FULL PAPER

T₂ relaxation times of macromolecules and metabolites in the human brain at 9.4 T

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Purpose: Relaxation times can contribute to spectral assignment. In this study, effective T₂ relaxation times (T₂eff) of macromolecules are reported for gray and white matter–rich voxels in the human brain at 9.4 T. The T₂eff of macromolecules are helpful to understand their behavior and the effect they have on metabolite quantification. Additionally, for absolute quantification of metabolites with magnetic resonance spectroscopy, appropriate T₂ values of metabolites must be considered. The T₂ relaxation times of metabolites are calculated after accounting for TE/sequence-specific macromolecular baselines.

Methods: Macromolecular and metabolite spectra for a series of TEs were acquired at 9.4 T using double inversion–recovery metabolite-cycled semi-LASER and metabolite-cycled semi-LASER, respectively. The T₂ relaxation times were calculated by fitting the LCModel relative amplitudes of macromolecular peaks and metabolites to a mono-exponential decay across the TE series. Furthermore, absolute concentrations of metabolites were calculated using the estimated relaxation times and internal water as reference.

Results: The T₂eff of macromolecules are reported, which range from 13 ms to 40 ms, whereas, for metabolites, they range from 40 ms to 110 ms. Both macromolecular and metabolite T₂ relaxation times are observed to follow the decreasing trend, with increasing B₀. The linewidths of metabolite singlets can be fully attributed to T₂ and B₀ components. However, in addition to these components, macromolecule linewidths have contributions from J-coupling and overlapping resonances.
1 | INTRODUCTION

Single-voxel proton MRS, a noninvasive technique, has complemented MRI by providing a means to detect and quantify concentrations of metabolites in the human brain. This has proven clinically useful, as shown in the review paper by Öz et al.1 in establishing biomarkers for pathologies in the brain.

Advantages of MRS using ultrahigh-field scanners (≥7 T) include higher SNR and increased spectral resolution.2 Hence, the macromolecular background signal (MMB) lying underneath the metabolites has to be characterized more precisely. Especially for short TE sequences, reliable metabolite quantification is more challenging without accounting for the MMB. Therefore, a measured macromolecular (MM) spectrum should be included in the fitting model.3 In addition, understanding macromolecules may help to identify valuable biomarkers for pathologies and several diseases of clinical relevance.3-6 The $T_2$ relaxation times of MM peaks have been reported in previous studies in rat brain7-10 and for the M0.92 peak in human brain at 2.1 T by Behar et al.11 However, $T_2$ relaxation times of multiple individual MM peaks in human brain have not been reported to the best of our knowledge. Estimating the $T_2$ relaxation times of individual macromolecules at 9.4 T could help in understanding and modeling their behavior.12

To derive absolute concentrations of metabolites in MRS, several different approaches exist.13-15 In one method, the unsuppressed internal water signal is used as a reference.16 However, the calculation of absolute concentrations from the apparent concentrations output by a quantification software requires a correction factor, which includes the $T_2$ relaxation times of the metabolites of interest.16 In addition, to record 2D-MRS data, it is useful to have a rough estimate of $T_2$ relaxation times to optimize TE range, such that one obtains the highest possible SNR.17,18 Additionally, altered $T_2$ relaxation times might provide information about evolving pathological or physiological states.19

Several studies have reported the $T_2$ relaxation time for metabolites in different regions of the human brain for a range of magnetic field strengths.20-22 The $T_2$ relaxation times were measured at 9.4 T for singlets, but not for J-coupled metabolites by Deelchand et al.23; however, this study did not consider the influence of $T_2$-dependent MM spectra, which affects the $T_2$ relaxation estimation of the singlets.

2 | METHODS

2.1 | Technical description and subjects

All measurements were performed on a 9.4 T Magnetom whole-body MRI scanner (Siemens Healthineers, Erlangen, Germany) using a home-built proton coil with 8 transmit and 16 receive channels.25 For single-voxel spectroscopy experiments, 3 channels at the bottom of the coil were driven using an unbalanced three-way Wilkinson splitter as previously described.26 Eleven healthy volunteers (8 males, 3 females, age: 26.3 ± 2.8 years) participated in this study for data acquisition in the GM-rich voxel. Data for the WM-rich voxels was acquired from 5 healthy volunteers (3 males, 2 females, age: 27.8 ± 1.9 years). The study was approved by the local ethics board, and written informed consent was given by all subjects before the examination.

2.2 | Data acquisition

Gradient-echo images were acquired using a 2D-FLASH sequence (in-plane resolution: 0.7 × 0.7 mm², 3.5-mm slice thickness, flip angle: 25°) along axial and sagittal orientations to facilitate placement of the spectroscopy voxel. A GM-rich voxel with the dimensions of 2 × 2 × 2 cm³ was chosen in the occipital lobe for $T_2$ measurement of metabolites, whereas in addition to the GM-rich voxel, a WM-rich voxel of the same size was chosen in the occipital–parietal transition for $T_2$ measurement of MM peaks. First-order and second-order
B₀ shimming was performed using FAST(EST)MAP,²⁷ and then voxel-based power calibration²⁸,²⁹ was executed.

Double inversion recovery (DIR) metabolite-cycled semi-LASER³⁰ and metabolite-cycled (MC) semi-LASER²⁶ spectra were acquired in the same 11 healthy volunteers. The TR was set to 10 seconds in the case of DIR-MC semi-LASER, and to 6 seconds for the MC semi-LASER, respectively, to ensure complete T₁ recovery of MM resonances and metabolites.³¹ A 16-step phase-cycling scheme³² was implemented for both spectroscopy sequences.

A series of DIR-MC semi-LASER spectra at different non-linearly spaced TEs (TE = 24 ms, 32 ms, 40 ms, 52 ms, and 60 ms; T₁₁/T₁₂ = 2360/625 ms; number of excitations = 32; transmit reference frequency (νref) = 2.4 ppm) was acquired to estimate the $T₂^*_{\text{eff}}$ of macromolecules. Setting νref to 2.4 ppm led to maximum chemical shift displacement effects of −11.5% for water and +2% for the acetyl moiety of NAA(CH₃) due to the large bandwidths of the corresponding RF pulses (excitation pulse and Adiabatic Full Passage pulse bandwidth of 8 kHz, creating a chemical-shift displacement effect of 5% per ppm).

