

## Errors in H-MRS estimates of brain metabolite concentrations caused by failing to take into account tissue-specific signal relaxation

Gasparovic, Charles; Chen, Hongji; Mullins, Paul G

### NMR in biomedicine

DOI:  
[10.1002/nbm.3914](https://doi.org/10.1002/nbm.3914)

Published: 01/06/2018

Peer reviewed version

[Cyswllt i'r cyhoeddiad / Link to publication](#)

*Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):*  
Gasparovic, C., Chen, H., & Mullins, P. G. (2018). Errors in H-MRS estimates of brain metabolite concentrations caused by failing to take into account tissue-specific signal relaxation. *NMR in biomedicine*, 31(6), e3914. <https://doi.org/10.1002/nbm.3914>

#### Hawliau Cyffredinol / General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

#### Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

# Errors in <sup>1</sup>H-MRS Estimates of Brain Metabolite Concentrations due to Ignoring Tissue-Specific Signal Relaxation

Charles Gasparovic<sup>†</sup>, Hongji Chen<sup>‡</sup> and Paul G. Mullins<sup>‡</sup>

Affiliations: <sup>†</sup>The Mind Research Network Albuquerque, NM, USA, <sup>‡</sup> Maryland Psychiatric Research Center, Department of Psychiatry, University of Maryland School of Medicine, MD, USA, <sup>‡</sup> School of Psychology, Bangor University, Gwynedd, United Kingdom

Corresponding author: Charles Gasparovic

[chuck@unm.edu](mailto:chuck@unm.edu)

Word count: 3988

Running Head: Correcting <sup>1</sup>H-MRS Brain Metabolite Estimates for Tissue Relaxation

## **Abstract**

Accurate measurement of brain metabolite concentrations with  $^1\text{H}$ -MRS can be problematic due to large voxels with mixed tissue composition, requiring adjustment for differing relaxation rates in each tissue if absolute concentration estimates are desired. Adjusting for tissue-specific metabolite signal relaxation, however, requires also knowing the relative gray (GM) and white matter (WM) concentrations of the metabolite within these compartments, which are not known *a priori*. Expressions for estimating the molality and molarity of brain metabolites with  $^1\text{H}$ -MRS are extended to account for tissue-specific relaxation of the metabolite signals and examined under different assumptions with simulated and real data. Though the modified equations have two unknowns and, hence, are unsolvable explicitly, they are nonetheless useful for estimating the effect of tissue-specific metabolite relaxation rates on concentration estimates under a range of assumptions and experimental parameters using simulated and real data. In simulated data using reported GM and WM  $T_1$  and  $T_2$  times for NAA at 3T and a hypothetical GM/WM NAA ratio, errors of 6.5-7.8% in concentrations resulted when TR was 1.5 s and TE was 0.144s, but reduced to less than 0.5% when TR was 6 s and TE was 0.006 s. In real data obtained at TR/TE=1.5/0.04s, the difference in the results (4%) was similar to that obtained with simulated data when assuming tissue-specific relaxation times rather than GM-WM averaged times. Using the expressions introduced in this report, these results can be extrapolated to any metabolite or set of assumptions regarding tissue-specific relaxation. Furthermore, while serving to bound the problem, this work underscores the challenge of correcting for relaxation effects given that relaxation times are generally not known and impractical to measure in most studies. To minimize such effects, the data should be acquired with pulse sequence parameters that minimize the effect of signal relaxation.

**Key Words:** brain, magnetic resonance spectroscopy, metabolite relaxation

## Introduction

Proton magnetic resonance spectroscopy (1H-MRS) in studies on the human brain requires relatively large voxels due to the low intensities of the metabolite signals. This poses a challenge for estimating metabolite concentrations in exclusively gray matter (GM) or white matter (WM) regions of interest (ROIs), since the voxels usually contain contributions from both GM and WM and, particularly in GM ROIs, cerebrospinal fluid (CSF). Several strategies exploiting spectroscopic imaging methods have been developed to estimate metabolite levels in pure GM or WM (1-7). However, these methods are not suitable to single voxel studies using conventional volume selection. The application of the many variations of these sequences, including popular spectral editing techniques that often require especially large voxels (8), by far dominates the use of 1H-MRS in studies on the brain.

