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Inversion recovery ultrashort echo time imaging of ultrashort $T_2^*$ tissue components in ovine brain at 3 T: a sequential D$_2$O exchange study

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Inversion recovery ultrashort echo time (IR-UTE) imaging holds the potential to directly characterize MR signals from ultrashort $T_2^*$ tissue components (STCs), such as collagen in cartilage and myelin in brain. The application of IR-UTE for myelin imaging has been challenging because of the high water content in brain and the possibility that the ultrashort $T_2^*$ signals are contaminated by water protons, including those associated with myelin sheaths. This study investigated such a possibility in an ovine brain D$_2$O exchange model and explored the potential of IR-UTE imaging for the quantification of ultrashort $T_2^*$ signals in both white and gray matter at 3 T. Six specimens were examined before and after sequential immersion in 99.9% D$_2$O. Long $T_2$ MR signals were measured using a clinical proton density-weighted fast spin echo (PD-FSE) sequence. IR-UTE images were first acquired with different inversion times to determine the optimal inversion time to null the long $T_2$ signals ($T_{\text{null}}$). Then, at this $T_{\text{null}}$, images with echo times (TEs) of 0.01–4 ms were acquired to measure the $T_2^*$ values of STCs. The PD-FSE signal dropped to near zero after 24 h of immersion in D$_2$O. A wide range of $T_{\text{null}}$ values were used at different time points (240–330 ms for white matter and 320–350 ms for gray matter at TR = 1000 ms) because the $T_1$ values of the long $T_2$ tissue components changed significantly. The $T_2^*$ values of STCs were 200–300 μs in both white and gray matter (comparable with the values obtained from myelin powder and its mixture with D$_2$O or H$_2$O), and showed minimal changes after sequential immersion. The ultrashort $T_2^*$ signals seen on IR-UTE images are unlikely to be from water protons as they are exchangeable with deuterons in D$_2$O. The source is more likely to be myelin itself in white matter, and might also be associated with other membranous structures in gray matter.

KEYWORDS
gray matter, inversion recovery ultrashort echo time imaging, inversion time, myelin, $T_2^*$, white matter

1 INTRODUCTION

The myelin sheath is a lipid-protein lamellar membranous structure. It envelops axons and is essential for the rapid propagation of electrical signals in the nervous system. In the human brain, myelin constitutes approximately 14% of the wet mass of white matter (WM); it is also present in gray matter (GM) in smaller quantities.$^{1,2}$ Loss of myelin integrity is an important biomarker for a variety of neurological diseases, such as multiple sclerosis (MS), which is a leading cause of disability in young and middle-aged adults.$^{1,3,4}$

Abbreviations used: FOV, field of view; $f_0$, fraction of ultrashort $T_2$ tissue components; FSE, fast spin echo; GM, gray matter; GM$_{\text{b}}$, long $T_2$ tissue components in gray matter; GM$_{\text{u}}$, ultrashort $T_2$ tissue components in gray matter; IR-UTE, inversion recovery ultrashort echo time; $T_2^*$, $T_2^*$ of the long $T_2$ tissue components; MRI, magnetic resonance imaging; MS, multiple sclerosis; NMR, nuclear magnetic resonance; PD, proton density; PD-FSE, proton density-weighted fast spin echo; RF, radiofrequency; ROI, region of interest; SNR, signal-to-noise ratio; $T_2^*$, $T_2^*$ of the ultrashort $T_2$ tissue components; STCs, ultrashort $T_2$ tissue components; TE, echo time; TE$_{0.6\text{ms}}$, TE = 0.6 ms; TE$_{\text{10}\mu\text{s}}$, TE = 10 μs; TI, inversion time; $T_{\text{null}}$, optimal inversion time for nulling long $T_2$ signals; UTE, ultrashort echo time; WM, white matter; WM$_{\text{b}}$, long $T_2$ tissue components in white matter; WM$_{\text{u}}$, ultrashort $T_2$ tissue components in white matter.
Myelin produces extremely short-lived magnetic resonance imaging (MRI) signals, which cannot be imaged directly in vivo using most currently available clinical MRI sequences, which typically have echo times (TEs) of several milliseconds or longer. Several relaxometry studies have characterized $T_2$ distributions in brain tissue, reporting values of 10–50 ms in water associated with myelin sheaths. Signals from these distributions are detectable with conventional clinical pulse sequences. These include $T_1$- and $T_2$-weighted fast spin echo (FSE) imaging, gadolinium-enhanced MRI, diffusion tensor imaging and magnetization transfer imaging. These techniques have all shown high sensitivity for MS lesions, but measures obtained with these techniques have not been strongly correlated with clinical manifestations. It is possible that this lack of correlation is because these measures cannot differentiate demyelination and remyelination from other pathological substrates, such as axonal loss and gliosis, which are associated with heterogeneous clinical manifestations of MS. Methods for direct myelin imaging in vivo provide a more specific and sensitive evaluation of myelin, and thus might be of considerable value.