### TABLE 1 Chemical shifts of the modeled MM peaks

| Macromolecule name | This work δ (ppm) | This work $\Delta v_{1/2}$ (Hz) | Lopez-Kolkovsky et al 2016⁷ δ (ppm) | Pfeuffer et al 1999⁸ δ (ppm) | Giapitzakis et al 2018³⁰ δ (ppm) |
|--------------------|-------------------|-------------------------------|-------------------------------|-----------------------------|-------------------------------|
| M₀.⁹² | 0.916 | 39.03 ± 4.70 | 41.87 ± 4.09 | 0.87 | 0.916 | 0.94 |
| M₁.₂₁ | 1.21 | 40.59 ± 5.53 | 38.67 ± 6.24 | 1.20 | 1.21 | 1.22 |
| M₁.₃⁹ | 1.39 | 43.75 ± 6.23 | 42.92 ± 5.67 | 1.39 | 1.39 | 1.43 |
| M₁.₆⁷ | 1.67 | 58.33 ± 8.10 | 58.33 ± 8.19 | 1.67 | 1.67 | 1.69 |
| M₂.₀⁴ | 2.04 | 59.28 ± 7.42 | 58.04 ± 7.30 | 1.91 | 2.04 | 2.04 |
| M₂.₂₆ | 2.26 | 62.73 ± 5.59 | 59.89 ± 5.04 | 2.26 | 2.26 | 2.27 |
| — — | — | — | — | 2.36 | 2.36 |
| — — | — | — | — | 2.46 | 2.46 |
| — — | — | — | — | 2.51 | 2.51 |
| M₂.₅₆ | 2.56 | 49.09 ± 2.93 | 47.46 ± 1.98 | 2.56 | 2.56 | 2.57 |
| M₂.₇₀ | 2.70 | 32.07 ± 2.37 | 31.80 ± 1.96 | 2.68 | 2.68 | 2.74 |
| M₂.₉₉ | 2.99 | 49.70 ± 1.53 | 49.28 ± 1.44 | 2.97 | 2.97 | 3.01 |
| M₃.₂¹ | 3.21 | 71.81 ± 0.83 | 71.76 ± 0.84 | 3.09 | 3.21 | 3.21 |
| — — | — | — | — | 3.22 | 3.22 |
| — — | — | — | — | 3.28 | 3.28 |
| M₃.₆₂ | 3.62 | 44.52 ± 0.65 | 44.21 ± 0.68 | 3.62 | 3.62 | 3.71 |
| M₃.₇₅ | 3.75 | 35.40 ± 0.49 | 35.20 ± 0.56 | 3.75 | 3.75 | 3.79 |
| M₃.₈₆ | 3.86 | 35.08 ± 2.05 | 34.79 ± 1.17 | 3.86 | 3.86 | 3.85 |
| M₄.₀₃ | 4.03 | 37.90 ± 0.90 | 37.53 ± 0.64 | 3.95 | 4.05 | 3.87 |
| — — | — | — | — | 4.17 | 4.20 |
| — — | — | — | — | 4.26 | 4.29 |
| — — | — | — | — | 4.33 | 4.37 |
| — — | — | — | — | 4.42 | 4.42 |

Note: The chemical shifts (in parts per million) of MM peaks modeled with Voigt lines from this work compared with studies on rat brain at 17.2 T,⁷ rat brain at 9.4 T,⁸ and human brain at 9.4 T.³⁰ The measured FWHM ($\Delta v_{1/2}$) of the MM peaks from this work is also reported.
To calculate the T$_2$ relaxation times of metabolites, a series of MC semi-LASER spectra at different nonlinearly spaced TEs (TE = 24 ms, 32 ms, 40 ms, 52 ms, and 60 ms; number of excitations = 96; $\nu_{\text{ref}}$ = 7.0 ppm) was acquired, originally measured to characterize the T$_2$ relaxation times of downfield metabolites/resonances in the same healthy volunteers. Setting $\nu_{\text{ref}}$ to 7.0 ppm led to maximum chemical-shift displacement effects of +11.5% for water and +25% for NAA(CH$_3$).

In this study, for absolute quantification of metabolites, MC semi-LASER spectra (TE/TR: 24/6000 ms; number of excitations = 32) were acquired, with $\nu_{\text{ref}}$ set to 2.4 ppm to minimize the chemical-shift displacement effect. To avoid any influence of MC pulses on quantification based on water, water-reference signals (number of excitations = 16) were acquired with semi-LASER (TE = 24 ms; $\nu_{\text{ref}}$ = 4.7 ppm) without metabolite cycling.

Finally, magnetization-prepared two rapid gradient-echo images were acquired using the same coil with RF transmission via all eight channels to calculate tissue-volume fractions for absolute quantification.

### 2.3 Data preprocessing

Raw data were analyzed with in-house-written software in MATLAB (version 2016a; MathWorks, Natick, MA). The metabolite and MM MRS data were processed as described previously. The following steps were used in processing the raw data: (1) truncation of FIDs at 250 ms for both metabolite and MM data; (2) frequency and phase alignment; (3) MC subtraction; (4) averaging; (5) zero-order phase and eddy current correction using the phase information from the MC water signal; (6) coil channel combination using a singular value decomposition method; (7) peak alignment in the frequency domain to 3.028 ppm and 3.925 ppm for the metabolite spectra and MM data, respectively; (8) residual water removal using a Hankel singular value decomposition method; and (9) truncation of FIDs at 150 ms for the MM data.

### 2.4 Macromolecule fitting

An MM basis set was created in LCModel V6.3-1L using simulated Voigt peaks, which is the best possible approximation used in literature and does not account for J-evolution of these MM peaks. The chemical shifts of the Voigt lines were varied systematically based on previously reported values, in which the ones reported by Pfeuffer et al were found to be the best; therefore, the reported chemical shifts were chosen for the MM peaks from 0.9 to 2.3 ppm and from 2.9 to 3.5 ppm. The values of M$_{2.56}$ and M$_{2.70}$ were first reported in a human brain study at 9.4 T. The chemical shifts of these two peaks were adapted to fit the shifts reported by the 17.2 T rat study. The chemical shifts for MM peaks between 3.5 ppm and 4.2 ppm were chosen based on a previously mentioned study, as the peaks were resolved more clearly in Lopez et al compared with the other two studies. All of these chemical shifts are summarized in Table 1.