Although mixed tissue contributions present a potential confound for interpreting single voxel 1H-MRS of the brain, determining the composition of the voxel by segmentation of a co-registered anatomical image allows correction for CSF, usually considered void of metabolites of interest at detectable levels, as well as the possibility of entering the tissue fraction of interest as a covariate in statistical analyses. Furthermore, if “absolute” concentrations are to be estimated, knowing the partial volume fractions allows the possibility of correcting for compartment-specific effects of relaxation on the observed signals (9-14). This is particularly important when using the water signal as a concentration standard since the water proton  $T_1$  and  $T_2$  relaxation times in GM, WM, and CSF differ markedly. Owing to the greater technical challenges of measuring the relaxation rates of metabolite signals (weak, overlapping, and often j-coupled), reports of their values have been inconsistent and, moreover, lacking for all metabolites of interest. However, when reported, the GM-WM differences have been small (reviewed by Di Costanzo et al. (15)). Consequently, these differences have been ignored in most studies reporting concentration estimates of neurometabolites.

A formula for estimating molal concentrations (moles/Kg of tissue water) of neurometabolites that incorporates the effects of compartment-specific relaxation on the water signal has been described (13). However, this formula does not account for tissue-specific effects of relaxation on metabolite signals but, instead, assumes that these rates are similar enough in GM and WM to incorporate them as a single factor. Here we extend the formula to include distinct

metabolite relaxation terms and propose a similar equation for concentration estimates based on molarity (moles/L tissue). To our knowledge, an expression that correctly accounts for GM- and WM-specific metabolite relaxation in concentration estimates has not been previously reported. The modified equation necessarily contains two unknown variables and, as such, is not solvable explicitly. Nonetheless, it is useful for examining the impact of differing GM and WM metabolite relaxation rates on the estimate of metabolite concentrations over a range of MRS parameters and assumptions.

## Theory

Molality is concentration expressed in moles of solute per Kg of solvent. In brain tissue the solvent is water. The advantages of measuring molality in brain 1H-MRS experiments have been discussed in prior publications (13,14), and include insensitivity to temperature, barometric pressure, and the contribution of other solutes or non-soluble material to the solution. If the 1H-MRS signals are acquire under fully relaxed conditions ( $TE \ll T_2$ ,  $TR \gg T_1$ ), the molality of the metabolite protons is directly proportional to the ratio of the metabolite signal intensity  $S_M$  to the water signal intensity  $S_{H_2O}$ , without assumptions about the water density in the tissue as is required with molarity. In the simplest case of a 1H-MRS voxel sampling only pure GM or pure WM molality is given by:

$$[M]_{\text{molal}} = \frac{S_M}{S_{H_2O}} \cdot \frac{2}{\#H_M} \cdot [H_2O]_{\text{molal}} \quad [1]$$

where  $[H_2O]_{\text{molal}}$  is the molality of pure water (55.49 moles/Kg water),  $\#H_M$  is the number of metabolite hydrogen atoms, 2 is the number of water protons, and both the water and metabolite fractions are assumed to be fully detected. If CSF is present in the voxel, the water signal is scaled by the tissue water mole fraction,  $f_{GM}$  or  $f_{WM}$ , or equivalently  $(1 - f_{CSF})$ , where  $f_{CSF}$  is the CSF water mole fraction.

If the signals were not acquired under fully relaxed conditions, they must also be scaled by the appropriate  $T_1$ - and  $T_2$ -weighted relaxation factors. If the initial pulse angle is  $90^\circ$  in a double spin echo or stimulated echo type of experiment with equal echo spacing, this factor is  $R_x = \exp[-TE/T_{2x,y}](1 - \exp[-TR/T_{1x,y}])$ , where the subscript x refers to either the metabolite or water signal and y refers to the compartment (GM, WM, or CSF). While different protons on a metabolite can

have different relaxation times, here we will assume that the times associated with the dominate peak approximately characterizes the entire signal (e.g. the N-acetyl peak of N-acetylaspartate). This is an important caveat, but one that could be avoided only with 1) knowledge of individual proton relaxation times which is, due to the difficulty of their measurement, lacking in the field, and 2) an accurate modeling of the total signal from each metabolite that takes into account any differing relaxation attenuation among its various spectral peaks. In the general case of partial volume contributions from GM, WM, and CSF, each with a compartment-specific water proton relaxation factor ( $R_{H2O\_GM}$ ,  $R_{H2O\_WM}$ ,  $R_{H2O\_CSF}$ ) but assuming a common GM and WM metabolite relaxation factor ( $R_M$ ), the molality is given by (13):