Previous nuclear magnetic resonance (NMR) spectroscopy studies have measured tissue myelin $T_2$ ex vivo and have reported values of approximately 50 μs in fixed human WM and 50 μs to 1 ms in freshly excited frog sciatic nerve. Inversion recovery ultrashort echo time (IR-UTE) sequences have the potential to selectively image short-lived MRI signals in vivo through the efficient suppression of long-lived water signals using an adiabatic inversion recovery pulse. Wilhelm et al first demonstrated IR-UTE imaging of myelin signals in excised rat spinal cord on a 9.4-T microimaging system. A more recent study explored the use of IR-UTE sequences to detect small MS lesions in cadaver brains, as well as to measure ultrashort $T_2^*$ values in WM both ex vivo and in vivo on a 3-T clinical scanner. The reported $T_2^*$ values (~110–330 μs) were comparable with those of myelin lipid powder when measured either alone or in a mixture with H2O or D2O. However, because brain tissue contains different water pools (termed as long $T_2$ tissue components in this article) that might have different $T_2$ values, it can be challenging to completely null water signals. Therefore, there is a possibility that the ultrashort $T_2^*$ signals seen on IR-UTE images are contaminated by water signals.

The present study aimed to explore myelin as a source of the ultrashort $T_2$ signals seen in WM on IR-UTE images, and to assess the feasibility of using IR-UTE to image the ultrashort $T_2$ tissue components (STCs) in GM on a 3-T clinical scanner in an ovine brain D2O exchange model. Water protons in these specimens were sequentially replaced by deuterons in D2O. If non-exchangeable protons, such as those in myelin, are the source of the ultrashort $T_2$ signals seen on IR-UTE images, the measured ultrashort $T_2^*$ value should remain constant before and after sequential D2O exchange.

## MATERIALS AND METHODS

### 2.1 Pulse sequences and contrast mechanisms

Figure 1 shows the two-dimensional IR-UTE pulse sequence used in this study (Figure 1A) and its contrast mechanism (Figure 1B, C). The sequence employs a half radiofrequency (RF) pulse excitation (pulse duration, 472 μs; bandwidth, ~2.7 kHz), followed by two-dimensional radial ramp sampling. An initial adiabatic inversion pulse (duration, 8.64 ms; bandwidth, ~1.5 kHz) was used to invert and null the longitudinal magnetization of long $T_2$ tissue components. To maximize the signal-to-noise ratio (SNR) and minimize eddy currents, slice-selective gradients were turned off to image the ~3–5-mm-thick specimens used in this study.

