contrast Enhancing and Non-enhancing T2 Lesions

13/89 patients had contrast enhancing lesions and the average volume of CE lesion load was 1.2 ml with a range of 0.1 – 4.9 ml. The average volume of non-enhancing T2 lesion load for all patients was 5.5 ml with a range of 0.0 – 36.9 ml.

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Myelin Water Fraction Values within Regions of Interests

A. Supratentorial NAWM Regions

The age adjusted mean MWF values within the WM/NAWM, non-enhancing T2 and CE lesions for all sub-groups are listed in Table 2. Figure 1 shows representative 8-slice of the MWF map from a control. As shown in Figure 1, short T2 components were mainly detected within the white matter. The mean MWF value within the WM region was 11.3 (0.2) % for the controls. It was observed that the mean MWF value was significantly decreased in the NAWM for all patients ($P = 0.004$) relative to the controls. Differences were observed for both patients with CIS/RRMS with $DD \leq 5$ years ($P = 0.05$) and for patients with SPMS/RRMS with $DD > 5$ years ($P = 0.0004$) relative to the controls. The mean MWF value within the NAWM for patients with SPMS/RRMS with $DD > 5$ years was also significantly lower than that for patients with CIS/RRMS with $DD \leq 5$ years ($P = 0.03$).

B. Contrast Enhancing Lesions

The mean MWF value was significantly reduced within the CE lesions for 13 patients relative to controls ($P < 0.0001$). The same findings were observed for both patients with CIS/RRMS with $DD \leq 5$ years ($P = 0.0001$) and for patients with SPMS/RRMS with $DD > 5$ years ($P = 0.0003$) relative to the controls. Both patient groups did not differ from each other. Figure 2 (a) shows a PD image ($TE = 20$ msec) from a control (left), the corresponding WM mask overlaid on the PD image (middle), and the corresponding MWF map overlaid on the PD image (right). Figure 2 (b) shows a PD image with T2 lesions (top left) and NAWM (top right) masks, and post contrast T1-weighted spin echo image with CE lesion mask (bottom left) in a patient with RRMS. The MWF

map was overlaid on the PD image (bottom right). Reduced MWF within the contrast enhancing MS lesion was observed compared to surrounding NAWM.

C. Non-enhancing T2 Lesions

A significant reduction of the mean MWF value was observed within the non-enhancing T2 lesions for all patients ($P < 0.0001$) relative to the controls. The same findings were observed for both patients with CIS/RRMS with $DD \leq 5$ years ($P < 0.0001$) and SPMS/RRMS with $DD > 5$ years ($P < 0.0001$) relative to controls. Both patient groups did not differ from each other.

Relationships between Lesion Load, Disease Duration, Disability and Myelin Water Fraction within the Regions of Interests

The volume of the non-enhancing T2 lesion for all patients was significantly correlated with the mean MWF values within the NAWM ($r = -0.3$, $P = 0.002$) and within the non-enhancing T2 lesions ($r = -0.4$, $P < 0.0001$). A weak correlation was observed between EDSS and the mean MWF within the non-enhancing T2 lesions ($r = -0.3$, $P = 0.02$) but not within the NAWM regions ($r = -0.1$, $P = 0.163$).

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DISCUSSION

To authors' knowledge, this is the first study evaluating a clinically relevant multi-slice nonlinearly sampled 12-echo MR imaging technique measuring multi-component T2 relaxation times at 3T with the objective of estimating myelin water fractions derived from large supratentorial brain regions of interest in MS patients. We first observed that the mean myelin water fraction was significantly reduced in NAWM regions of MS patients and found that our

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method was sensitive enough to detect changes in patients within five years of disease onset. The second finding was that the mean myelin water fractions within both CE and non-enhancing T2 lesions were even more profoundly reduced for all patients relative to the controls. Lastly, only modest relationships were observed between T2 lesion volume and myelin water fraction within NAWM regions, EDSS and myelin water fraction within T2 lesions.