$$[M]_{\text{molal}} = \frac{S_{M\_obs} \cdot (f_{GM} \cdot R_{H2O\_GM} + f_{WM} \cdot R_{H2O\_WM} + f_{CSF} \cdot R_{H2O\_CSF})}{S_{H2O\_obs} \cdot (1 - f_{CSF}) \cdot R_M} \cdot \frac{2}{\#H_M} \cdot [H_2O]_{\text{molal}} \quad [2]$$

The fractions in Eq. [2] are not the volume fractions estimated by image segmentation, but the mole fractions of water. They can be related to the volume fractions by taking into account the relative water fraction in each segmentation fraction:

$$f_{GM} = \frac{f_{GM\_vol} \cdot d_{GM}}{f_{GM\_vol} \cdot d_{GM} + f_{WM\_vol} \cdot d_{WM} + f_{CSF\_vol} \cdot d_{CSF}} \quad [3]$$

$$f_{WM} = \frac{f_{WM\_vol} \cdot d_{WM}}{f_{GM\_vol} \cdot d_{GM} + f_{WM\_vol} \cdot d_{WM} + f_{CSF\_vol} \cdot d_{CSF}} \quad [4]$$

and

$$f_{CSF} = \frac{f_{CSF\_vol} \cdot d_{CSF}}{f_{GM\_vol} \cdot d_{GM} + f_{WM\_vol} \cdot d_{WM} + f_{CSF\_vol} \cdot d_{CSF}} \quad [5]$$

where  $f_{GM\_vol}$ ,  $f_{WM\_vol}$ , and  $f_{CSF\_vol}$  are the GM, WM, and CSF volume fractions, respectively, estimated by segmentation, and the density factors ( $d$ ) are the water densities associated with them.

Accounting for tissue-specific metabolite signal relaxation in the equation for molality is analogous to accounting for compartment-specific water signal relaxation (13). However, a critical difference is that while the water molal concentration is the same in all compartments (i.e., the

concentration of water in pure water, 55.49 moles/Kg water) the metabolite concentrations are not. The metabolite mole fractions are not simply determined by the volume fractions and water tissue densities but also by the different metabolite concentrations in the GM and WM water fractions. The metabolite mole fractions,  $f_{M\_GM}$  and  $f_{M\_WM}$ , are related to the metabolite concentrations in GM and WM,  $[M]_{GM}$  and  $[M]_{WM}$ , respectively, as

$$f_{M\_GM} = \frac{f_{GM} \cdot [M]_{GM}}{f_{GM} \cdot [M]_{GM} + f_{WM} \cdot [M]_{WM}} \quad [6]$$

and

$$f_{M\_WM} = \frac{f_{WM} \cdot [M]_{WM}}{f_{GM} \cdot [M]_{GM} + f_{WM} \cdot [M]_{WM}} \quad [7]$$

where the fractions  $f_{GM}$ ,  $f_{WM}$ , and  $f_{CSF}$  are the mole fractions of water given by Eqs. [3-5]. The observed metabolite signal is equal to the sum of the mole fractions of the fully relaxed signal  $S_{M\_R}$ , with each fractional signal weighted by its compartment-specific relaxation factor:

$$S_{M\_obs} = f_{M\_GM} \cdot S_{M\_R} \cdot R_{M\_GM} + f_{M\_WM} \cdot S_{M\_R} \cdot R_{M\_WM} \quad [8]$$

Here R has been added to the subscript  $S_M$  of Eq. [1] to emphasize that it is the total signal intensity (from all compartments) that would be observed under fully relaxed acquisition conditions. Solving for  $S_{M\_R}$  we obtain:

$$S_{M\_R} = \frac{S_{M\_obs}}{f_{M\_GM} \cdot R_{M\_GM} + f_{M\_WM} \cdot R_{M\_WM}} \quad [9]$$