To measure the ultrashort $T_2^*$ signals in WM, the inversion time (TI) is chosen to null the long $T_2$ tissue components in WM (WM$_L$) (Figure 1B). At the time at which UTE acquisition starts (TE = 10 μs, i.e. $T_{E0.010}$), the STCs in WM (WM$_S$) and GM (GM$_L$) have positive magnetization, whereas the long $T_2$ tissue components in GM (GM$_S$) have negative magnetization. The addition of the negative magnetization from GM$_L$ and the positive magnetization from GM$_S$ on the $T_{E0.010}$ image leads to a smaller magnitude of the total GM signal, relative to that of GM$_L$ alone. At a relatively later TE (e.g. TE = 0.6 ms, i.e. $T_{E0.006}$), the positive magnetization of GM$_S$ decays to zero or near zero (so does that of WM$_S$), whereas the negative magnetization of GM$_L$ decays very little. As a result, the magnitude of the total GM signal on $T_{E0.006}$ is nearly the same as that of GM$_L$ alone. Consequently, subtraction of the magnitude signal on the $T_{E0.006}$ image from that on the $T_{E0.010}$ image leads to a negative signal for GM and positive signal for WM$_L$, i.e. WM$_L$ is selectively imaged.

With TI chosen to null GM (Figure 1C), both WM$_S$ and GM$_S$ have positive magnetization at $T_{E0.010}$, which decays to zero or near zero at $T_{E0.006}$. WM$_L$ has positive magnetization at $T_{E0.010}$, which only decays slightly at $T_{E0.006}$. Subtraction of the magnitude signal on the $T_{E0.006}$ image from that on the $T_{E0.010}$ image highlights both WM$_S$ and GM$_S$, with WM generally showing higher signal intensity, possibly as a result of its higher myelin content than GM.$^6,21$

### 2.2 Ovine brain specimen preparation

Six specimens containing cerebral hemisphere ($n = 5$) and cerebellum ($n = 1$) were prepared from three freshly frozen ovine brains. All specimens were stored at 4°C and pretreated with broad-spectrum antibiotics in saline before the initial imaging (an initial image for cerebral hemisphere specimen no. 4 was not available).

Each cerebral hemisphere or cerebellum specimen (~3 mm thick) was immersed in 10 mL D2O (99.9%, Sigma-Aldrich, St. Louis, MO, USA) in a 6.4-cm covered and sealed plastic dish to allow exchange. Four cerebral hemisphere specimens were imaged with IR-UTE sequences before and after 90-min, 150-min, 8-h and 24-h immersion intervals in D2O (for details, please see Table 1). One cerebral hemisphere specimen was imaged with both UTE and IR-UTE sequences before and after 24 h of immersion in D2O. The cerebellum specimen (~3 mm thick) was imaged after...
8-h and 32-h immersion intervals in D$_2$O. All specimens were allowed to reach room temperature before imaging. At each immersion time point, specimens were removed from the D$_2$O incubation container and flushed with fresh D$_2$O for 5 s prior to imaging. They were immersed in fresh D$_2$O again after each imaging experiment.

2.3 Imaging experiments

All specimens were imaged using a GE 3-T Signa TwinSpeed MR scanner (GE Healthcare Technologies, Milwaukee, WI, USA) and a 7.6-cm receive-only coil. A conventional proton density-weighted fast spin echo (PD-FSE) sequence (TR/TE = 8000/13.5 ms) was used with a fixed receiver gain at all imaging time points to measure long $T_2$ proton signals and thereby to assess exchange between tissue protons and deuterium in D$_2$O. An IR-UTE sequence with TR/TE = 1000/2.2 ms and variable TIs (20, 100, 300, 500 and 800 ms) was used to determine the optimal TI for nulling long $T_2$ signals in WM (WML $T_{Inull}$). Other parameters included a bandwidth of 166.6 kHz, a field of view (FOV) of 12 cm and an acquisition matrix of 96 × 96, providing a nominal voxel size of 0.94 × 0.94 × 3 mm$^3$, with a scan time of 96 s per acquisition. The same IR-UTE sequence was then used with TI = WM$ _L$ $T_{Inull}$ and variable TEs (0.01, 0.1, 0.2, 0.4, 0.6, 2 and 4 ms) to measure $T_2^*$ of WM$ _S$ in all specimens. The $T_2^*$ value of GM$ _S$ was also measured in the cerebellum specimen using the same IR-UTE sequence with TI set to null long $T_2$ signals in GM (GM$ _L$ $T_{Inull}$). A UTE sequence without inversion preparation was performed (TR = 1000 ms; flip angle, 65°; 11 TEs ranging from 0.01 to 15 ms) in one cerebral hemisphere specimen to quantitatively estimate the fraction of STCs ($f_5$) before and after a 24-h immersion in D$_2$O.