Multi-component T2 relaxation imaging techniques are being investigating intensively since they have the potential to provide the most specific *in vivo* MRI marker of myelin integrity. Beaulieu et al. demonstrated three-component T2 relaxation times (i.e. myelin-, axonal- and interaxonal-water) *in vitro* for cranial nerves of the garfish at 2.35T⁸. In their study, the authors concluded that the short T2 components (T2 < 50 msec) were assigned to myelin water because it was present in myelinated trigeminal and optic nerves, but absent in non-myelinated olfactory nerves. In our current study, we observed two- or three-component T2 relaxation times mainly in white matter with an estimated myelin water fraction for controls of 11.3 % as calculated from the multiple slices acquired. Laule et al. showed that mean myelin water fraction from several white matter structures was 11.2 % with a range of 7 – 16 % for controls using a single-slice 32-echo spin echo sequence¹². This myelin water fraction value is consistent with our results using the multi-slice nonlinearly spaced 12-echo T2 prep spiral sequence. Laule et al. also showed that the reduction of the myelin water fraction in NAWM for patients with MS was dominated by myelin loss of integrity rather than increased diffuse edema or inflammation¹².

Although a study involving the peripheral nervous system, Does et al. demonstrated three-component T2 relaxation times (mean T2 values of 19, 63 and 241 msec) in the sciatic nerve of

the amphibian *Xenopus laevis* at 2.35T³² and also showed that after crash injury these three components were reduced remote from the crash due to Wallerian degeneration, reflecting collapse and loss of myelinated fibers⁹. In the current study, we observed a 6 % reduction of the mean myelin water fraction within the NAWM for all patients relative to controls. The same metric within the NAWM was significantly lower for patients with SPMS/RRMS with DD > 5 years (9 % reduction) than that for patients with CIS and RRMS with DD ≤ 5 years (4 % reduction). This likely reflects more loss of myelin integrity with more advanced disease and/or more effective remyelination which may be prominent at the early stages of the disease³³. Moreover, we found a relationship, although modest, between T2 lesion volume and reduced MWF within NAWM regions representing damage that may be due to Wallerian degeneration caused by distant MS lesions.

The inflammatory infiltration within the active MS lesion (contrast enhancing lesions) is composed of active T cells, macrophages and microglial cells which are all involved in the pathogenesis of demyelination in MS^{1,34}. It was observed that there was a 26% reduction of mean myelin water fraction within the CE lesions for 13/89 patients in the current study relative to controls, which may indicate active demyelination within inflammatory lesions. Bruck et al. also showed that the highest number of macrophages was observed in actively demyelinating as well as early remyelinating lesions and the acute stage inflammatory macrophage markers were selectively expressed in early and late active lesions³⁵. In the current study, although significant reduction of the mean myelin water fraction was observed within the CE lesion, the variation of the same value was large (range of 4.0 – 13.0 %, N = 13). This may suggest that there is a mixture of active demyelination and early remyelination within the inflammatory lesions.

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It was observed that there was a 29 % reduction, relative to controls, of the mean myelin water fraction within the non-enhancing T2 lesions for all patients. Wide heterogeneity of the myelin water fraction within the non-enhancing T2 lesions (0.0 – 15.9 %) was also observed. This may be because non-enhancing T2 lesions were at different stages of demyelination or, possibly, remyelination.

In most previous studies the relationships between EDSS and metrics derived from several *in vivo* MRI techniques were found to be modest^{36, 37}. A similar pattern was observed between EDSS and the myelin water fraction in this current cross sectional study ($r = -0.3$, $P = 0.02$). This is not surprising considering that MWF, although specific to myelin integrity, does not take into account axonal injury, which is more likely to contribute to disability in MS.