To account for the GM and WM metabolite signal fractions that are weighted by different relaxation factors, the term  $R_M$  in Eq. [2] is substituted by the denominator of Eq. [9]. This term, which we label  $R_{M\_GM\_WM}$ , can be expanded in terms of Eqs. [3], [4], [6], and [7]. Further, the unknown GM and WM concentrations can be expressed as a single ratio to obtain:

$$R_{M\_GM\_WM} = \frac{f_{GM\_vol} \cdot d_{GM} \cdot ([M]_{GM}[M]_{WM}) \cdot R_{M\_GM} + f_{WM\_vol} \cdot d_{WM} \cdot R_{M\_WM}}{f_{GM\_vol} \cdot d_{GM} \cdot ([M]_{GM}[M]_{WM}) + f_{WM\_vol} \cdot d_{WM}} \quad [10]$$

Inserting this term into Eq. 2 we obtain:

$$[M]_{\text{molal}} = \frac{S_{M\_obs} \cdot (f_{GM} \cdot R_{H_2O\_GM} + f_{WM} \cdot R_{H_2O\_WM} + f_{CSF} \cdot R_{H_2O\_CSF})}{S_{H_2O\_obs} \cdot (1 - f_{CSF}) \cdot R_{M\_GM\_WM}} \cdot \frac{2}{\#H_M} \cdot [H_2O]_{\text{molal}} \quad [11]$$

Note that if the water and metabolite signals are acquired under fully relaxed conditions, Eq. [11] reduces to Eq. [1] with the water signal scaled by the tissue fraction, i.e.,  $[M]_{\text{molal}}$  is independent of the metabolite concentrations in each tissue. As with the water mole fractions, the metabolite mole fractions in the calculation of molality serve only to account for how much of the total metabolite signal is scaled by each compartment-specific relaxation factor. Hence, at very short TE and sufficiently long TR (when  $R \sim 1$ ), the observed signal is simply proportional to the number of moles of metabolite and tissue water in the voxel. Also, when the metabolite relaxation times are the same in GM and WM, Eq. [11] reduces to Eq. [2]. These results are expected, of course: under simplifying conditions, Eq. [11] must reduce to the simpler expressions. The validity of alternative strategies for including differing GM-WM metabolite relaxation rates in partial volume corrections (16,17) should also be evaluated with this requirement in mind.

An expression similar to Eq. [2] can be written for molarity (moles per volume of tissue) by taking into account that the fractional contribution to  $S_{H_2O}$  from each compartment is weighted not only by the compartment-specific relaxation terms when calculating moles per volume of tissue, but by the relative water densities in each compartment, as well. This effectively scales the molarity of water in pure water  $[H_2O]_{\text{molar}}$  (55.49 moles/L water) to the number of moles of water in the voxel volume. Again, starting with the simplest case of either pure GM or pure WM under fully relaxed conditions, molarity is given by:

$$[M]_{\text{molar}} = \frac{S_M}{S_{H_2O}} \cdot d_{\text{issue}} \cdot \frac{2}{\#H_M} \cdot [H_2O]_{\text{molar}} \quad [12]$$

In a voxel with a mixture of GM, WM, and CSF not sampled under fully relaxed conditions, the observed water signal  $S_{H_2O\_obs}$  is the sum of the volume fractions of the fully relaxed signal  $S_{H_2O\_R}$  from each compartment, with each fractional signal weighted by the relaxation factor and the water density of the compartment:

$$S_{H2O\_obs} = f_{GM\_vol} \cdot d_{GM} \cdot S_{H2O\_R} \cdot R_{H2O\_GM} + f_{WM\_vol} \cdot d_{WM} \cdot S_{H2O\_R} \cdot R_{H2O\_WM} + f_{CSF\_vol} \cdot d_{CSF} \cdot S_{H2O\_R} \cdot R_{H2O\_CSF}$$

[13]

As with the derivation of Eq. [2], we solve for  $S_{H2O\_R}$  and substitute the resulting expression for  $S_{H2O}$  in Eq. [12]. Assuming equal GM and WM metabolite signal relaxation times at this point, substituting  $S_{M\_obs}/R_M$  for  $S_M$ , and scaling  $S_{H2O}$  by the tissue volume fraction to correct for the CSF inclusion yields:

$$[M]_{molar} = \frac{S_{M\_obs} \cdot (f_{GM\_vol} \cdot d_{GM} \cdot R_{H2O\_GM} + f_{WM\_vol} \cdot d_{WM} \cdot R_{H2O\_WM} + f_{CSF\_vol} \cdot d_{CSF} \cdot R_{H2O\_CSF})}{S_{H2O\_obs} \cdot (1 - f_{CSF\_vol}) \cdot R_M} \cdot \frac{2}{\#H_M} \cdot [H_2O]_{molar}$$

[14]

where  $[H_2O]_{molar}$  is the molar concentration of pure water (55.49 moles/L).