2.4 Data analysis

Regions of interest (ROIs) were carefully chosen in WM and GM to avoid partial volume effects. $T_1$ and $T_{Inull}$ were measured in each ROI offline using Matlab (Mathworks Inc., Natick, MA, USA) by fitting the IR-UTE DICOM images obtained with variable TIs using a single-component,
three-parameter fitting model. The $T_{2}^*$ of STCs was quantified by fitting the IR-UTE DICOM images obtained with variable TEs using a single-component, three-parameter fitting model, or fitting the UTE DICOM images with a previously reported bi-component fitting model. Changes in PD-FSE signals, $T_2$ and $T_{2}^*$ values were plotted against the immersion time in D$_2$O. SNR was calculated as the ratio of the mean signal intensity inside an ROI to the standard deviation of the background noise. All images shown and used for analysis were magnitude and not phase sensitive.

3 | RESULTS

Figure 2B shows representative two-dimensional IR-UTE images acquired with variable TIs (TR/TE = 1000/2.2 ms) from cerebral hemisphere specimen no. 1 before immersion in D$_2$O. At TI = 20 and 100 ms, there was little or no contrast between WM and GM. At TI = 300 ms, there were near-zero signals in WM and a relatively high signal in GM, suggesting dramatic suppression of signals from WM$_L$ and insufficient suppression of signals from GM$_L$. At TIs of 500 and 800 ms, WM and GM signals both increased, with the WM signal being greater than that from GM. Figure 2C shows the $T_2$ map of WM$_{L}$ and GM$_{L}$ calculated from the images shown in Figure 2B. Figure 2D shows typical $T_2$ fitting curves for a WM ROI and a GM ROI (red boxes). $T_{1\text{null}}$ values of ~300 and ~370 ms (TR = 1000 ms) were found for WM$_{L}$ and GM$_{L}$, respectively (Figure 2D).

Figure 3A shows representative IR-UTE images with variable TEs acquired from cerebral hemisphere specimen no. 1 at WM$_L$ $T_{1\text{null}}$ and the $T_{2}^*$ map calculated from these images. WM had relatively lower signals than the surrounding GM, and these signals dropped progressively with the increase in TE. As shown on the $T_{2}^*$ map, the WM$_L$ $T_{2}^*$ was 200–300 µs. Figure 3B shows a subtraction image (the $T_{E=0.6\text{ms}}$ image minus the $T_{E=0.6\text{ms}}$ image) revealing a positive signal for WM and negative signal for GM. Figure 3C shows a representative mono-exponential $T_{2}^*$ fitting curve for the ROI shown in Figure 3B (yellow box).

Table 1 lists $T_{2}^*$ values (µs) measured from four cerebral hemisphere specimens before and after different periods of immersion in D$_2$O. $T_{2}^*$ was derived from mono-exponential decay fitting of IR-UTE magnitude images acquired at WM$_L$ $T_{1\text{null}}$ with TR = 1000 ms and a series of TEs (i.e. 0.01, 0.1, 0.2, 0.4, 0.6, 2 and 4 ms).

| Specimen | 0 min | 90 min | 150 min | 8 h | 24 h |
|----------|-------|--------|---------|-----|-----|
| 1        | 200   | 215    | 226     | n.a.| 225 |
| 2        | 197   | 196    | 209     | n.a.| n.a.|
| 3        | 244   | n.a.   | 248     | n.a.| n.a.|
| 4        | n.a.  | n.a.   | 218     | n.a.| n.a.|