Comparative studies using different quantitative or semi-quantitative MR modalities (magnetization transfer imaging, diffusion tensor imaging, T2 and T1 quantification, magnetic resonance spectroscopy) should be performed on the same subjects to test the relative sensitivity of each method to detect disease related white matter changes. Additionally, combining MRI

metrics to assess the major CNS tissue elements such as N-acetyl-aspartate for neuro-axonal integrity, myo-inositol for glial content, and MWF measurements for myelin integrity may improve our ability to define stronger relationship with clinical measures of disability. For the same reason the relationship with brain atrophy determinants will need to be evaluated with such MRI markers, such studies are now underway at our institution.

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CONCLUSION

This study demonstrated that it is possible to use a multi-slice nonlinearly spaced 12-echo T2 prep spiral imaging sequence to quantify *in vivo* multi-component T2 relaxation times using the NNLS algorithm for patients with MS at 3T in clinically acceptable scan times. It was observed that the mean myelin water fraction was 11.3 (%) for controls and significantly reduced in NAWM for patients. Myelin water was even more profoundly reduced within both CE and non-enhancing T2 lesions for all patients relative to controls. Longitudinal studies following the temporal evolution of myelin water fractions in newly developed lesions are needed, which may improve our ability to monitor lesion severity and/or myelin repair.

ACKNOWLEDGMENT

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Table 1

Clinical characteristics for individual sub-groups

	Number of subjects	Age (year)	Disease Duration (year)	EDSS
Controls	28	34.2 (9.7) [18.0 – 51.0]	N/A	N/A
All CIS/MS	89	43.4 (9.5) [24.0 – 66.0]	9.7 (9.7) [1.0 – 45.0]	1.7 (1.4) [0.0 – 6.0]
CIS/RRMS with DD ≤ 5y	41	40.0 (9.0) [24.0 – 60.0]	2.7 (2.0) [1.0 – 10.0]	1.3 (1.2) [0.0 – 6.0]
SPMS/RRMS with DD > 5y	48	46.4 (9.0) [31.0 – 66.0]	15.7 (10.0) [4.0 – 45.0]	2.0 (1.5) [0.0 – 6.0]

Note - Data are the mean (standard deviation) [range].
EDSS (Expanded disability status scale), CIS (Clinically isolated syndrome), MS (Multiple sclerosis), RRMS (Relapsing remitting MS), SPMS (Secondary progressive MS), DD (Disease duration).

Table 2

Age adjusted least square mean values of myelin water fraction for sub-groups

Sub-groups	Myelin water fraction (%)		
	WM/NAWM	Non-enhancing	CE lesions
	T2 lesions		
Controls	11.3 (0.2)	N/A	N/A
	[N = 28]		
CIS/MS	10.6 (0.1) *	8.0 (0.3) *	8.4 (0.4) *
	[N = 89]	[N = 88]	[N = 13]
CIS/RRMS with DD ≤ 5y	10.8 (0.2) *	8.0 (0.4) *	8.5 (0.6) *
	[N = 41]	[N = 40]	[N = 7]
SPMS/RRMS with DD > 5y	10.3 (0.2) *	8.1 (0.4) *	8.4 (0.6) *
	[N = 48]	[N = 48]	[N = 6]

Note - Data are the age adjusted least square mean (standard error) [Number of subject].

* P < 0.05 relative to controls.

WM (White matter), NAWM (Normal appearing white matter).

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FIGURE LEGEND

Figure 1 Representative 8-slice of MWF map from a control.

Figure 2 (a) PD image (TE = 20 msec, left) and overlaid WM mask on the same image (middle) for a control. The myelin water fraction map was overlaid on the same PD images (right).

Figure 2 (b) PD image with MS lesion (TE = 20 msec, left) for a patient with RRMS. The NAWM mask was overlaid on the post contrast T1-weighted spine echo image (middle). The myelin water fraction map was overlaid on the PD images (right). Note reduced myelin water fraction within the contrast enhancing MS lesion compared to surrounding NAWM.