The simulated basis set consisted of the following MM peaks: M$_{0.95}$ (0.916 ppm), M$_{1.21}$ (1.21 ppm), M$_{1.39}$ (1.39 ppm), M$_{1.67}$ (1.67 ppm), M$_{2.04}$ (2.04 ppm), M$_{2.26}$ (2.26 ppm), M$_{2.56}$ (2.56 ppm), M$_{2.70}$ (2.7 ppm), M$_{2.99}$ (2.99 ppm), M$_{3.21}$ (3.21 ppm), M$_{3.62}$ (3.62 ppm), M$_{3.75}$ (3.75 ppm), M$_{3.86}$ (3.86 ppm), M$_{4.03}$ (4.03 ppm), and M$_{4.17}$ (4.17 ppm). The same basis set was used throughout the TE series, with the M$_{2.70}$ peak being simulated with a negative amplitude for TE = 52 and 60 ms, as this peak was observed to be fully inverted due to J-evolution. A much narrower Voigt line was added to fit the residual creatine at 3.925 ppm. The chemical shifts, $\Delta \nu_{1/2s}$, and amplitudes were constrained in the LCModel as described in Supporting Information Annex B.

Each individual MM data set and the sum of all data sets were fitted to the simulated MM basis set while enforcing a flat baseline by setting the LCModel parameter DKNTMN to 99.

### 2.5 Metabolite fitting

The metabolite basis set was simulated in Vespa (ver. 0.9.3) using full quantum mechanical density matrix calculations for the semi-LASER sequence, including the actual complex excitation and adiabatic RF pulse shapes for all TEs specified in section 2.2. The following 17 metabolites were simulated: NAA, NAA glutamate (NAAG), γ-aminobutyric acid (GABA), aspartate (Asp), creatine (Cr), glutamate (Glu), glutamine (Gln), glutathione, glycerophosphocholine, glycine (Glyc), myo-inositol (ml), scyllo-inositol (Scy), lactate (Lac), phosphocreatine (PCr), phosphocholine (PCho), phosphoethanolamine (PE), and taurine (Tau). Their chemical shifts and coupling constants were taken from Govindaraju et al, except for the coupling constant of GABA, for which the values from near et al were chosen.

Selected metabolites were split into moieties for fitting the TE series to calculate the metabolites’ T$_2$ times. As previous studies have shown, different moieties of the same metabolite have different relaxation times. NAA was split into an NAA–acetyl moiety [NAA(CH$_3$)] and an NAA–aspartyl moiety [NAA(CH$_2$)], whereas instead of Cr and PCr metabolites, the singlets at 3.925 ppm total creatine [tCr(CH$_2$)] and at 3.028 ppm [tCr(CH$_3$)] were included in the basis set. The metabolites were used in their entirety to calculate absolute concentrations.

Because of the strong overlap and ill-posed problem of fitting PCho and glycerophosphocholine separately, these were combined to a total choline metabolite (tCho) with corresponding volume fractions of 0.6 mM PCho and 1 mM.
glycerophosphocholine based on the mean concentration values from de Graaf. Similarly, PCho, glycerophosphocholine, and PE were combined with Chol (corresponding to volume fractions of 0.6 mM PCho, 1.0 mM glycerophosphocholine, and 1.5 mM PE), to obtain a robust metabolite fit for the \( T_2 \) calculations across the TE series.

Then, all individual metabolite spectra were fitted using the LCModel with the simulated baseline set, including the respective MMB summed across subjects for each TE. The metabolite spectra summed across subjects were fitted similarly. Because the summed MMB spectra were included in the fit, DKNTMN was set to 0.25, resulting in a stiffer LCModel baseline compared with the default LCModel value of 0.15.

To further improve the fitting procedure, two soft constraints were introduced into the echo series fit, namely, NAA(CH\(_3\))/NAA(CH\(_2\)) = 1.2 ± 0.02 (to account for possible faster decay of NAA(CH\(_2\)) and mI/Glyc = 5 ± 0.5 (value extracted from the TE = 24 ms fit) (Supporting Information Annex B). No soft constraints were used for GABA or other metabolites.

### 2.6 \( T_2 \) relaxation calculations

The concentrations of MM peaks and metabolite peaks from the individual data and the summed spectra were fit to a mono-exponential decay across the TE series to yield \( T_2 \) relaxation-time estimates. The mean coefficient of determination (\( R^2 \)) value was calculated for the exponential fit across the individual subject data and was used to evaluate the quality of the exponential fits. Relaxation times with a mean \( R^2 \) smaller than 0.5 were discarded. The \( T_2 \) times for Lac, Tau, and Scy are not reported, as they did not satisfy the \( R^2 \) criterion.

### 2.7 Linewidth calculations

After quantification and extracting the fitted lineshapes of MM peaks and metabolite singlets from the .coord files of LCModel quantification, the \( \Delta v_{1/2} \) were measured. The contribution of \( T_2 \) relaxation to \( \Delta v_{1/2} \) was calculated according to \( (\pi T_{2eff}^2)^{-1} \), using the calculated \( T_2 \) values.

The residual linewidth was defined as

\[
\Delta v_{\text{residual}} = \Delta v_{1/2} - (\pi T_{2eff}^2)^{-1} - \Delta v_{\text{single}}
\]

The \( B_0 \) components were calculated from NAA(CH\(_3\)) and tCr(CH\(_2\)) as

\[
\Delta v_{\text{single}} = \Delta v_{1/2} - (\pi T_{2eff}^2)^{-1} \approx \Delta v_{\text{micro}} + \Delta v_{\text{macro}}
\]

respectively. The values of \( \Delta v_{\text{micro}}, \Delta v_{\text{macro}} \) represent the micro-susceptibility and macrosusceptibility, respectively.

### 2.8 Absolute quantification

Using Statistical Parametric Mapping 12 software (Wellcome Trust Centre for Neuroimaging, London, United Kingdom), the acquired magnetization-prepared two rapid gradient-echo images were segmented into the following tissues: WM, GM, and CSF. Final tissue compositions were calculated using an in-house written Python (ver. 3.7) method and were used for absolute quantification of metabolites. Obtained tissue compositions for the GM-rich voxels were GM: 69.2% ± 3.9%, WM: 25.8% ± 5.0%, and CSF: 4.9% ± 3.0%, and the WM-rich voxels were GM: 32.9% ± 9.3%, WM: 64.7% ± 11.1%, and CSF: 2.4% ± 3.7%.