IR-UTE, inversion recovery ultrashort echo time; n.a., not assessed; TE, echo time; $T_{1\text{null}}$, optimal inversion time for nulling long $T_2$ signals; WM$_L$, long $T_2$ tissue components in white matter; WM$_S$, ultrashort $T_2$ tissue components in white matter.
**FIGURE 2**  A, longitudinal magnetization ($M_z$) of white matter (WM) and gray matter (GM) long $T_2$ tissue components (i.e. WML and GML) plotted against the inversion time (TI) in the inversion recovery ultrashort echo time (IR-UTE) sequence. B, two-dimensional IR-UTE images of one cerebral specimen (no. 1) acquired with different TIs before exchange with D$_2$O (TR/TE = 1000/2.2 ms). It should be noted that, at TE = 2.2 ms, the signals of WM and GM ultrashort $T_2$ tissue components (WMS and GMS) become negligible because of their ultrashort $T_2^*$ values. At shorter TIs (TI = 20 and 100 ms), there is little or no contrast between WM and GM. With TI increased to 300 ms, WML signals were largely suppressed and GML signals were moderately suppressed, as evidenced by the near-zero signals in WM and dramatic signal reduction in GM. A further increase in TI resulted in a signal increase in both WM and GM, with WM showing a higher signal than GM. C, $T_1$ map calculated from the images shown in (B). The three purple boxes represent three regions of interest (ROIs) defined in the WM for quantification of the average WML $T_1$. D, $T_1$ fitting curves of a WM ROI (left, red box) and a GM ROI (right, red box) shows that, with TR = 1000 ms, the TI for optimal nulling of long $T_2$ signals ($T_{1null}$) was ~300 ms for WML and ~377 ms for GML. SI, signal intensity.

**FIGURE 3**  A, inversion recovery ultrashort echo time (IR-UTE) images from one cerebral specimen (no. 1) acquired before exchange with D$_2$O with the inversion time (300 ms) chosen to null white matter (WM) long $T_2$ tissue components (TR = 1000 ms). $T_2^*$ values of WM ultrashort $T_2$ tissue components were 200–300 μs as shown on the $T_2^*$ map (bottom panel, right corner). B, the magnitude subtraction image (TE$_{10μs}$ – TE$_{0.6ms}$) provides positive signal for WM and negative signal for gray matter (GM). C, mono-exponential fitting of the IR-UTE images in (a) showed a $T_2^*$ value of 206 μs in a WM ROI (yellow box, B). SI, signal intensity.
FIGURE 4  A, proton density-weighted fast spin echo (PD-FSE) images of one cerebral hemisphere specimen (no. 1) acquired before (0 min) and after sequential immersion in D$_2$O for 90 min, 150 min and 24 h. PD signals progressively decreased with increasing immersion time. B, corresponding inversion recovery ultrashort echo time (IR-UTE) images (TE = 10 μs) of the same specimen acquired with an inversion time that was chosen to null white matter (WM) long T$_2$ tissue components. C, quantitative sequential signal intensity changes of the PD-FSE images in (a), as measured in three regions of interest (ROIs) in WM (small yellow boxes inside the tissue area, 0-min PD-FSE and IR-UTE images). D, corresponding average T$_1$ changes in these WM ROIs. E, relatively constant T$_2^*$ values were obtained from these ROIs at corresponding time points. The signal-to-noise ratios (SNRs) of the PD-FSE image were 166 and 11.3 for a WM ROI (the small yellow box labeled with a red star in the 0-min PD-FSE and IR-UTE images) before and after exchange, respectively. The SNRs of the corresponding IR-UTE image were 20.5 and 21.4 before and after exchange, respectively (TE = 10 μs). White arrowheads show the margin (sealed by 3 M Micropore® surgical tape) of the 6.4-cm plastic dish containing the samples. The margin of the plastic dish was immediately next to the 7.6-cm surface coil. The ROI representing background noise was placed outside of the coil area (a, B; large yellow boxes in the top right-hand corner labeled with red stars).