To account for different metabolite signal relaxation rates in GM and WM, the term  $R_M$  is replaced by  $R_{M\_GM\_WM}$  to yield:

$$[M]_{molar} = \frac{S_{M\_obs} \cdot (f_{GM\_vol} \cdot d_{GM} \cdot R_{H2O\_GM} + f_{WM\_vol} \cdot d_{WM} \cdot R_{H2O\_WM} + f_{CSF\_vol} \cdot d_{CSF} \cdot R_{H2O\_CSF})}{S_{H2O\_obs} \cdot (1 - f_{CSF\_vol}) \cdot R_{M\_GM\_WM}} \cdot \frac{2}{\#H_M} \cdot [H_2O]_{molar}$$

[17]

## Methods

Equations [11] and [17] contain two unknown variables: the total tissue (GM+WM) metabolite concentration  $[M]$  and the ratio of GM to WM metabolite concentrations (inherent in  $R_{M\_GM\_WM}$ ). As such, they cannot be solved explicitly. However, approximations of the GM/WM metabolite ratio for various metabolites in healthy or in diseased tissue can be obtained from many published reports. In most of these studies, spectroscopic imaging data was used to estimate concentrations in several voxels with mixed GM, WM, and CSF content. Hypothetically pure GM and WM concentrations were estimated by regressing the concentrations against the fractional GM content of the voxel, normalized by the total tissue fraction, and extrapolating to fractions of 0 and

1 to estimate WM and GM concentrations, respectively(1). To demonstrate the impact of differing GM and WM metabolite  $T_1$  and  $T_2$  times on concentration estimates in the present report, we examine real and simulated spectroscopic imaging data, allowing us to display the effect over a range tissue compositions typically encountered in brain 1H-MRS.

Simulated data were generated with programs written with Matlab (version R2014A, The MathWorks Inc., [www.matlab.com](http://www.matlab.com)) by scaling hypothetical water and metabolite signals by the mole fractions and relaxation factors that would be associated with them in GM, WM, and CSF. The GM and WM molal concentrations assigned to the metabolite signal fractions were 18 and 15 moles/Kg tissue water, respectively, drawing from previous work (18), with each signal attenuated by relaxation factors based on  $T_1$  and  $T_2$  times previously reported for the N-acetyl protons of N-acetylaspartate (NAA) in GM and WM (19). (See the caption for Fig. 1 for more details.) To better mimic the situation of a slice transecting the inter-hemispheric fissure, an increasing fraction of CSF was included in the hypothetical voxels after the GM fraction exceeds the WM fraction, reaching a maximum of 15% when the WM is zero. In other words, voxels with no WM have 85% GM and 15% CSF, reflecting the common overlap of predominantly GM voxels with CSF spaces. Molal concentrations are plotted versus normalized GM mole fraction ( $f_{GM}/f_{GM+f_{WM}}$ ) and molar concentrations versus the normalized GM volume fraction ( $f_{GM\_vol}/f_{GM\_vol+f_{WM\_vol}}$ ).

NAA measurements were obtained from a healthy human subject in a previously reported study (18). Briefly, MRI anatomical and spectroscopic imaging data were acquired with a Siemens 3T Tim Trio scanner. 1H-MRS imaging was performed with a phase-encoded version of a double spin echo sequence, both with and without water presaturation (TE = 40 ms, TR = 1500 ms, slice thickness = 15mm, FOV = 220 x 220 mm, circular k-space sampling (radius = 12), total scan time = 582s). The MRS data were initially analyzed with LCModel (<http://s-provencher.com>) and then with Matlab programs that applied either Eqs. [11] or [17]. GM, WM, and CSF segmentation maps were generated from a  $T_1$ -weighted MPRAGE image using SPM5 (<http://www.fil.ion.ucl.ac.uk/spm>). The study was conducted with the approval of the Institutional Review Board of the University of New Mexico Health Sciences Center.