The unsuppressed water-reference signal from the voxel was used as the internal concentration reference for absolute quantification calculated in millimolar values (millimoles per kilogram of solvent [mmol/kg]). The concentrations of the metabolites were absolutely quantified using the formula (Supporting Information Annex A) given by Gasparovic et al. for TE = 24 ms, including corrections for the \( T_1 \) and \( T_2 \) relaxation times of water in different compartments and the metabolite relaxation times. More specifically, the \( T_1 \) and \( T_2 \) relaxation times of water at 9.4 T in GM (\( T_1^\text{GM} = 2120 \) ms; \( T_2^\text{GM} = 37 \) ms) and WM (\( T_1^\text{WM} = 1400 \) ms; \( T_2^\text{WM} = 30 \) ms) were taken from Hagberg et al. Relaxation times for CSF were calculated based on data from the same work (\( T_1^\text{CSF} = 4800 \) ms; \( T_2^\text{CSF} = 181 \) ms). The \( T_1 \) relaxation times of metabolites were taken from Wright et al. The \( T_2 \) relaxation times of the metabolite peaks calculated in this study were used. For the metabolites, for which \( T_2 \) relaxation times could not be estimated, the mean \( T_2 \) relaxation time of all other metabolites was used.

### 3 RESULTS

Spectra without major artifacts were obtained for all subjects: The NAA(CH\(_3\)) SNR ranged from 526 ± 130 to 334 ± 82 for TE = 24-60 ms, respectively. However, between 0.9 and 1.8 ppm, some spectra, especially MM spectra from WM voxels (Figure 1 and Supporting Information Figure S1), presented outer-volume lipid impurities for some subjects, as lipid suppression techniques such as outer-volume saturation were not used. The value of \( \Delta v_{1/2} \) of the unsuppressed water signal was 17.6 ± 1.3 Hz. The TE series of MM and metabolite spectra from the GM-rich voxel are shown in Figure 1, where the shaded area depicts the SD, illustrating the reproducibility of the data quality. Supporting Information Figure S1 shows the TE series of WM-MM spectra. No data sets were excluded.
3.1 Macromolecule fitting

The spectrum from the GM-rich voxel summed across subjects, together with all the fitted Voigt lines for the MM peaks, is shown in Figure 2 for TE = 24 ms and in Supporting Information Figure S2 for the other TEs. Supporting Information Figures S3 and S4 show the fit of the WM-TE series spectra summed across subjects. The fit residual is minimum without structured noise, indicating a high fit quality. Similar fit quality was achieved for all data sets across all TEs. The M2.70 peak is observed to undergo a full inversion due to J-evolution over the TE steps. More precisely, the full-inversion M2.70 occurred...
The residual CH$_2$ resonance of total creatine [tCr(CH$_2$)] was modeled by fitting a sharper Voigt line at 3.925 ppm with a measured $\Delta v_{1/2}$ of around 0.035 ppm (14 Hz) across subjects. Next, the residual Cr was extracted from the LCModel fit, and was subtracted from the MM spectra to yield a more appropriate MMB for the metabolite fits (Figure 3). The resulting MMB was included in the basis set to fit the metabolite spectra for $T_2$ relaxation-time calculations as well as for absolute quantification.

### 3.2 Metabolite fitting

The fit of the metabolites to the summed metabolite spectra is shown in Figure 4. The fit residual is small, indicating a high fit quality. For TE = 32, 40, 52 and 60 ms, the basis set modeled the J-evolution of mI, NAA(CH$_2$), Asp, Glu, and Tau well (Supporting Information Figure S5).

Adding ascorbic acid or glucose to the simulated basis set did not improve the fit in terms of Cramer-Rao lower bounds, $T_2$-fit result confidences, and residual artifacts. These resonances were not found by LCModel in most cases. Hence, these metabolites were not included in the final basis set.

### 3.3 $T_2$ results

The calculated $T_2^{\text{eff}}$ and $T_2$ relaxation times (Figure 5) for MM peaks and metabolites, respectively, were overall in good agreement for the fits of individual subject data and the summed spectra. Box plots of the resulting $T_2$ relaxation times are shown in decreasing order for metabolites and MM peaks in Figure 5. The $T_2$ relaxation time of the residual tCr(CH$_2$) peak in the MM spectra was also in agreement with the relaxation time of the tCr(CH$_2$) peak in the metabolite spectra.

The $T_2$ relaxation times of metabolites and MM peaks from the summed spectra and individual data, together with the mean and SD of $R^2$ of the corresponding exponential fits, are listed in Table 2.

The $T_2$ relaxation times of metabolites were found to lie between 55 ms and 105 ms, except for NAAG with approximately 40 ms. In contrast, the values of $T_2^{\text{eff}}$ for all MM resonances were between 13 ms and 40 ms in WM and between 13 ms and 37 ms in GM.

The $R^2$ for the metabolite exponential decay fits were all above 0.70, except for Asp and Glyc. The mean $R^2$ of the exponential decay fits was above 0.70 for all MM peaks as well, except for M$_{4.03}$, which was subjected to water residuals and noise at longer TEs, hence the lower $R^2$ value. Furthermore, the uncertainties in the $T_2^{\text{eff}}$ relaxation times of the M$_{1.21}$ and M$_{1.39}$ were influenced by lipid contaminations.

### 3.4 Linewidth calculations

The value of $\Delta v_{1/2}$ of the MM resonances was measured to vary between 35 Hz and 85 Hz across all TEs (Table 1), whereas $\left(\pi T_2^{\text{eff}}\right)^{-1}$ was calculated to be between 4 Hz and 30 Hz (Figure 6). In the case of metabolite resonances, and for the residual tCr(CH$_2$) in the MM spectra, a $\Delta v_{1/2}$ of 11 Hz to 20 Hz was found, whereas $\left(\pi T_2^{\text{eff}}\right)^{-1}$ ranged between 2 Hz to 10 Hz.
and 5 Hz. This led to a \( \Delta v_{\text{singlet}} \) value of approximately 12 Hz for the metabolite singlets.

Supporting Information Figure S7 shows the \( \Delta v_{\text{residual}} \) values of metabolites and MM peaks, which were calculated using the \( \Delta v_{\text{singlet}} \) of NAA(CH\(_3\)) and tCr(CH\(_2\)) for the metabolite and MM spectra, respectively. The values of \( \Delta v_{\text{residual}} \) of metabolites were around zero after applying the correction. In contrast, MM peaks had \( \Delta v_{\text{residual}} \) values ranging between 10 Hz and 60 Hz.