FIGURE 5  Proton density-weighted fast spin echo (PD-FSE) (TR/TE = 8000/13.5 ms) and ultrashort echo time (UTE) (TR/TE = 1000/0.01 ms) images, as well as UTE bi-component T$_2^*$ fitting results, of one cerebral hemisphere specimen (no. 5) before (top panel) and after (bottom panel) it was immersed in D$_2$O for 24 h. $f_S$ is the fraction of ultrashort T$_2$ tissue components, $sT_2^*$ is T$_2^*$ of the ultrashort T$_2$ tissue components and $lT_2^*$ is T$_2^*$ of the long T$_2$ tissue components. Insets in the bi-component T$_2^*$ fitting plots are inversion recovery ultrashort echo time (IR-UTE) images (TR/TE = 1000/0.01 ms) before and after exchange with D$_2$O, with long T$_2$ signals from gray matter (GM) being suppressed by the adiabatic inversion pulse. The red circle in the top inset shows the definition of the region of interest (ROI) in white matter (WM) for T$_2^*$ fitting at both time points.
panel). Figure 6C shows representative $T_2^*$ fitting curves in a WM ROI (red box in the inset) and a GM ROI (yellow box in the inset). Figure 6D plots the changes in PD‐FSE signal intensity, $T_1$ of WML and GML, and $T_2^*$ of WM and GM against immersion time. PD signals showed minimal differences between WM and GM after 8 h of immersion in D2O. PD signals and $T_1$ of WML and GML decreased in parallel with increasing immersion time. The WMS and GMS $T_2^*$ values remained relatively constant before and after exchange, with mean values of 209 ± 9 $\mu$s and 258 ± 4 $\mu$s, respectively.

4 | DISCUSSION

This study employed half‐pulse IR‐UTE for the first time to estimate the value of $T_{I_{null}}$ needed to null long $T_2$ water signals in the brain on a 3‐T clinical scanner. Long $T_2$ suppression is critical for the selective imaging of myelin as water protons contribute more than 90% of the detectable UTE signal (our preliminary bi‐component analysis of UTE images of fresh WM showed that only about 4% of the UTE signal has a $T_2^*$ of ~0.3 ms, as presented in Figure 5). As suggested by the non‐zero signal in the spinal cord WM on the IR‐UTE image with TE/TI = 1.2/500 ms in Wilhelm et al, an empirically selected TI is unlikely to be adequate for selective myelin imaging. By using the same sequence for $T_{I_{null}}$ estimation and $T_2^*$ measurement, we were able to minimize potential $T_1$ measurement inaccuracies which could have led to insufficient nulling of long $T_2$ signals. This is also the most efficient method to obtain the best estimate of WM $T_{I_{null}}$ for the measurement of $T_2^*$ of the STCs using IR‐UTE sequences. It is simple and straightforward without requiring complicated fitting models or technically demanding corrections.

