Relaxation-corrected macromolecular model enables determination of $^1$H longitudinal $T_1$-relaxation times and concentrations of human brain metabolites at 9.4T

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Purpose: Ultrahigh field MRS has improved characterization of the neurochemical profile. To compare results obtained at 9.4T to those from lower field strengths, it is of interest to quantify the concentrations of metabolites measured. Thus, measuring $T_1$-relaxation times is necessary to correct for $T_1$-weighting that occurs in acquisitions for single-voxel spectroscopy and spectroscopic imaging. A macromolecule (MM) simulation model was developed to fit MM contributions to the short TE inversion series used to measure $T_1$-relaxation times.

Methods: An inversion series with seven time points was acquired with metabolite-cycled STEAM to estimate $T_1$-relaxation times of metabolites. A short TE was employed in this study to retain signals from metabolites with short $T_2$-relaxation times and J-couplings. The underlying macromolecule spectrum was corrected by developing a sequence-specific, relaxation-corrected simulated MM model. Quantification of metabolite peaks was performed using internal water referencing and relaxation corrections.

Results: $T_1$-relaxation times for metabolites range from approximately 750 to approximately 2000 ms and approximately 1000 to approximately 2400 ms in gray matter (GM)- and white matter (WM)- rich voxels, respectively. Quantification of metabolites was compared between GM and WM voxels, as well as between results that used a simulated MM spectrum against those that used an experimentally acquired MM spectrum. Metabolite concentrations are reported in mmol/kg quantities.

Conclusion: $T_1$-relaxation times are reported for nonsinglet resonances for the first time at 9.4T by use of a MM simulation model to account for contributions from the macromolecular environment.
1 | INTRODUCTION

Molecular imaging using proton magnetic resonance spectroscopy (1H MRS) offers a noninvasive means to investigate the neurochemical profile. As static magnetic field strengths have increased over time, so have the detection capabilities of MRS.\(^1\)\(^,\)\(^2\) A specific advantage that accompanies ultrahigh field MRS is the capability to distinguish more metabolite peaks due to increased spectral resolution. In combination with an increase in signal-to-noise, this effect allows for more accurate and precise metabolite quantification.

Often, metabolite ratios have been used to evaluate spectroscopy results quantitatively.\(^3\) However, metabolite ratios are not sufficient in pathologies where there may not be a stable reference metabolite, such as creatine.\(^4\) Therefore, concentrations of metabolites should be reported in comparable units (ie, molar or molal quantities) to most accurately compare results between measurement techniques, field strengths, research sites, and vendors.

A convenient method for calculating concentrations of metabolites in healthy subjects and a wide range of disorders is to use the internal water signal as a reference standard.\(^5\)\(^,\)\(^7\) When using the internal water as reference, the pure concentration of water (55.126 M or 55.510 mol/kg) is used in conjunction with the MR visible fractions of water protons\(^6\) in gray matter (GM), white matter (WM), and CSF. Additionally, concentrations must be corrected for longitudinal T\(_1\)- and transversal T\(_2\)-relaxation effects that arise from the sequence used to measure the spectrum, as well as the tissue type. Thus, knowledge of tissue-type–specific T\(_1\) and T\(_2\)-relaxation times of both water and metabolites is necessary to perform a complete set of relaxation corrections on apparent concentration values obtained with spectral fitting.

Previous work at 9.4T\(^8\) characterized the T\(_1\)-relaxation times of the singlet resonances of NAA, tCho, and tCr from a GM-rich voxel using a STEAM inversion recovery (IR) series with a long TE of 80 ms that minimized the macromolecule (MM) contribution. In this work, we aimed to measure the T\(_1\)-relaxation times of both singlets and J-coupled resonances of a wide range of metabolites for both GM and WM tissues in the human brain at 9.4T. Furthermore, T\(_1\)-relaxation times of pure GM- and WM-tissue are estimated by linear extrapolation.

A short TE was applied in this study to characterize T\(_1\)-relaxation times of a wide range of metabolites, including those with short T\(_2\)-relaxation times. However, the broad underlying MM spectrum present in short TE spectra complicates metabolite quantification and must be considered as part of the spectral model during fitting. An IR approach was used to measure T\(_1\)-relaxation times in this work; however, it would have been practically impossible to acquire MM spectra with matching T\(_1\)-weightings at all TIs because of the need to suppress metabolite signal contributions to MM spectra. Thus, a T\(_1\) and T\(_2\)-MM relaxation–corrected simulated spectral model (MMAXIOM) was developed to account for MM contributions in metabolite spectra acquired with different TIs. The simulation model considers T\(_1\) and T\(_2\)-relaxation times of MMs previously reported at 9.4T\(^9\)\(^,\)\(^10\) and simulates the MM spectrum by use of prior information from in vivo-acquired MM spectra from GM and WM regions for each TI. This approach enables reliable estimates of T\(_1\)-relaxation times for metabolites.

Concentrations of metabolites were calculated using internal water referencing with full consideration of voxel tissue composition, specific water concentration, and water relaxation time corrections.\(^7\)\(^,\)\(^11\) Metabolite concentrations were corrected for tissue-type–specific T\(_1\) and T\(_2\)-relaxation effects of the metabolites themselves (T\(_1\)-relaxation times from this work and previously determined 9.4T\(^9\) T\(_2\)-relaxation times\(^9\)).

The MM simulation model was evaluated by direct comparison against experimental MM spectra acquired with two different sequences and metabolite concentration estimates obtained when using simulated or experimentally acquired MM spectra to account for the MM contribution. Concentrations were compared against each other and against previous results from 9.4T\(^9\) to assess the effects of using the MMAXIOM spectrum against an experimentally acquired MM spectrum (MM\(_\text{exp}\)).

2 | THEORY

In short TE spectra, MM contributions\(^12\) have to be accounted for to accurately fit and thus quantify metabolite concentrations. Often, it is necessary to measure a MM spectrum from the same region with similar sequence parameters and include
the respective $\text{MM}_{\text{exp}}$ signal into the spectral fitting model. However, when acquiring a metabolite inversion series to determine $T_1$-relaxation times of metabolites, it is practically impossible to acquire MM spectra with matching $T_1$-weightings. Measuring MM spectra is a time-consuming process; thus, it is often not performed in the context of clinical studies. Furthermore, MM spectra are prone to errors arising from metabolite contaminations or $T_1$-weighting. Therefore, we propose a novel method to simulate $T_1$- and $T_2$-relaxation-corrected MM models that account for sequence-specific parameters for 13 MM peaks reported at 9.4T$^{13}$ and an additional three peaks reported at 17.2T. The MM peaks are abbreviated as $M_{XX \alpha}$, where $XX \alpha$ is the chemical shift in ppm. The following algorithm workflow is depicted in Figure 1.