### 3.5 Concentrations

Absolute concentrations in millimoles per kilogram with and without \( T_2 \) correction are shown in Figure 7. The values for the metabolites are reported in Table 3 with and without the \( T_2 \) correction factor. In Supporting Information Figure S8 and Supporting Information Table S1, absolute concentrations are given in millimoles per tissue volume in a liter (mmol/L).

### 4 DISCUSSION

In this study, transverse relaxation times of a wide range of MM peaks and metabolites at 9.4 T are reported. Additionally, in vivo absolute concentrations of metabolites for single-voxel MRS acquisitions from the occipital lobe are reported. The resulting \( T_2 \) values and absolute concentrations were largely in line with the literature.

#### 4.1 Spectral quality and fitting of MM spectra

The DIR technique sufficiently nulled all of the metabolites except tCr(CH\(_2\)) for the chosen TI\(_1\) and TI\(_2\), as the \( T_1 \) relaxation time of this resonance is the shortest among the singlets, as reported by Deelchand et al.\(^{23}\) Indeed, the difference between the \( T_1 \) relaxation times of some of the MM peaks\(^{44}\) and the CH\(_2\) group of Cr is not large; thus, there is a residual peak present in the MM spectra. The residual tCr(CH\(_2\)) was subtracted using the fit of the Cr singlet fit from the LCModel. The resulting spectra (Figure 3) after the residual subtraction visually showed no leftover Cr.

For the fitting of the MM spectra, the chemical shifts and \( \Delta v_{1/2} \) were systematically varied to achieve the lowest SD of the \( T_2 \) results among subjects, to maximize the \( R^2 \) values, and to minimize the mean Cramer-Rao lower bounds. These systematic variations supported that the values chosen by Lopez et al\(^{7}\) were among the most suitable choices, and these values were also well justified by peak characteristics seen at 17.2 T.
Hence, these values were chosen, with minor deviations as indicated in Table 1.

For the first time, the J-evolution of the M 2.7 resonance (Figures 1 and 3, Supporting Information Figure S1) was investigated in this study. This resonance was preliminarily assigned to β-methylene protons of aspartyl groups.30 The Biological Magnetic Resonance Bank amino acid database45 lists the following coupling constants for the β-methylene protons (δ2.7 ppm) of aspartate amino acids: approximately 5 and 8 Hz between α-methylene and β-methylene protons, and 17.5 Hz between β-methylene protons. These coupling constants are comparable to those of Asp and the aspartate moieties of NAA and NAAG,40 which experience full inversion between TEs of 52 and 60 ms. All observations support the preliminary attribution of the M 2.7 resonance to the aspartyl groups. Nevertheless, the possibility cannot be excluded that the MM spectra also included NAA(CH2) residuals, provided that this moiety has a short enough T1 relaxation time. In particular, at longer TEs (52 and 60 ms), the contribution of NAA(CH2) could be more significant, as metabolites have longer T2 relaxation times than macromolecules.

### 4.2 Metabolite fitting

Previous work at 9.4 T reported concentrations of 18 metabolites in the human brain using an MC semi-LASER sequence.26 However, due to the complexity of the adiabatic pulses and their spin locking effect,46 the simulated basis set was approximated using a spin-echo sequence with TE = 6.5 ms.30 In this study, the basis set for MC-semi-LASER was simulated using actual adiabatic RF pulse shapes. In addition, the same TEs were used in the acquisition of both metabolite and MM spectra. This allowed to individually fit the metabolite spectra with the matching simulated basis set and the corresponding MMB for each TE. The fit results (Figure 4 and Supporting Information Figure S5) show that the simulated basis set represents the J-evolution patterns of the acquired spectra well.

### 4.3 T2 results

This study reports the \( T^*_{2} \) of individual MM peaks as well as T2 relaxation times of both singlets and J-coupled metabolites at 9.4 T in human brain. The values of \( T^*_{2} \) of MM peaks are in agreement with previous work. Behar et al reporting 44-ms T2 at 2.1 T for M 0.92 in human brain,11 which is in agreement with a slow decay across field strengths, as shown in the rat brain studies.7,10

The T2 of metabolite singlets, such as NAA(CH3), tCr(CH2), tCr(CH3) and tCho, are higher than those previously reported at 9.4 T.23 The higher values in this work can be attributed to the fact that a TE-specific experimentally measured MMB was included, which is a faster-decaying component. The reported T2 relaxation times of the metabolites appear to follow the same trend compared with previous results,7,20,21,47 with the longest relaxation times found for the singlets NAA(CH3), tCho and tCr(CH3) [the T2 of tCr(CH3) larger than for tCr(CH2), difference decreasing with increasing field strength],7,20,21,23,47 and significantly shorter T2 for NAAG than for NAA moieties.20 All reported T2 relaxation times show the expected negative correlation with increasing field strength. Glutamate T2 is higher than that of Gln, in agreement with previous studies.7,20 However, the difference is unexpected, considering the similar molecular weight of the two metabolites and the similar distribution within the brain48; hence, most likely the T2 of Gln is underestimated. Nevertheless, differences could arise from the presence in different compartments, or bindings to different transporters or enzymes.

The summed and individual fits are in good agreement for T2 relaxation times for both MM resonances and metabolites.
### Table 2: T₂ relaxation times for metabolites and macromolecules