The deuterium in D2O is an isotope of hydrogen. Its MR frequency (which scales with the gyromagnetic ratio) is 6.5 times lower than that of protons. As a result, deuterium is not detectable using conventional 1H MRI techniques. After sequential exchange with D2O, the specimens in this study showed a gradual decrease in PD‐FSE signals. The PD‐FSE sequence used in this study had a TE > 10 ms and could only detect signals from long $T_2$ (several milliseconds or longer) tissue components. After a 24‐h D2O exchange, the specimens had near‐zero long $T_2$ signals on the PD‐FSE image, and the SNR in a WM ROI decreased by about 15‐fold. The UTE signal also dropped significantly, and the bi‐component $T_2^*$ analyses showed a water fraction (relative to STCs, i.e. $1 - f_2$) of approximately 59% in WM, which suggests that approximately 90% of the
WM water had been replaced by D$_2$O considering the innately low $f_h$ in brain tissue. However, with TI set to TI$_{null}$ for WM$_s$, SNR of the WM$_s$ signals seen on the IR-UTE images was relatively unchanged after exchange. Furthermore, $T_2^*$ values obtained with the IR-UTE sequence remained in the range 200–300 $\mu$s, and were independent of the duration of exchange. The $sT_2^*$ values obtained with bi-component $T_1^*$ analysis of the UTE images were also constantly in the range 200–300 $\mu$s before and after D$_2$O exchange. These $T_2^*$ values were comparable with those measured in myelin extract powder, as well as in mixtures of the powder with D$_2$O and H$_2$O using the IR-UTE sequence in one previous study.\textsuperscript{31} Despite the incomplete H$_2$O/D$_2$O exchange in the present ovine brain D$_2$O exchange model, our results suggest a minimal contribution from exchangeable long $T_2$ protons to the ultrashort $T_2^*$ signals seen on IR-UTE images acquired both before and after D$_2$O exchange. These results support the view that the ultrashort $T_2^*$ signals seen on IR-UTE images are unlikely to be generated from water or residual water in the tissue. They are more likely to be associated with non-aqueous protons.

The STC-invisible PD-FSE images always showed higher signal in GM and lower signal in WM, suggesting that there was more long $T_2$ water in GM than in WM both before and after D$_2$O exchange (Figures 4 and 5). Like the PD-FSE images, the UTE images (TR = 1000 ms) acquired at an ultrashort TE (TE = 0.01 ms) were also mainly PD weighted. As a result of the large FA used (FA = 65$^\circ$), the UTE image also showed $T_1$ weighting, leading to a higher signal in WM than in GM (if there was no difference in PD) because WM$_d$ had a shorter $T_1$ than GM$_d$ (Figure 2D, 6D). Unlike the PD-FSE image, the ‘STC-sensitive’ UTE image showed weaker WM/GM contrast before D$_2$O exchange and largely no WM/GM contrast after D$_2$O exchange (Figure 5). This can be explained as follows. In fresh brain tissue, GM had a higher water PD than WM, and WM had a higher non-aqueous PD than GM.\textsuperscript{1,2,4} The total number of protons, including all water protons and solid mass protons, might not be significantly different between WM and GM.\textsuperscript{26} However, not all of the solid mass proton signals were detectable with the UTE sequence with a TE of 0.01 ms.\textsuperscript{2} Therefore, the WM/GM contrast induced by the water content difference might not be completely canceled by the WM/GM non-aqueous proton content difference and the $T_1$ weighting of the UTE sequence, leading to a higher signal in GM than in WM on the UTE image (Figure 5B). After 24-h D$_2$O exchange, the signal on the PD-FSE image dropped by ~90%, suggesting significant exchange of H$_2$O with D$_2$O (Figure 5A, D). The PD-FSE image still showed higher signal in GM than in WM, suggesting that there was more residual water in GM than in WM after 24-h D$_2$O exchange (Figure 5D), but the absolute WM/GM water PD difference should be much smaller than that before D$_2$O exchange. Meanwhile, methylene protons in the brain non-aqueous tissue are thought to be the major non-exchangeable protons in nervous tissue.\textsuperscript{4} As a result, the difference between WM/GM non-aqueous PDs was largely reserved after D$_2$O exchange. In addition, WM$_d$ still had a shorter $T_1$ than GM$_d$ after D$_2$O exchange (Figure 6D). These factors, altogether, could have made the contrast between WM and GM undiscernible on the UTE image after D$_2$O exchange. Because of the presence of residual water, the WM/GM contrast on the IR-UTE images after incomplete D$_2$O exchange was affected by many factors. These factors included the differences in the amount of WM/GM residual water and WM/GM $T_1$ differences of the WM/GM STCs (which needs further investigation).