**FIGURE 1** The $\text{MM}_{\text{AXIOM}}$ algorithm diagram. Lorentzian components are constructed by using measured $T_2$-relaxation times and are convoluted with Gaussian components using the measured linewidths of MM peaks at 9.4T$^{9}$ to yield MM Voigt lineshapes. The Voigt linearity is then scaled to match summed $\text{MM}_{\text{exp}}$ based on peak integrations.$^{10}$ Following the scaling simulated spectrum, Bloch simulations provide the magnetization state in the $\text{MM}_{\text{exp}}$ spectrum ($M_{\text{Bloch simulation}}$). The next step is to define the universal base MM spectrum, $M_0$. The universal base spectrum is then able to be attenuated by sequence-specific relaxation effects to yield a final $\text{MM}_{\text{AXIOM}}$ spectrum. Further detail is described in section 2. Theory. MM, macromolecule.
MM spectra previously acquired$^{10}$ at 9.4T with a double inversion recovery (DIR)-metabolite cycled (MC)-semiLASER$^{13}$ ($T_{1i}/T_{1j}/T_{E}/T_{R} = 2360/625/24/8000$ ms) sequence were summed and used to determine the frequency offsets of individual MM peaks as prior knowledge. Residual metabolites in MM$_{\text{exp}}$ spectra were fitted with either narrow Voigt lines or metabolite-basis vectors where appropriate, and is described in more detail by Murali-Manohar et al.$^{10}$ Simulated MM peaks were modeled as Voigt lines by using $T_{2}$-relaxation times of MM peaks$^{9}$ to estimate the FWHM of Lorentzian components. The Gaussian components of each peak were estimated by assuming a global $B_0$ constant linewidth of 30 Hz and summing this with the overlap component of each peak as reported by Murali-Manohar et al.$^{9}$ To define the relative areas of the MM peaks in the MM$_{\text{AXIOM}}$, each MM peak area from the experimentally acquired summed spectrum was referenced to the integration of $M_{0.92}$. Reported J-coupling constants$^{13,15}$ were included for $M_{0.92}$, $M_{1.21}$, $M_{1.39}$, and $M_{2.70}$ to account for J-evolution that is obvious at longer $T_E$ by estimating the J-evolution for a peak as $\cos(J \times \pi \times T_E)$.

As a next step, Bloch simulations were performed to calculate the $T_1$- and $T_2$-weighting present when using $T_{1i}/T_{1j} = 2360/625$ ms as in the experimental MM input data set and to correct MM$_{\text{AXIOM}}$ for these relaxation effects, that is to create a MM model basis spectrum not confounded by relaxation- or sequence-specific effects. To achieve this, a DIR block (Supporting Information Annex A)$^{1}$-$T_2$-relaxation times of individual MM peaks,$^{9,10}$ the transmit field strength $B_1^+$ achieved during the actual experiment, and pulse-specific frequency offset. This DIR block preceded a localization block for the semiLASER sequence ($T_E = 24$ ms) used to acquire MM$_{\text{exp}}$ spectra. This approach calculated the state of signal attenuation, $M_{\text{Bloch simulation}}^i$, and was used to scale each individual MM peak to a state of equilibrium magnetization without relaxation and sequence timing induced signal attenuation

$$M_0 = \frac{\sum_{i=1}^{N=16} M^i_{\text{measured}}}{M^i_{\text{Bloch simulation}}}$$

where, $M^i_{\text{measured}}$ is the observed magnetization of each MM peak and $M_0$ is the equilibrium magnetization representing a MM spectrum corrected for relaxation effects (referred to as universal base MM spectrum in Figure 1). The entire procedure yielded a MM-model–basis spectrum without relaxation bias that can be generally used to simulate sequence and tissue-type–specific MM spectra.

The universal base MM spectrum was then attenuated according to relaxation effects induced by localization schemes and sequence timings used for metabolite $T_1$-relaxation time calculations (IR-STEAM) and for metabolite quantification trials (STEAM and semiLASER). The MM-peak–specific attenuation factors can be calculated as

$$S_{\text{MM peak}} = R_{\text{sequence}} (\eta, T_1(GM, WM), T_2) \times M_0.$$

$R_{\text{sequence}}$ are the relaxation effects calculated for a given sequence $\eta$ (STEAM, spin echo, semiLASER, etc.) considering the sequence specific timings ($T_E, T_R, T_I$, etc.), $T_1$-relaxation times $T_1(GM, WM)$ calculated based on the voxel tissue composition and regional $T_2$-relaxation times. $S_{\text{MM peak}}$ is then the final signal vector for a specific MM peak. After $S_{\text{MM peak}}$ is calculated for each peak, the signal vectors are combined and yield a sequence specific MM spectrum with corresponding amplitudes relative to $M_{0.92}$. This MM$_{\text{AXIOM}}$ spectrum can then be used in metabolite spectral fitting.

The performance of MM$_{\text{AXIOM}}$ was evaluated by fitting a respective MM$_{\text{AXIOM}}$ spectrum to MM$_{\text{exp}}$ spectra with different sequence settings using LCModel (V6.3-1L).$^{16}$ For this work, the quality of fit was evaluated for both DIR-semiLASER and DIR-STEAM sequences (Figure 2). Further discussion of this process follows in section 3.1. MM$_{\text{AXIOM}}$ was also evaluated for determination of metabolite $T_1$-relaxation times and metabolite concentrations as detailed in section 3.2.

3 | METHODS

In vivo measurements were performed on a 9.4T Siemens Magnetom whole-body MRI scanner (Siemens Healthineers) with a coil with 8 transmission (Tx) and 16 reception (Rx) channels$^{17}$ driven in volume coil mode using all eight Tx channels for MP2RAGE imaging experiments and driven in surface coil mode by a three-way splitter to drive all power to the bottom three channel elements for all MRS measurements.$^{13}$ A $2 \times 2 \times 2$ cm$^3$ voxel was placed spanning the longitudinal fissure in the occipital lobe for GM measurements, whereas WM measurements were acquired with a voxel positioned in the right occipital-parietal transition region (Figure 3A) for determining both $T_1$-relaxation times and concentrations. All volunteers participated with the approval of the local institutional review board and provided written informed consent.

3.1 | Evaluation of the MM simulation model

3.1.1 | Data acquisition

Previously acquired MM spectra (DIR-MC-semiLASER$^{13}$; $T_{1i}/T_{1j}/T_E/T_R = 2360/625/24/8000$ ms) from Murali-Manohar et al.$^{10}$ measured MM spectra from both GM- and WM-rich voxels acquired with a DIR-MC-semiLASER
sequence and were used in this work as initial input to generate the MM model-basis spectrum as described above. These spectra have also been used for evaluation of the simulation model for the semiLASER sequence. Additionally, a DIR-MC-STEAM MM spectrum (TI1/TI2/TE/TR = 2360/625/8/8000 ms) was acquired from one volunteer from the GM-rich region to evaluate the simulation model for a STEAM sequence. All MM data were processed as described in Murali-Manohar et al.\textsuperscript{10}

### 3.1.2 Evaluation of the MM model

To evaluate \(\text{MM}_{\text{AXIOM}}\), the simulated \(\text{MM}_{\text{AXIOM}}\) spectra were fitted to matching \(\text{MM}_{\text{exp}}\) spectra in LCModel. First, the aforementioned \(\text{MM}_{\text{exp}}\) spectra from a GM-rich voxel in the occipital lobe (DIR-MC-semiLASER; TI1/TI2/TE/TR = 2360/625/24/8000 ms) were summed across 11 subjects. The summed spectrum was fitted using the respective \(\text{MM}_{\text{AXIOM}}\) spectrum (Figure 2A). A second evaluation step was performed by fitting the DIR-MC-STEAM (TI1/TI2/TE/TR = 2360/625/8/8000 ms) \(\text{MM}_{\text{exp}}\) spectrum with an \(\text{MM}_{\text{AXIOM}}\) spectrum (Figure 2B). The LCModel parameter DKNTMN was set to 99 to enforce a stiff spline baseline. Residual metabolites in \(\text{MM}_{\text{exp}}\) spectra (tCr-CH\(_3\), tCr-CH\(_2\), NAA-CH\(_3\), NAA-CH\(_2\), and mI) were handled by including them as simulated basis vectors during fitting.