| Metabolite | T₂ (ms) | T₂ ± SD (ms) | R² ± SD | Macromolecule T₂ eff | T₂ eff, sum (ms) | T₂ eff ± SD (ms) | R² ± SD | White matter T₂ eff | T₂ eff ± SD (ms) | R² ± SD |
|------------|---------|--------------|---------|----------------------|-----------------|-----------------|---------|---------------------|-----------------|---------|
| Asp        | 72      | 54.4 ± 11.5  | 0.72 ± 0.12 | M₀ 0.92             | 36.9            | 36.4 ± 7.0      | 0.91 ± 0.07 | 31.3                | 35.1 ± 10.0     | 0.86 ± 0.09 |
| tCr(CH₃)   | 95.2    | 100.2 ± 16.0 | 0.90 ± 0.10 | M₁ 1.21             | 37.4            | 34.3 ± 13.1     | 0.79 ± 0.13 | —                   | 25.3 ± 0.0      | 0.98 ± 0.00 |
| tCr(CH₂)   | 95.4    | 81.8 ± 10.8  | 0.83 ± 0.08 | M₁ 1.39             | 36.7            | 31.5 ± 7.4      | 0.83 ± 0.08 | 31                  | 39.6 ± 24.2     | 0.75 ± 0.13 |
| Glu        | 76.8    | 87.0 ± 23.3  | 0.73 ± 0.10 | M₁ 1.67             | 15.9            | 17.6 ± 3.6      | 0.92 ± 0.08 | 17.4                | 17.5 ± 7.5      | 0.89 ± 0.11 |
| Gln        | 65.9    | 47.0 ± 8.6   | 0.81 ± 0.06 | M₂ 2.04             | 15.9            | 15.8 ± 2.1      | 0.98 ± 0.03 | 17.1                | 18.9 ± 1.5      | 0.98 ± 0.02 |
| GSH        | 70.7    | 74.8 ± 34.2  | 0.73 ± 0.09 | M₃ 2.26             | 19.6            | 18.4 ± 1.5      | 0.96 ± 0.03 | 19.1                | 18.1 ± 1.0      | 0.96 ± 0.02 |
| Glyc       | —       | 60.9 ± 11.2  | 0.60 ± 0.07 | M₃ 2.56             | 18.7            | 20.1 ± 3.2      | 0.93 ± 0.04 | 19.3                | 23.9 ± 3.4      | 0.84 ± 0.10 |
| mI         | 84.5    | 90.0 ± 18.0  | 0.70 ± 0.09 | M₄ 2.70             | 11.6            | 13.9 ± 2.9      | 0.93 ± 0.08 | 11.6                | 13.4 ± 1.9      | 0.94 ± 0.03 |
| NAA(CH₂)   | 90.4    | 102.2 ± 23.8 | 0.92 ± 0.12 | M₅ 2.99             | 18.9            | 18.8 ± 1.6      | 0.94 ± 0.06 | 18                  | 19.1 ± 1.3      | 0.93 ± 0.03 |
| NAA(CH₃)   | 104.9   | 110.5 ± 27.7 | 0.93 ± 0.12 | M₆ 2.31             | 18.4            | 18.4 ± 3.2      | 0.93 ± 0.08 | 13.9                | 16.3 ± 5.4      | 0.91 ± 0.06 |
| NAAG       | 37.7    | 45.0 ± 16.3  | 0.87 ± 0.09 | M₇ 3.62             | 15.5            | 13.9 ± 6.4      | 0.90 ± 0.07 | 12.4                | 15.9 ± 4.8      | 0.85 ± 0.06 |
| tCho+      | 80.4    | 90.1 ± 23.5  | 0.92 ± 0.11 | M₈ 3.75             | 23.3            | 25.4 ± 6.8      | 0.91 ± 0.08 | 25.6                | 24.5 ± 6.8      | 0.82 ± 0.11 |
| tNAA+      | 93.2    | 100.6 ± 21.8 | 0.93 ± 0.11 | M₉ 3.86             | 19.5            | 22.0 ± 5.3      | 0.91 ± 0.12 | 18.5                | 24.2 ± 5.2      | 0.84 ± 0.10 |
| mI+Glyc    | 77.8    | 84.4 ± 16.8  | 0.77 ± 0.12 | M₄ 4.03             | 11.5            | 20.7 ± 8.2      | 0.71 ± 0.10 | —                   | —               | —       |
| Glx        | 72.8    | 71.9 ± 10.5  | 0.76 ± 0.09 | tCr(CH₃) 65.3       | 71.3 ± 7.9      | 0.75 ± 0.11     | 67.3        | 61.7 ± 3.1          | 0.84 ± 0.12     |

*Note:* Calculated T₂ relaxation times for metabolites (left), T₂ eff for MM gray matter (middle), and MM white matter (right) are shown for fitting the spectra summed across subjects (T₂ eff, sum) and per-subject fits (mean and SD) with the confidence of the exponential fit (R²). Some exponential decay fits are shown in Supporting Information Figure S6. The T₂ relaxation times of GABA, Lac, Scy, and PE are not included, as they did not satisfy the imposed R² > 0.5 criterion.

*Abbreviation:* GSH, glutathione.
(Table 2). It can also be noted that the relaxation time of the tCr(CH$_2$) in the metabolite spectra and the residual present in the MM spectra are in good agreement. The differences between $T_2^{eff}$ relaxation times of MM peaks in WM-rich and GM-rich voxels were never investigated previously, nor were the relaxation times calculated for the peaks individually. Therefore, an attempt was made to calculate $T_2^{eff}$ for both tissue compositions in this work. The $T_2^{eff}$ relaxation times found in this study are comparable between these voxels.

However, it is known that the region of interest in the human brain is crucial when applying $T_2$ correction for absolute concentrations of metabolites. Hence, no attempt was made to report the $T_2$ relaxation times of metabolites separately for GM and WM, as the selected voxel was neither...
| Metabolite                  | Asp        | Asc        | Cr          | PCr         | GABA       | Glucose     | Gln          | Glu         | Glyc       | GSH         | Lac        | mI         | NAA         | NAAG        | tCho        | PE          | Scy         | Tau         | NAA+NAAG    | mI+Glyc     | Cr+PCr      | Glu+Gln     |
|---------------------------|------------|------------|-------------|-------------|------------|-------------|---------------|-------------|------------|-------------|------------|------------|------------|-----------|-----------|-----------|-------------|-------------|-----------|------------|-------------|------------|
| This work with T<sub>2</sub> correction | 4.84 ± 1.15<sup>b</sup> | 3.12 ± 0.74 | 5.70 ± 0.85 | 4.43 ± 0.83 | 1.87 ± 0.92<sup>d</sup> | —           | 7.61 ± 0.91<sup>b</sup> | 10.90 ± 0.80 | 1.11 ± 0.28<sup>b</sup> | 1.72 ± 0.16 | 5.22 ± 0.45 | 12.61 ± 1.02 | 1.42 ± 0.19<sup>a</sup> | 1.03 ± 0.12 | 2.28 ± 0.95<sup>d</sup> | 0.13 ± 0.06<sup>d</sup> | 1.58 ± 0.37 | 13.78 ± 1.01<sup>a</sup> | 6.34 ± 0.52 | 10.10 ± 0.46<sup>a</sup> | 17.96 ± 1.49<sup>b</sup> |
| This work without T<sub>2</sub> correction | 3.12 ± 0.4 | 1.1 ± 0.3  | 4.38 ± 0.65 | 3.40 ± 0.64 | 1.39 ± 0.68 | —           | 4.56 ± 0.54  | 8.27 ± 0.61  | 0.75 ± 0.19  | 1.25 ± 0.12 | 4.00 ± 0.34 | 12.06 ± 0.81 | 0.83 ± 0.11  | 0.79 ± 0.09 | 1.75 ± 0.73  | 0.09 ± 0.05<sup>c</sup> | 1.17 ± 0.27 | 10.86 ± 0.79 | 4.77 ± 0.39 | 7.76 ± 0.36 | 12.86 ± 1.07 |