GM$_d$ also showed ultrashort submillisecond $T_2^*$ values, which were slightly but consistently higher than those of WM$_d$ in the same specimen at all imaging time points and, like WM$_s$, showed minimal change with increasing immersion time in D$_2$O. The ultrashort $T_2^*$ signals seen in nerve tissues and bovine brain myelin extract after D$_2$O exchange are thought to predominantly arise from carbon-bound methylene protons.\textsuperscript{4} However, methylene protons also exist in other non-myelin membranous structures of cells.\textsuperscript{27} Because GM contains more cellular and subcellular structures than WM, GM$_s$ might be composed of a much higher fraction of other methylene-containing macromolecules than myelin. Protons in these macromolecules might have different $T_2^*$ values from those in myelin, which warrants more sophisticated investigation with a larger sample size and the analysis of different WM and GM regions. The IR-UTE sequences may therefore have applications in the characterization of GM abnormalities, and the characterization of GM$_s$ also potentially provide an important biomarker for MS.\textsuperscript{28}

The $T_1$ values of GM$_d$ and WM$_d$ varied significantly with increasing immersion time in D$_2$O; therefore, a wide range of TIs (240–330 ms for WM and 320–350 ms for GM) were used to null signals from these components. The $T_1$ decrease with D$_2$O exchange may be a result of gradual tissue water loss. In native tissue, the fast-relaxing non-aqueous protons would accelerate $T_1$ relaxation of aqueous protons through magnetization transfer.\textsuperscript{29} In deuterated tissue, the ratio of exchangeable aqueous protons to non-exchangeable non-aqueous protons was greatly reduced. The magnetization transfer effects could be more prominent and lead to further reduced $T_1$ of the residual aqueous protons. Another possibility is that the relatively free water might be first replaced by D$_2$O; the remaining signal on IR-UTE images would be dominated by bound water, such as that trapped in macromolecules, and could have a relatively short $T_1$.\textsuperscript{5,23} Prolonged D$_2$O exchange for up to 7 days led to a $T_1$ increase (data not shown here), possibly because of a loss of macromolecular peptides as a result of tissue degeneration.\textsuperscript{30} The $T_2^*$ values remained relatively constant despite changes in $T_1$ of GM$_d$ and WM$_d$.

There are several limitations of this study. First, in a clinical setting with slice-selective gradients switched on, eddy currents, which occur commonly with MRI scanners, can cause distortion of the combined $k$-space signal profile. This was not investigated in this study. If strong eddy currents exist, it might be necessary to take measures, such as dedicated prescans, to mitigate against this problem.\textsuperscript{31} Second, different forms of tissue water may have different exchange rates with D$_2$O. This was not explored in this study. Nevertheless, the relatively constant $T_2^*$ values suggest minimal water contamination in the IR-UTE $T_2^*$ measurements. Third, myelin $T_1$ and PD were not measured. It is possible to probe the fraction of exchangeable myelin protons by comparing the IR-UTE signals before and after D$_2$O exchange. This needs $T_1$ correction as the $T_1$ value of myelin protons may change significantly in a deuterated environment, and this warrants further investigation. Last, although this study was performed within 32 h of tissue thawing, the specimens may still have undergone natural degradation and their myelin properties might have changed after D$_2$O exchange. Histology is required in future studies for the assessment of any such effect.
5 | CONCLUSION

In conclusion, our results suggest that the IR-UTE sequence can be applied on a clinical scanner to directly detect signals from non-aqueous protons, presumably myelin protons in WM, as well as protons in other STCs in GM. This requires an accurate estimation of WM, and GM: T1null values, which can be determined by fitting the IR-UTE images acquired with variable T1s to a standard single-component T1 measurement fitting model. This technique can also be used to measure myelin T2 and PD. Such quantitative measures may provide a new opportunity to characterize demyelinating diseases.

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