### 3.2 Metabolite T\(_1\)-calculation and quantification

#### 3.2.1 Study design

Eleven healthy volunteers (eight males, three females, aged 26.9 ± 2.8 years) were recruited to participate in metabolite
T1-relaxation measurements. All 11 of these participants completed GM and WM measurements.

Another 11 healthy volunteers (six males, five females, aged 27.7 ± 2.3 years) were recruited for the acquisition of data for metabolite quantification (without a preinversion pulse). All 11 volunteers completed quantification measurements for GM-rich voxels; however, only nine volunteers completed measurements for WM-rich voxels. Voxels were placed as described in section 3.1.1

### 3.2.2 | Anatomical imaging

High-resolution MP2RAGE\(^1\) images (0.6 × 0.6 × 0.6 mm\(^3\)) were acquired at the beginning of the scan during the first visit for volunteers for both T1-relaxation and quantification measurements (Figure 3A). Following MP2RAGE acquisition, an intervention was required to alter the coil setup to the surface coil mode using only 3 Tx and all 16 Rx channels for the MRS scans; volunteers remained on the table and were instructed to remain stationary during the procedure.\(^1\)

A localizer was then reacquired, and two FLASH images were acquired to position the spectroscopy voxel. On the second visit, only FLASH images were acquired, and these were later coregistered via rigid-body transformation to the previously acquired MP2RAGE.

### 3.2.3 | Metabolite T1-relaxation measurements—data acquisition

Data for GM- and WM-rich voxels were acquired using an IR-MC-STEAM sequence with parameters such that metabolites with short T2-relaxation times could be measured.
and with the TR sufficiently long to ensure full relaxation between averages (TE/TM/TR = 8/50/10,000 ms, NEX:32). The TIs were chosen to encompass a range of magnetizations for all metabolites (TI = 20, 100, 400, 700, 1000, 1400, and 2500 ms). All metabolite acquisitions were performed with the transmit reference frequency set to 2.4 ppm to reduce the chemical shift displacement effect experienced by the upfield metabolite spectrum.

3.2.4 | Metabolite concentration measurements—data acquisition

MC-STEAM$^{18}$ (TR/TE: 6000/8 ms, NEX:32) and MC-semiLASER$^{18}$ (TR/TE: 6000/24 ms, NEX:32) data were acquired from the same healthy volunteers in both GM- and WM-rich voxels during the same scan session. Additionally, corresponding water reference signals (NEX:16) were acquired with the transmit reference frequency set at 4.7 ppm and without metabolite cycling to avoid any effect of the MC pulse on the quantification using internal water referencing. This also ensured acquisition of water signal from the same voxel as the metabolite spectrum.

3.2.5 | MRS data preprocessing

All spectroscopy raw data were reconstructed using an in-house MATLAB (version 2016a; MathWorks) tool. Data were reconstructed in a similar fashion to Giapitzakis et al.$^{18}$ All acquired FIDs were first truncated and zero-filled from 250 ms, and then were frequency and phase aligned based on the water signal. The two distinct MC subtraction schemes split the data into two spectral mixes (water and metabolite mixes), which then were averaged. Spectra were $0^\text{th}$ order phase, and eddy current corrected$^{19}$ using the MC water mix. Singular value decomposition (SVD) was used to combine the data from all 16 receive channels. The residual water signal in the metabolite mixes was removed using Hankel singular value decomposition (HSVD), and the resulting FIDs were again zero-filled from 250 ms because of an artifact that occurs at the end of the FID from doing HSVD.

3.2.6 | Spectroscopy fitting

All TI-series data were fitted using LCModel with basis sets simulated using the VeSPA tool.$^{20}$ The metabolites basis set included NAA, tCr, aspartate (Asp), γ-aminobutyric acid (GABA), taurine (Tau), phosphoethanolamine (PE), glutamine (Gln), glutamate (Glu), glutathione (GSH), glycine (Glyc), mI, scyllo-inositol (Scyllo), N-acetylaspartylglutamate (NAAG), and phosphocholine + glycerophosphocholine (tCho). MM$^{\text{AXIOM}}$ spectra were simulated for each TI (Figure 3) and used to fit MM contributions throughout the TI series. While fitting metabolites, manual phase corrections for TI = 1000 ms and 1400 ms were required where the phase for metabolites was neither uniformly positive nor negative. Furthermore, for TI = 1000 ms in WM-rich voxels a ppmgap was given from 1.1 to 1.8 ppm, which minimized the influences from lipid contamination at this TI. The LCModel parameter, dkntmn, was set to be 0.25 for fitting for all TI. However, data reported with default LCModel MMs were fitted with the default dkntmn (0.15) and without an MM$^{\text{AXIOM}}$ spectrum.

Data for metabolite quantification were also fitted using LCModel with corresponding metabolite basis sets simulated with the VeSPA tool for semiLASER and STEAM. MC-semiLASER data were fitted twice in LCModel to compare quantification results when using a MM$^{\text{exp}}$ spectrum to those using an MM$^{\text{AXIOM}}$ spectrum. MC-STEAM data were fitted with an MM$^{\text{AXIOM}}$ spectrum (Figure 3B) for a respective STEAM sequence to verify the performance of the MM$^{\text{AXIOM}}$ for another sequence in addition to semiLASER. The MM$^{\text{exp}}$ spectra used for quantification used metabolite subtraction to remove the residual tCr-CH$_2$ signal as described in previous work.$^9$ The MM$^{\text{AXIOM}}$ spectra for semiLASER and STEAM were specific to the average voxel tissue-content across subjects and to the sequence acquisition parameters. This is further discussed in section 3.2.7. Fit-setting files are provided in Supporting Information Annex B.

3.2.7 | MP2RAGE segmentation

MP2RAGE images were segmented into GM, WM, and CSF probability maps with SPM12.$^{21}$ The 2D FLASH images used for voxel placement were coregistered to the MP2RAGE image using SPM12, which returned an affine transformation between the image spaces. The affine matrix relating the two images was used in a home-built Python (v3.7)$^{22,23}$ tool to determine the tissue fractions in spectroscopy voxels.

3.2.8 | T$_1$ calculation

Following spectral fitting, calculated metabolite peak areas from the entire TI series were fitted to a biexponential model:

$$S = A \ast \left( 1 - 2e^{-\frac{\text{TI}}{\text{T}_1}} + e^{-\frac{\text{TR}}{\text{T}_1}} \right)$$

(1)

Here $S$ refers to the LCModel water normalized peak area of a specific metabolite. The metabolite specific T$_1$-relaxation times were then calculated using a linear model curve-fitting optimization (optimizing $A$ and T$_1$). The constant, $A$, is a term that
contains constants related to spin density, the Boltzmann constant, temperature, coil loading, and receiver bandwidth. The calculation was performed using the SciPy\textsuperscript{24} optimize toolkit in Python, and figures were created using the matplotlib\textsuperscript{25} library.

Because $T_1$-relaxation times of water have been reported to vary predominantly by tissue type,\textsuperscript{11,26} an attempt to further estimate the relaxation of theoretically pure GM and WM voxels, $T_{\text{pure voxel}}^{GM}$ and $T_{\text{pure voxel}}^{WM}$ respectively, was made. To that, $T_1$-relaxation times from GM- and WM-rich voxels with known tissue type compositions from this work were regressed against the relative GM fraction of each voxel for NAA, Glu, and water (Figure 4); respective regression curves for further metabolites are reported in Supporting Information Figure S1. Thus, in accordance with previous literature\textsuperscript{26} and results from Supporting Information Figure S1, we assumed a linear relationship between metabolite $T_1$-relaxation times and voxel tissue compositions. Calculation for these times is detailed in Supporting Information Annex C.