(Continues)
### TABLE 3

**Absolute concentrations (mmol/kg ± SD)**

|                          | This work with T<sub>2</sub> correction | This work without T<sub>2</sub> correction | Terpstra et al 2009 | Mangia et al 2006 | Mekle et al 2009 | Mekle et al 2009 | Deelchand et al 2010 | Marjańska et al 2012 |
|--------------------------|----------------------------------------|------------------------------------------|-------------------|------------------|------------------|------------------|----------------------|---------------------|
|                          | Occipital lobe                         | Occipital lobe                           | T<sup>51</sup>     | T<sup>50</sup>     | T<sup>29</sup>   | T<sup>29</sup>   | T<sup>23</sup>        | T<sup>11</sup>       |
| Included MMB             | measured                               | measured                                 |                   |                  |                  |                  |                      |                     |
| Metabolite T<sub>2</sub> | yes                                    | no                                       | no                | no               | no               | no               | no                   | yes                 |
| correction               |                                        |                                          |                   |                  |                  |                  |                      |                     |
| Tissue fraction,         | yes                                    | yes                                      | Assuming 80%      |                  |                  |                  |                      |                     |
| H<sub>2</sub>O T<sub>1</sub>, T<sub>2</sub> |                                        |                                          | water content     |                  |                  |                  |                      |                     |
| corrections              |                                        |                                          |                   |                  |                  |                  |                      |                     |
| Sequence                 | semi-LASER                             | semi-LASER                               | STEAM             | STEAM            | SPECIAL          | SPECIAL          | STEAM                | LASER               |
| TE                       | 24 ms                                  | 24 ms                                    | 6 ms              | 6 ms             | 6 ms             | 6 ms             | 8 ms                 | 35 ms               |

**Note:** Absolute concentrations for the metabolites are shown (in mmol/kg). Concentrations for this work are reported with the correction of the metabolite T<sub>2</sub> values and without this correction. Both reported values use the T<sub>1</sub>, T<sub>2</sub>, and tissue-compartment corrections of the water signal. The concentrations without metabolite T<sub>2</sub> correction are all in good agreement with the millimolar concentrations reported in the literature at different field strengths from different studies (sequence and fitting configurations are also summarized). Absolute concentrations for the metabolites (in mmol/L) are found in Supporting Information Table S1 together with the literature comparison.

**Abbreviation:** Asc, ascorbic acid; GSH, glutathione.

<sup>a</sup>Concentrations that are higher than the literature values after metabolite T<sub>2</sub> correction (small changes).

<sup>b</sup>Concentrations that are higher than the literature values after metabolite T<sub>2</sub> correction (large changes).

<sup>c</sup>The Scy concentration is marked because it is possibly underestimated in this study. However, it is not clear whether the other literature used 1 or 6 proton resonances (1<sup>4</sup>CH) contributing to their Scy basis set simulation.

<sup>d</sup>Metabolites for which the mean T<sub>2</sub> relaxation time of all other metabolites was taken.
purely WM nor GM. This difference in $T_2$ relaxation times is highly influenced by the iron concentrations across the human brain, as shown by Hasan et al.\textsuperscript{49} Hence, the $T_2$ relaxation times of metabolites reported in this work are specific to the region of the GM-rich occipital lobe.

The TE values of 24, 32, 40, 52, and 60 ms were chosen for the calculation of $T_2$ relaxation times, while keeping the shorter MM $T_2^0$ in mind. The MM signals were almost entirely decayed at $TE = 60$ ms. Having a corresponding MMB for the metabolite spectra improves the quantification of metabolite concentrations, hence the choice of identical TEs. The chosen TEs were sufficient to estimate the $T_2$ relaxation times of MM peaks and metabolites with shorter $T_2$ times. However, including some longer TE values would improve the accuracy of the $T_2$ relaxation times of metabolites with longer $T_2$s.

4.4 \quad Linewidth calculations

For any given voxel, $B_0$ inhomogeneities originate from a mixture of effects of $\Delta\nu_{\text{micro}}$, $\Delta\nu_{\text{macro}}$ as well as tissue compartment effects.\textsuperscript{23} This $B_0$ effect experienced by spins is identical, whereas $T_2$ relaxation time is metabolite/resonance-specific. The $\Delta\nu_{1/2}$ of metabolite singlets, both in MC-semi-LASER and $tCr(CH_2)$ in DIR MC-semi-LASER, clearly show the two components of the linewidth (Figure 6). The $\Delta\nu_{1/2}$ of $tCr(CH_2)$ is in line with the ones previously reported.\textsuperscript{23} The large differences between the $\Delta\nu_{1/2}$ of MM peaks and $(1/T_2^g)^1$ (Figure 6, Table 1) indicate that these resonances are potentially composed of unresolved multiplets and/or different protons resonating at similar chemical shifts, which are strongly overlapping. Also, Supporting Information Figure S7 shows that the values of $\Delta\nu_{\text{residual}}$ for MM peaks are between 10 Hz and 60 Hz, whereas for metabolites they are closer to 0 Hz. The $\Delta\nu_{\text{residual}}$ value is consistent between GM and WM MM peaks, indicating that the magnitude of potential overlap and/or J-evolution\textsuperscript{11} component for each MM peak is similar between different tissue types. These peaks could originate from amino acids\textsuperscript{45} which, depending on the larger protein structure they belong to, can have different chemical shifts, but are distributed around a main resonance frequency for the bulk of protein peaks.