3.2.9 | Quantification of metabolite concentrations

Quantification of metabolite concentrations was performed for GM- and WM-rich voxels acquired with MC-STEAM (TE/TM/TR = 8/50/5000 ms) and MC-semiLASER (TE/TR = 24/6000 ms) sequences. Along with the comparison of tissue types and sequences, the impact of using a MM\textsubscript{AXIOM} spectrum instead of an MM\textsubscript{exp} spectrum was investigated by comparing fit results from identical spectra with either an MM\textsubscript{AXIOM} or MM\textsubscript{exp} spectrum as described further in sections 3.2.6 and 5.5.

Internal water referencing was performed by acquiring non-water–suppressed spectra. Metabolite concentrations, $M$, were calculated by correcting the LCModel concentrations as described in Supporting Information Annex D.

The corrections presented in Supporting Information Annex D were carried through fully for semiLASER data. STEAM data was quantified using $T_2$ relaxation of metabolites acquired using a semiLASER acquisition\textsuperscript{9}; although $T_2$-relaxation has been shown to be sequence dependent, it was assumed that there would be a minor error in quantitation caused by the short TE used for STEAM acquisitions.

3.2.10 | Statistics

Differences between metabolite $T_1$-relaxation times measured in GM- and WM-rich voxels were assessed with Welch’s $t$ test ($\alpha = 0.05$) for all metabolites. Control for multiple comparisons was performed by calculating an adjusted $P$ value using the Bonferroni correction \( \frac{\alpha}{N} = \frac{0.05}{20} = 0.0025 \).

Differences between WM- and GM-rich regions for metabolite concentrations were evaluated using the Mann-Whitney $U$ test. Multiple comparisons were controlled for by the Bonferroni correction \( \frac{\alpha}{N} = 0.0033 \).

4 | RESULTS

4.1 | Evaluation of the MM model

Figure 2 shows the fit of an MM\textsubscript{AXIOM} spectrum to summed MM\textsubscript{exp} spectra acquired using MC-semiLASER and to an MM\textsubscript{exp} spectrum from a subject using MC-STEAM. The residual for both fits are minimal and show good quality of the fit. However, because of lipid contamination and lineshape

![Figure 4](image-url)
effects arising from the complex structures of the MMs, some structure is apparent in the residual between 1.3 and 1.9 ppm. The fit of metabolite residuals is also shown.

4.2 Inversion series of spectra and voxel content

The TI series of short TE MC-STEAM data for GM and WM voxels are displayed in Figure 3. The subject-wise summed spectra display good spectral quality between 0.8 and 4.2 ppm. Metabolites that were analyzed include Asp, NAA, tCr, GABA, Tau, PE, Gln, Glu, ml, Scyllo, NAAG, Glx, tCho, tCho + PE, and ml + Glyc. In addition to NAA and tCr resonances, T1-relaxation time measurements were also performed for the moieties NAA-CH2, NAA-CH3, tCr-CH2, and tCr-CH3.

The average voxel content for TI-series data in GM-rich voxels and WM-rich voxels were GM/WM/CSF = 69 ± 4/24 ± 7/7 ± 6% and 32 ± 8/66 ± 9/2 ± 2%, respectively.

4.3 Spectral fitting

Spectral fitting with dkntmn = 0.25 yielded a relatively flat spline baseline with good fits with small residuals among the TI-series data when a MM spectrum was included in the spectral basis set. The simulated MM spectrum for each TI (Figure 3C) resulted in good fitting of the metabolites. However, misestimations of MM contributions were evident at TI = 400, 700, 1000, and 1400 ms when LCModel default MM were included, and the spline baseline was left more flexible in place of including an MMAXIOM spectrum. Supporting Information Figure S2 shows a comparison between using a flexible spline baseline with default LCModel MM and fits with a stiffer spline baseline plus an MMAXIOM spectrum basis vector.

4.4 T1-relaxation times

Following LCModel spectral fitting, resulting metabolite peak areas were fitted to Equation (1). Relaxation curves for T1-relaxation time calculations are shown in Figure 5 and Supporting Information Figure S3. Figure 6 shows the T1-relaxation times of metabolites with the extrapolation to GM- and WM-pure voxels.

In addition to Figures 5 and 6, Table 1 reports the T1-relaxation times for GM- and WM-rich voxels and GM- and WM-pure voxels with a simulated MM spectrum used in spectral fitting. Additionally, the T1-relaxation times for GM- and WM-rich voxels with the LCModel default MM settings instead of an MMAXIOM spectrum used in spectral fitting are reported on the right-hand side of the table.

Significant differences between GM- and WM-rich voxel T1-relaxation times were found for Glu, GSH, Glyc, NAA-CH3, Tau, Glx, and ml+Glyc and are denoted in Table 1 and Figure 6 by an asterisk: (*) P value < .0025. Results from Welch’s t test and the P values are reported in Supporting Information Table S1.

4.5 Metabolite quantification

The voxel content differed slightly in data used for quantification, and the average voxel content for GM- and WM-rich data used in quantification was GM/WM/CSF = 72 ± 4/22 ± 2/6 ± 4% and 35 ± 6/62 ± 7/3 ± 3%, respectively. Data fitted in LCModel from MC-semiLASER acquisitions show residual with little structure when fitted with both MMAXIOM spectrum and with MM exp spectrum (Figure 7). The spline baseline is slightly flatter from 1.5 to 2.5 ppm for fits with MMAXIOM spectra in comparison with fits with MM exp (Figure 7 and Supporting Information Figure S4). The data acquired with MC-STEAM show good fits from 1.8 to 4.0 ppm, but there was lipid contamination evident in individual fits between 1.1 and 1.7 ppm (Supporting Information Figure S4). Figure 8 shows quantification results from GM- and WM-rich voxels for semiLASER acquisitions fitted with either an MM exp spectrum or an MMAXIOM spectrum. Concentration values for metabolites are reported in millimole per kilogram in Table 2. Supporting Information Figure S5 displays STEAM quantification results from GM-rich voxels fitted with either an MM exp spectrum or MM AXIOM spectrum. Significant differences (P < .0033) assessing concentration differences are listed as superscripts a through e in Table 2 and Figure 8; refer to the Table 2 footnote for superscript assignments.

5 DISCUSSION

5.1 MM model

MMAXIOM was proposed to account for MM contributions throughout an IR series in short TE spectra. Figure 2 shows that it is possible to use MMAXIOM spectrum to fit MM exp spectra provided one has T1-10 and T2-9 relaxation times. Because MMAXIOM spectra replicated the MM exp spectra well, MMAXIOM spectra were used for fitting the inversion series spectra acquired in this study. Using the MMAXIOM spectrum reduced the acquisition time greatly by removing the need to measure an MM exp spectrum for each subject along the TI series. Furthermore, measuring an MM exp spectrum at every step of the inversion series is not achievable because of the need to null metabolite signals and also match T1-weightings.

In contrast to the proposed MMAXIOM, Xin et al.27 used a single MM exp spectrum to account for MM peaks throughout
his entire inversion series. Lopez et al.\textsuperscript{14} followed a similar method to handle the MM spectrum for the inversion series. However, these MM\textsubscript{exp} spectra may have led to $T_1$-estimation errors as spectra from each TI are $T_1$-weighted differently for metabolites and macromolecules. In this work, $T_1$-weighting errors were avoided by simulating the magnetization for each MM peak corresponding to the individual TI steps.

The region around 1.3 ppm in individual spectra is typically influenced from dephased lipid signals that arise from unwanted coherence pathways present from outside the volume of interest. Both Figure 2 and Supporting Information Figure S4 show imperfections in fit performance around 1.3 ppm. This might be caused by the (cancellation and hence) absence of lipid contaminations in the summed MM\textsubscript{exp} spectrum, which was used to simulate the MM\textsubscript{AXIOM} spectrum. Also, the spurious signals from skull lipids might be more prominent in the WM-rich voxels as the necessary $B_1$-field strength is more difficult to achieve deeper in the brain while driving power to the bottom three channels in the coil. This problem can be avoided in the future by using lipid suppression techniques such as outer-volume saturation or inner-volume saturation in the localization sequence. During the time of this work the available, vendor-implemented outer-volume suppression (OVS) bands did not improve the data quality because of poor localization efficiency of the OVS bands.

Furthermore, spurious signals caused by skull lipids will vary between volunteers, and the severity of lipid contamination will vary from differences in $B_1$ performance. Thus, the imperfections in fit performance seen in Figure 2 can be partly attributed to the use of average $T_1$- and $T_2$-relaxation times to create Voigt lines to fit the MM spectrum, which will not capture variations in lipid signals. The MM spectrum has been shown to be comprised of many overlapping peaks that increase in complexity as field strength increases.\textsuperscript{12,14} In this work, 16 MM peaks have been considered in the MM\textsubscript{AXIOM}. A more accurate representation of the MM spectrum, with more peaks included, either requires higher field strengths,\textsuperscript{14} $T_1$- and $T_2$-relaxation times, and coupling constants for peaks or requires dedicated studies invested into measuring MM spectra with high SNR and good localization efficiency.

As can be observed from Figure 3C, MMs between 2.5 and 4.0 ppm have almost no signal contribution in the spectrum with TI = 400 ms, whereas the other peaks maintain a positive longitudinal magnetization that is opposite in phase to that of the metabolites. This occurs because of the difference in their $T_1$-relaxation times\textsuperscript{10} and the $T_1$-weighting from the inversion pulse. To the best of our knowledge, this work is the first attempt to handle the MM spectrum through an inversion series correctly by simulating the $T_1$-weighting present for each MM peak.
Overall data quality of MC-STEAM metabolite inversion series spectra was good from 1.9 to 4.0 ppm. However, there was lipid contamination visible between 1.3 and 1.8 ppm in individual subjects. Individual spectra from GM-rich voxels had better SNR and less lipid contamination in comparison with spectra from WM-rich voxels. This was expected because WM-rich voxels were placed further away from the power-transmitting coil elements; thus, adiabatic pulses were slightly less efficient. Figure 3B shows inversion series spectra from both GM- and WM-rich voxels summed across subjects covering a range of magnetizations for the metabolites (positive, almost nulled, and negative).

5.3 | Spectral fitting

While fitting inversion series spectra, a TI-specific MMAXIOM spectrum was included in the basis set, which represented the correct amount of magnetization present in the acquired spectra (Figure 3). Additionally, care was taken to account for the correct positive and negative magnetizations for the metabolites in the basis set depending on the inversion spectrum to be fit. However, in data sets with complex phases or poorer data quality the simulated MM spectrum could fail to be fitted by LCModel. T1-relaxation times from Table 1, that were estimated with default LCModel MM, were fitted (Supporting Information Figure S2) using the default dkntmn value (0.15). However, when fitting the inversion series data with MMAXIOM spectra, it was possible to stiffen the dkntmn (0.25) that allowed for a better spline baseline and spectral fit. Using default LCModel MM settings resulted in unreliable fits for metabolites, thereby leading to T1 curves with poor R2 values resulting in unreliable T1-relaxation times for most of the metabolites. As evidenced in Table 1, including the MMAXIOM spectra performed significantly better in terms of fitting both in LCModel and in calculating the T1-relaxation times of metabolites.

5.4 | T1-relaxation times

T1-relaxation times were calculated by fitting the LCModel determined peak areas to the exponential curve as shown in Figure 5. Figure 6 shows T1-relaxation times of metabolites in GM-pure, GM-rich, WM-rich and WM-pure voxels. The values range from approximately 750 to approximately 2000 ms in GM-rich voxels and approximately 1000 to approximately 2400 ms in WM-rich voxels. Tau had the longest...
measured $T_1$-relaxation time (~1890 and ~2340 ms in GM- and WM-rich voxels, respectively) as also shown by Lopez et al.\textsuperscript{14} NAA-CH$_3$ was measured to be 1701 ± 47 ms in GM-rich voxel in the occipital lobe, which agrees with the value reported by Deelchand et al.\textsuperscript{8} for a similar voxel. $T_1$-relaxation times of tCr-CH$_3$, tCr-CH$_2$, and tCho are also in the range reported by Deelchand et al.\textsuperscript{8} and the values reported in the current study have a reduced SD compared with the previous study at 9.4T.\textsuperscript{8}

$T_1$-relaxations times for coupled metabolites have only been measured in the rat brain at 9.4T.\textsuperscript{28,29} The rat brain is primarily comprised of GM; thus, the most appropriate comparison would be to look at GM-rich voxels from the human brain when performing this comparison. Interestingly, we find agreement between the $T_1$-relaxation times of Tau, NAA-CH$_3$, tCr-CH$_3$, mI, tCho, and tCr-CH$_2$. Glx results from this work fall between those measured by Cudalbu et al.\textsuperscript{28} and de Graaf et al.\textsuperscript{29}, with a measured a $T_1$-relaxation time of approximately 1500 ms.

Indeed, the values reported at 7T are longer\textsuperscript{27} than the values reported in this study, even though that is not the expected field strength trend.\textsuperscript{8} This can possibly be because of the differences in the magnetization of individual MM peaks, which need to be taken into account for reliable calculation of the $T_1$-relaxation times. This study used TI-specific MM$_{AXIOM}$ spectra for each inversion spectrum, which accounted for the