4.5 \quad Concentrations

Table 3 provides a consolidated comparison of absolute concentrations (millimoles per kilogram) of metabolites from literature\textsuperscript{21,23,29,50,51} and this work. Metabolite concentrations from this study are reported with and without $T_2$ correction for a fair comparison between the other studies and this work, as most of the other studies did not include $T_2$ correction due to the use of ultrashort TEs ($<10$ ms). The concentrations of NAA, NAAG, tCho, PE, Tau, Glu, GABA, and mI with $T_2$ correction are overall in good agreement with the literature.\textsuperscript{21,23,29,50,51}

Concentrations of Asp, Gln, Glyc, and Glu are significantly higher after including the $T_2$ correction factor. However, their concentration values match those from the literature when considered without $T_2$ correction. Potential overestimations in this study could also arise due to an underestimation of the $T_2$ relaxation times of Gln, Glu and Asp, which exhibit strong J-evolution effects at $TE = 52$ ms and $TE = 60$ ms. In particular, the Gln concentration is likely overestimated, as the spline baseline exhibits negative behaviour in that area, which could not be compensated in the LCModel fits. Improved spectral resolution at ultrahigh field between Glu and Gln also influenced Gln concentrations in the current study. Furthermore, the loss of magnetization for individual MM components because of $T_1$ differences in DIR semi-LASER compared with semi-LASER could also influence our results. All referenced literature used single inversion recovery sequences to measure the MMB component. Similarly, Cr and PCr concentrations match the literature without $T_2$ correction, but are somewhat higher (~25%) when the $T_2$ correction is applied. This deviation could indicate that including the $T_2$ correction factor in the absolute quantification yields higher concentrations compared with the literature, as in most studies, no correction for the corresponding $T_2$ relaxation times of the metabolites was applied, or the Cr + PCr concentration was set to 8 mmol/kg. The shorter TEs used in the aforementioned studies, however, should not generally have a significant impact on metabolite concentrations.

Concentration of Scy, on the other hand, is lower in this study compared with other studies. However, it remains unclear whether care was taken to use all six carbon atoms, written as $1^{16}$CH in Govindaraju et al.\textsuperscript{40} each having a proton resonating at the same frequency, thus contributing to their Scy basis set simulation.

Absolute concentrations in millimoles per liter, given in Supporting Information Figure S8 and Supporting Information Table S1, are in excellent agreement with the concentrations that corrected for metabolite relaxations given by Penner et al\textsuperscript{52} and with the literature comparison presented in the same article.

5 \quad CONCLUSIONS

In this study, for the first time, $T_2$ relaxation times of 14 individual macromolecule peaks ranging from 13 ms to 45 ms are measured in both GM-rich and WM-rich voxels.

In addition, in vivo transverse relaxation times of 12 metabolites and metabolite moieties in a GM-rich voxel in the occipital lobe at 9.4 T are reported. The $T_2$ relaxation values...
ranged from 40 ms to 110 ms and were used as a correction factor for the absolute quantification of metabolites. The $T_2^D$ and $T_2$ values for MM peaks and metabolites, respectively, confirm the decreasing trend of transverse relaxation times with increasing static magnetic field. Finally, this work quantitatively shows the contribution of $T_2$ relaxation times and $B_0$ components to the linewidth of MM peaks. The residual linewidth includes not only components of J-coupling, but also chemical-shift distributions of amino acid proton groups.

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SUPPORTING INFORMATION

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

FIGURE S1 Echo time series (TE = 24, 32, 40, 52, and 60 ms) for MM spectra (right) from WM, with solid lines showing the mean spectra and the shaded area indicating the SD across subjects.

FIGURE S2 Gray Matter MM spectra summed across subjects (TE = 32, 40, 52 and 60 ms) are shown with the fitted macromolecules and tCr(CH2) moiety. The basis set configuration shows the setup for inverting the M2,70 peak at TE = 40, 52, and 60 ms.

FIGURE S3 White matter MM spectrum summed across subjects (TE = 24 ms) is shown with the fit using simulated Voigt lines. The residual tCr(CH2) peak in the spectra is modeled with a significantly narrower linewidth.

FIGURE S4 White matter MM spectra summed across subjects (TE = 32, 40, 52, and 60 ms) are shown with the fitted macromolecules and tCr(CH2) moiety. The basis set configuration shows the setup for inverting the M2,70 peak at TE = 40, 52, and 60 ms.

FIGURE S5 Metabolite spectra summed across subjects (TE = 32, 40, 52, and 60 ms) are shown with the fitted metabolites and measured MMB. The basis set configuration shown here with the tCr split to its moieties tCr(CH3) and tCr(CH2), NAA split to NAA(CH2) and NAA(CH3) moiety, and tCho and PE combined with tCho+ was used to estimate the T2 relaxation times. For each TE, a corresponding basis set was simulated with Vespa.
FIGURE S6 Exponential decay plots of M_{0.92}, M_{2.04}, M_{2.26}, and M_{2.99} from summed GM and WM spectra. The lines show the fit, whereas the scatter shows the quantified data points. The y-axis is reported in arbitrary units, and no conclusions should be drawn between GM and WM concentration differences.

FIGURE S7 The FWHMs for the singlets of the metabolite spectra (left) and individual MM peaks and tCr(CH_{2}) peak of the MM spectra (right) after correcting for the T2 component of corresponding metabolite/macromolecule, and the B0 components from NAA(CH_{3}) and tCr(CH_{2}), respectively. The metabolite FWHM of tCho+ and tCr(CH_{2}) reflects the multiple components contributing to the singlet peak. The crosses (X) indicate the calculations for the spectra summed across subjects, whereas the box plots show the per-subject fits. 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.

FIGURE S8 Calculated absolute concentrations for the metabolites for the different subjects (in mmol/L). The blue box plots show the concentrations when metabolite T2 correction is applied, while the green box plots show the concentrations without applying this correction factor. 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.

TABLE S1 Absolute concentrations for the metabolites (in mmol/L)

Note: Concentrations for this work are reported with the correction of the metabolite T2 values. The concentrations are all in good agreement with the millimolar concentrations reported by Penner et al (sequence and fitting configurations are also summarized).

\(^a\)Concentrations that are higher than the literature values (small changes).

\(^b\)Concentrations that are higher than the literature values (large changes).

\(^c\)The Scy concentration is marked, because it is possibly underestimated in this study. However, it is not clear whether the other literature used 1 or 6 proton resonances (^15NH) contributing to their Scy basis set simulation.

\(^d\)Metabolites for which the mean T2 relaxation time of all other metabolites was taken.

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