| TABLE 1 | $T_1$-relaxation times for 19 metabolites and metabolite combinations for GM-/WM-rich and GM-/WM-pure voxels are reported when the MM contribution in the TI series spectra was handled with TI specific MM$_{AXIOM}$ spectra (dkntmn = 0.25) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Metabolite** | **GM-rich** | **WM-rich** | **GM-pure** | **WM-pure** | **GM-rich** | **WM-rich** |
| **$T_1$ [ms]** | **$R^2$** | **$T_1$ [ms]** | **$R^2$** | **$T_1$ [ms]** | **$R^2$** | **$T_1$ [ms]** | **$R^2$** |
| Asp | 1300 ± 75 | 0.92 | 1385 ± 72 | 0.86 | 1247 ± 143 | 0.86 | 1579 ± 199 | 0.48 | 1432 ± 145 | 0.72 |
| tCho | 1161 ± 47 | 0.97 | 1154 ± 31 | 0.97 | 1165 ± 86 | 0.86 | 1051 ± 59 | 0.86 | 1084 ± 25 | 0.98 |
| tCr-CH2 | 1118 ± 30 | 0.96 | 1110 ± 29 | 0.97 | 1123 ± 57 | 0.97 | 1128 ± 49 | 0.91 | 1074 ± 29 | 0.97 |
| tCr-CH3 | 1678 ± 34 | 0.97 | 1719 ± 29 | 0.98 | 1653 ± 64 | 0.98 | 1521 ± 102 | 0.78 | 1666 ± 21 | 0.99 |
| GABA | 1240 ± 91 | 0.78 | 1194 ± 109 | 0.74 | 1269 ± 180 | 0.74 | 1158 ± 165 | 0.74 | 1203 ± 156 | 0.56 |
| Gln | 1214 ± 75 | 0.84 | 1246 ± 50 | 0.91 | 1194 ± 137 | 0.91 | 1271 ± 127 | 0.91 | 1595 ± 194 | 0.65 |
| Glu | 1343 ± 40 | 0.96 | 1471 ± 28 | 0.98 | 1263 ± 74 | 0.98 | 1572 ± 68 | 0.98 | 1210 ± 94 | 0.79 |
| GSH | 1505 ± 58 | 0.92 | 1265 ± 46 | 0.93 | 1654 ± 108 | 0.93 | 1076 ± 100 | 0.93 | 1465 ± 163 | 0.55 |
| Glyc | 739 ± 103 | 0.69 | 988 ± 131 | 0.77 | 584 ± 207 | 0.77 | 1184 ± 189 | 0.77 | 1065 ± 238 | 0.35 |
| mI | 1449 ± 43 | 0.95 | 1441 ± 29 | 0.98 | 1535 ± 79 | 0.98 | 1395 ± 73 | 0.98 | 1277 ± 44 | 0.93 |
| NAA-CH2 | 1137 ± 26 | 0.97 | 1151 ± 27 | 0.97 | 1128 ± 50 | 0.97 | 1162 ± 46 | 0.97 | 958 ± 58 | 0.85 |
| NAA-CH3 | 1701 ± 47 | 0.95 | 1796 ± 31 | 0.98 | 1642 ± 86 | 0.98 | 1871 ± 80 | 0.98 | 1434 ± 112 | 0.72 |
| NAAG | 1185 ± 236 | 0.45 | 1167 ± 69 | 0.86 | 1196 ± 419 | 0.86 | 1153 ± 390 | 0.86 | 955 ± 129 | 0.46 |
| PE | 1305 ± 129 | 0.81 | 1417 ± 127 | 0.78 | 1235 ± 247 | 0.78 | 1505 ± 227 | 0.78 | 1321 ± 89 | 0.84 |
| Scyllo | 1632 ± 99 | 0.79 | 1730 ± 149 | 0.64 | 1571 ± 208 | 0.64 | 1807 ± 188 | 0.64 | 1196 ± 203 | 0.45 |
| Tau | 2063 ± 135 | 0.87 | 2428 ± 154 | 0.87 | 1836 ± 265 | 0.87 | 2715 ± 242 | 0.87 | 1609 ± 173 | 0.74 |
| NAA | 1385 ± 24 | 0.98 | 1390 ± 24 | 0.98 | 1382 ± 46 | 0.98 | 1394 ± 42 | 0.98 | 1139 ± 73 | 0.82 |
| tCr Combined | 1528 ± 31 | 0.97 | 1546 ± 27 | 0.97 | 1517 ± 58 | 0.97 | 1560 ± 54 | 0.97 | 1405 ± 70 | 0.88 |
| Glx | 1366 ± 38 | 0.95 | 1467 ± 25 | 0.95 | 1303 ± 70 | 0.95 | 1546 ± 64 | 0.95 | 1402 ± 98 | 0.85 |
| tCho + PE | 1241 ± 65 | 0.92 | 1251 ± 52 | 0.92 | 1235 ± 121 | 0.92 | 1259 ± 112 | 0.92 | 1242 ± 63 | 0.92 |
| mI + Glyc | 1540 ± 37 | 0.96 | 1450 ± 29 | 0.96 | 1596 ± 69 | 0.96 | 1379 ± 63 | 0.96 | 1251 ± 56 | 0.92 |

Note: In the right-hand columns $T_1$-relaxation times for GM- and WM-rich voxels are reported for 11 metabolites when default LCModel MMs were included (dkntmn = 0.15) to fit the MM contribution in the TI-series data.

Abbreviations: ASP, aspartate; GABA, γ-aminobutyric acid; GLN, glutamine; Glu, glutamate; Glyc, glycine; GM, gray matter; GSH, glutathione; LASER, locating ancestry using sequencing reads; MM, macromolecule; mL, myo-inositol; NAA, N-acetyl aspartate; NAAG, N-acetylaspartylglutamate; PE, phosphoethanolamine; Scyllo, scyllo-inositol; Tau, taurine; tCho, total choline; tCr, total creatine; WM, white matter.

*Significant $T_1$-relaxation times differences between WM-rich and GM-rich regions ($P < .0024$). Results from Welch's $t$ test and the $P$ values are reported in Supporting Information Table S1.
magnetization of individual MM peaks in contrast to the static MM\textsubscript{exp} spectrum included at 7T\textsuperscript{27}. Table 1 reports the T\textsubscript{1}-relaxation times of metabolites in GM-rich, WM-rich, GM-pure, and WM-pure voxels. From the T\textsubscript{1}-relaxation times of pure WM and GM voxel, T\textsubscript{1}-relaxation times for voxels with arbitrary tissue composition can be calculated. This in turn gives the potential to correct for heavy T\textsubscript{1}-weighting that is apparent in MRSI acquisitions\textsuperscript{30-32} when matched with tissue segmentation.

Table 1 compares T\textsubscript{1}-relaxation times for GM- and WM-rich voxels when accounting for MM contributions using MM\textsubscript{AXIOM} spectra and default LCModel MM. T\textsubscript{1}-relaxation times of metabolite singlets (NAA-CH\textsubscript{3}, tCr-CH\textsubscript{3}, and tCr-CH\textsubscript{2}) calculated after using MM\textsubscript{AXIOM} spectra agree with Deelchand et al.\textsuperscript{8} However, T\textsubscript{1}-relaxation times calculated when including default LCModel MM do not agree with the literature, and relaxation times could not be reliably measured for metabolites such as PE, NAAG, and Scyllo without including TI-specific MM\textsubscript{AXIOM} spectra. Therefore, handling MM contributions corresponding to each TI was essential for reliable calculation of T\textsubscript{1}-relaxation times in this work.

Several metabolites were found to have significantly different T\textsubscript{1}-relaxation times between GM- and WM-rich voxels. As can be seen in Table 1, Glu, GSH, Glyc, NAA-CH\textsubscript{3}, Tau, Glx, and ml+Glyc vary significantly between GM and WM. Results in Supporting Information Table S1 report the P values and adjusted P values for GM and WM T\textsubscript{1}-relaxation comparisons.

5.5 Metabolite quantification

Quantification of MC-semiLASER data was performed twice: once, using an MM\textsubscript{exp} and a second time with a MM\textsubscript{AXIOM}. The fit results for a representative spectrum are shown in Figure 7. Both the MM\textsubscript{exp} and MM\textsubscript{AXIOM} spectra resulted in residuals with little structure when fitting metabolite spectra. The most notable difference between the MM spectra is the flatter baseline for metabolite data fitted with MM\textsubscript{AXIOM}.

FIGURE 7 Comparison of a representative subject GM semiLASER spectrum fit with an MM\textsubscript{exp} spectrum and fit with an MM\textsubscript{AXIOM} spectrum. The fit residue are reduced in both cases. However, the spline baseline is more stable in the fit with the MM\textsubscript{AXIOM} spectrum.

The concentration values (Table 2 and Supporting Information Table S2) for all metabolites match well with previous work at 9.4T\textsuperscript{9} measured in the occipital lobe (GM-rich). NAAG, Gln, and Asp show elevated
concentrations compared with previous literature\textsuperscript{8,33-36} both when using an MM\_exp spectrum or MM\_AXIOM spectrum for fitting the MM contribution. Most previous studies\textsuperscript{33-35} did not include T\textsubscript{2}-correction for metabolites. However, the concentrations of Asp, Gln, and NAAG are in agreement with a previous study\textsuperscript{9} at 9.4T, which included T\textsubscript{2}-correction for metabolite concentrations. Most notable from the quantification results would be the elevated concentrations of multiple low-concentration metabolites from STEAM data, but not in semiLASER data. This could be from MM\_AXIOM being restricted to simulating peaks visible at 9.4T and not simulating peaks such as M\textsubscript{2,0} as a combination of subpeaks that would address line-shape inaccuracies in MM\_AXIOM. Future work would benefit by using data from higher field strengths\textsuperscript{14} (>9.4T) to simulate the MM peaks in MM\_AXIOM and assigning relaxation times to groups of peaks for the relevant field strength being simulated.

A primary step of metabolite quantification is correcting for sequence-specific relaxation effects. Previous work by Gasparovic et al\textsuperscript{7} used a mean T\textsubscript{1}-relaxation time for each metabolite to correct for T\textsubscript{1}-relaxation effects. However, in this work a voxel tissue-composition–specific T\textsubscript{1}-relaxation time was calculated; this method can be further extended to MRSI data that has the potential to be heavily influenced by T\textsubscript{1}-weightings because of the short TRs used in FID-MRSI.\textsuperscript{37} T\textsubscript{2}-relaxation times\textsuperscript{9} reported for the MC-semiLASER sequence from a GM-rich occipital lobe was used to correct the T\textsubscript{2}-weighting in MC-semiLASER and MC-STEAM data in both GM- and WM-rich voxels in this work. However, T\textsubscript{2}-relaxation times are sequence-dependent and region-specific.\textsuperscript{38} Therefore, this correction might have led to an error in concentration estimates from both GM- and WM-rich MC-STEAM data, and also from WM-rich MC-semi-LASER data. Future work investigating the effects of basis sets on metabolite quantification would benefit the MRS community. Concentrations should agree when data are acquired from the same brain region and should be independent of field strength and chosen localization technique. Therefore, a full validation incorporating comparisons between simulated basis sets to measured basis sets with known concentrations of metabolites would be beneficial to identify potential issues either with simulated basis sets or the individual settings being used in fitting software (ie, parameters in LCModel such as RFWHM, CHNOT, DKNTMN, etc).

**FIGURE 8** Boxplots showing metabolite concentrations in mmol/kg from GM- and WM-rich voxels depending on the used MM spectrum: MM\_exp or MM\_AXIOM. Horizontal lines inside the boxes indicate median values (50% quartile), whereas the bottom and top box boundaries illustrate 25% and 75% quartiles, respectively. Plus signs (+) show outliers. Significant differences were assessed using Mann-Whitney U tests and assigned for \( P < .0033 \) and are marked to specify to test groups. A, WM-rich and GM-rich regions for semiLASER fitted with an MM\_exp spectrum. B, WM-rich and GM-rich regions for semiLASER fitted with an MM\_AXIOM spectrum. C, MM\_exp spectrum and MM\_AXIOM spectrum fit results for GM-rich regions for semiLASER. D, MM\_exp spectrum and MM\_AXIOM spectrum fit results for GM-rich regions for STEAM. E, Between MM\_exp spectrum and MM\_AXIOM spectrum fit results for GM-rich regions for STEAM. ASP, aspartate; GABA, γ-aminobutyric acid; GLN, glutamine; Glu, glutamate; Glyc, glycine; GM, gray matter; GSH, glutathione; LASER, locating ancestry using sequencing reads; MM, macromolecule; mI, myo-inositol; NAA, N-acetyl aspartate; NAAG, N-acetylaspartylglutamate; PE, phosphoethanolamine; ScyIlo, scyllo-inositol; Tau, taurine; tCho, total choline; tCr, total creatine; WM, white matter.
CONCLUSION

A relaxation-corrected MM-simulation model, MM_{AXIOM}, that yields sequence- and tissue-type–specific, T\textsubscript{1} and T\textsubscript{2}, relaxation–corrected MM spectra for STEAM and semi-LASER sequences at 9.4T is described. This simulation model can be extended to different field strengths, provided the relaxation times of individual MM peaks are used. The performance of MM_{AXIOM} model is similar to the conventional method of including MM_{exp} in the basis set. Therefore, MM_{AXIOM} could prove to be a useful tool by saving valuable data acquisition time or being applied where MM_{exp} acquisition was not possible with short TE and MRSI acquisitions. Also in this study, TI-specific MM_{AXIOM} spectra were simulated to improve accuracy of T\textsubscript{1}-relaxation time estimates of metabolites. Longitudinal relaxation times of both singlets and J-coupled resonances of a wide range of metabolites are reported for both GM- and WM- voxels at 9.4T in the human brain for the first time. The values range from approximately 750 to approximately 2000 ms and approximately 1000 to approximately 2400 ms in GM- and WM-rich voxels, respectively. T\textsubscript{1}-relaxation times were found to vary significantly for GSH, Glu, NAA-CH\textsubscript{3}, Tau, Glx, Glyc, and ml + Glyc between GM- and WM-rich voxels. Concentrations of the metabolites are calculated using respective relaxation correction and using MM_{exp} spectra versus MM_{AXIOM} spectra. While reported concentrations are similar to previously reported literature values, our findings suggest methodological differences (section 5.5) that we hope will provide insights into the challenges surrounding brain metabolite quantification and progress the field.

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**REFERENCES**

1. Henning A. Proton and multinuclear magnetic resonance spectroscopy in the human brain at ultra-high field strength: a review. *Neuroimage*. 2017;168:181-198.

2. Öz G, Deelchand DK, Wijnen JP, et al. Advanced single voxel $^1$H magnetic resonance spectroscopy techniques in humans: experts’ consensus recommendations. *NMR Biomed*. 2021;34. https://doi.org/10.1002/nbm.4236

3. Near J, Harris AD, Juchem C, et al. Preprocessing, analysis and quantification in single-voxel magnetic resonance spectroscopy: experts’ consensus recommendations. *NMR Biomed*. 2021;34. https://doi.org/10.1002/nbm.4257

4. Li BSY, Wang H, Gonen O. Metabolite ratios to assumed stable creatine level may confound the quantification of proton brain MR spectroscopy. *Magn Reson Imaging*. 2003;21:923-928.

5. Kreis R, Ernst T, Ross BD. Absolute quantification of water and metabolites in the human brain. II. Metabolite concentrations. *J Magn Reson Ser B*. 1993;102:9-19.

6. Ernst T, Kreis R, Ross B. Absolute quantification of water and metabolites in the human brain. I. Compartments and water. *J Magn Reson Ser B*. 1993;102:1-8.

7. Gasparovic C, Song T, Devier D, et al. Use of tissue water as a concentration reference for proton spectroscopic imaging. *Magn Reson Med*. 2006;55:1219-1226.

8. Deelchand DK, Moortelet P-F, Adriany G, et al. In vivo $^1$H NMR spectroscopy of the human brain at 9.4 T: initial results. *J Magn Reson Med*. 2010;206:74-80.

9. Murali-Manohar S, Borbath T, Wright AM, Sober B, Mekle R, Henning A. T2 relaxation times of macromolecules and metabolites in the human brain at 9.4 T. *Magn Reson Med*. 2020;84:542-558.

10. Murali-Manohar S, Wright AM, Borbath T, Avdievich NI, Henning A. A novel method to measure T1-relaxation times of macromolecules and quantification of the macromolecular resonances. *Magn Reson Med*. 2021;85:601-614.

11. Hagberg GE, Bause J, Ethofer T, et al. Whole brain MP2RAGE-based mapping of the longitudinal relaxation time at 9.4T. *Neuroimage*. 2017;144:203-216.

12. Cudalbu C, Behar KL, Bhattacharyya PK, et al. Contribution of macromolecules to brain $^1$H MR spectra: experts’ consensus recommendations. *NMR Biomed*. 2021;34. https://doi.org/10.1002/nbm.4393

13. Giapitzakis I, Avdievich N, Henning A. Characterization of macromolecular baseline of human brain using metabolite cycled semi-LASER at 9.4T. *Magn Reson Med*. 2018;80:462-473.

14. Lopez-Kolkovsky AL, Mériaux S, Boumezbeur F. Metabolite and macromolecule T1 and T2 relaxation times in the rat brain in vivo at 17.2T. *Magn Reson Med*. 2016;75:503-514.

15. Behar KL, Rothman DL, Spencer DD, Petroff OAC. Analysis of macromolecule resonances in $^1$H NMR spectra of human brain preparation of brain tissue. *Magn Reson Med*. 1994;32:294-302.

16. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson Med*. 1993;30:672-679.

17. Avdievich NI, Giapitzakis I-A, Pfrommer A, Henning A. Decoupling of a tight-fit transceiver phased array for human brain imaging at 9.4T: loop overlapping rediscovered. *Magn Reson Med*. 2018;79:1200-1211.

18. Giapitzakis I-A, Shao T, Avdievich N, Mekle R, Kreis R, Henning A. Metabolite-cycled STEAM and semi-LASER localization for MR spectroscopy of the human brain at 9.4T. *Magn Reson Med*. 2018;79:1841-1850.

19. Klose U. In vivo proton spectroscopy in presence of eddy currents. *Magn Reson Med*. 1990;14:26-30.

20. Soher B. VeSPA—Versatile Simulation, Pulses, and Analysis. https://scion.duhs.duke.edu/vespa, Published 2008. Accessed May 28, 2019.

21. Ashburner J, Barnes G, Chen C, et al. *SPM12 Manual*. Wellcome Trust Centre for Neuroimaging; 2014.

22. van Rossum G. Python Tutorial, Technical Report CS-R9526. 1995.

23. Oliphant T. *A Guide to NumPy*. Vol. 1. Trelgol Publishing USA; 2006.

24. Jones E, Oliphant E, Peterson P, Al E. *SciPy: Open Source Scientific Tools for Python*. 2001.

25. Hunter JD. Matplotlib: a 2D graphics environment. *Comput Sci Eng*. 2007;9:90-95.

26. Ethofer T, Mader I, Seeger U, et al. Comparison of longitudinal metabolite relaxation times in different regions of the human brain at 1.5 and 3 Tesla. *Magn Reson Med*. 2003;50:1296-1301.

27. Xin L, Schaller B, Mlynarik V, Lu H, Gruetter R. Proton T1 relaxation times of metabolites in human occipital white and gray matter at 7 T. *Magn Reson Med*. 2013;69:931-936.

28. Cudalbu C, Mlynárik V, Xin L, Gruetter R. Comparison of T1 relaxation times of the neurochemical profile in rat brain at 9.4 tesla and 14.1 Tesla. *Magn Reson Med*. 2009;62:862-867.

29. De Graaf RA, Brown PB, McIntyre S, Nixon TW, Behar KL, Rothman DL. High magnetic field water and metabolite proton T1 and T2 relaxation in rat brain in vivo. *Magn Reson Med*. 2006;56:386-394.

30. Nassirpour S, Chang P, Henning A. High and ultra-high resolution metabolite mapping of the human brain using 1H FID MRSI at 9.4T. *Neuroimage*. 2016;168:211-221.

31. Henning A, Fuchs A, Murdoch JB, Boesiger P. Slice-selective FID acquisition, localized by outer volume suppression (FIDLOVS) for 1H-MRSI of the human brain at 7 T with minimal signal loss. *Magn Reson Biomed*. 2010;23:227-232. https://doi.org/10.1002/nbm.1423

32. Bogner W, Gruber S, Trattnig S, Chmelik M. High-resolution mapping of human brain metabolites by free induction decay 1H MRSI at 7 T. *Magn Reson Med*. 2006;56:386-394.

33. Terpstra M, Ugurbil K, Tkac I. Noninvasive quantification of human brain ascorbate concentration using 1H NMR spectroscopy at 7 T. *Magn Reson Biomed*. 2012;25:873-882.

34. Mangia S, Tkáč I, Gruetter R, et al. Sensitivity of single-voxel 1H-MRSI at 9.4T. *Magn Reson Med*. 2017;168:181-198.
35. Mekle R, Mlynárík V, Gambarota G, Hergt M, Krueger G, Gruetter R. MR spectroscopy of the human brain with enhanced signal intensity at ultrashort echo times on a clinical platform at 3T and 7T. *Magn Reson Med*. 2009;61:1279-1285.

36. Marjańska M, Auerbach EJ, Valabrègue R, Van de Moortele P-F, Adriany G, Garwood M. Localized 1H NMR spectroscopy in different regions of human brain in vivo at 7 T: T2 relaxation times and concentrations of cerebral metabolites. *NMR Biomed*. 2012;25:332-339.

37. Nassirpour S, Chang P, Henning A. High resolution maps of individual macromolecule components in the human brain at 9.4T. In: The ISMRM 25th Annual Meeting & Exhibition. 2017. p. 619. http://www.ismrm.org/17/program_files/O46.htm. Accessed December 16, 2020.

38. Hasan KM, Walimuni IS, Kramer LA, Narayana PA. Human brain iron mapping using atlas-based T2 relaxometry. *Magn Reson Med*. 2012;67:731-739.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

**FIGURE S1** Linear regressions were performed assessing T1-relaxation dependence on relative GM fractions. Black dots represent calculated T1-relaxation times for individual volunteers with a blue line representing the fit to the data. Pearson's correlation coefficient, r, is listed in each plot.

**FIGURE S2** Comparison of a representative subject spectrum from GM-rich region with TI = 400 and 700 ms fitted with default LCModel MM and with MMAXIOM.

**FIGURE S3** Normalized signal amplitudes determined from the inversion series for NAA-CH3, tCr-CH2, and Gln fitted by Equation 1 for GM- and WM-rich voxels. Points represent the data points from 11 healthy volunteers while the solid black line is the fitted solution. Boxplots are included at each TI displaying the interquartile range and median line for each box. The y-axis is normalized to the minimum signal observed at TI = 20 ms. R^2 is reported below the listed T1-relaxation time calculation as well as in Table 1. Supporting Information Figure S3.

**TABLE S1** T1-relaxation comparisons between GM- and WM-rich voxels. After Bonferroni correction, significant differences were assigned at P < .0024. Significant differences are noted with an asterisk.

**TABLE S2** Mann-Whitney U tests were used to assess for differences of metabolite concentrations, and controlled for multiple comparisons using a Bonferroni correction (α/N = 0.0033). The tests performed are organized into the following columns with significant results in bold font: a) WM-rich and GM-rich regions for semiLASER fitted with an MMexp spectrum; b) WM-rich and GM-rich regions for semiLASER fitted with an MMAXIOM spectrum; c) MMexp spectrum and MMAXIOM spectrum fit results for GM-rich regions for semiLASER; d) MMexp spectrum and MMAXIOM spectrum fit results for WM-rich regions for semiLASER; e) between MMexp spectrum and MMAXIOM spectrum fit results for GM-rich regions for STEAM.

Annex A – Bloch simulation code
Annex B – Example Control files
Annex C – Calculation of T1-relaxation times for pure GM and WM voxels
Annex D – Quantification of metabolites

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