## Results

To illustrate the effect of differing GM and WM metabolite relaxation times, we show only the case of molal concentrations, since the term  $R_{M\_GM\_WM}$  is a scaling factor that will affect the

molarity and molality expressions similarly. To this end, the GM-WM averaged  $T_1$  and  $T_2$  metabolite times in Eq. [11] were replaced by the tissue-specific times reported in reference 19 ( $T_{1GM\_NAA} = 1.47$  s,  $T_{1WM\_NAA} = 1.35$  s,  $T_{2GM\_NAA} = 247$  ms,  $T_{2WM\_NAA} = 295$  ms). Data were simulated for 5 sets of TR and TE times: TE/TR = 0.006/3 s, TE/TR = 0.006/2 s, TE/TR = 0.006/1.5 s, TE/TR = 0.020/1.5 s, and TE/TR = 0.040/1.5 s. For comparison, data were also simulated with a single average  $T_1$  and  $T_2$  for both GM and WM. The plots of these data are shown in Fig. 1. Along the bold line are overlaid the plots of data corrected with Eq. [11] using the correct relaxation times across the range of TE and TR, yielding the correct results in each case. The thinner lines that deviate from the bold line illustrate the errors that arise when using the same averaged  $T_1$  and  $T_2$  values for both GM and WM signals. As expected, the error increases at longer TE and shorter TR and with a greater fraction of either WM or GM. This error ranged from approximately 1.6% (TE/TR = 0.006 s/3 s) to 3.7% (TE/TR = 0.040 s/1.5 s) in the estimate of the concentration in either pure WM or GM. This trend will continue, of course, as TE lengthens and/or TR becomes shorter, which can be verified simply by examining the case of either a pure GM or a pure WM voxel. For example, assuming the relaxations times used here for the example of NAA and a TR of 1.5 s and a TE of 144 ms, adjustment with a single tissue-averaged metabolite relaxation term would produce an estimate of pure GM NAA concentration that would be 7.8% lower than the estimate using tissue-specific relaxation times. In pure WM, this estimate would be 6.5% higher than the estimate using tissue-specific relaxation times. At TE = 0.006 s and TR = 6 s, on other hand, the maximum errors will be less than 0.5%.

Estimating absolute metabolite concentrations while accounting for compartment-specific GM and WM signal relaxation times requires an assumption about the GM/WM metabolite ratio in order to assign each fraction of the total metabolite signal to its distinct relaxation factor. The dotted line in Fig. 2 illustrates the error in the concentration estimate when there is an error in this ratio. For the representative case shown, the corrected data were simulated with TE=40 ms and TR=1.5 s. The dotted line shows the deviation from the correct concentrations when the GM/WM metabolite ratio is assumed to be 1.5 rather than the correct ratio of 1.2 (18/15). The errors in the concentrations will depend not only on the error in the ratio but on the tissue composition and assumed relaxation times. The error will be zero for voxels containing only WM or only GM and CSF and will be maximal at an intermediate mix of GM and WM. For the example shown the maximum error was less than 0.5%.

\*\*\*Fig. 2 appears near here\*\*\*

Illustrated in Fig. 2A is the application of Eqs. [11] and [17] to NAA measurements obtained from a healthy human subject with TE=40 ms and TR=1.5 s and corrected using the averaged  $T_1$  and  $T_2$  values for GM and WM used in the simulations. The regression plots of the corrected NAA concentrations versus normalized GM fraction demonstrate the basic differences between molarity and molality illustrated by the simulated data.

In Fig. 2B, the same raw data are plotted after correction with Eq. [11] assuming different metabolite signal  $T_1$  and  $T_2$  values in GM and WM (the same values used in the simulation) and a GM/WM NAA concentration ratio of 1.3. The latter factor was derived from the regression involving the data corrected with the same, averaged  $T_1$  and  $T_2$  times for both GM and WM (shown in Fig. 2A). Notable is the lower WM and higher GM extrapolated endpoints of the regression relative to the endpoints of the regression involving the molal data shown in Fig. 2A. The “100% WM” endpoint is 3.7% lower and the “100% GM” endpoint is 3.8% higher than these values when assuming single, averaged  $T_1$  and  $T_2$  values in both tissues, resulting in a higher GM/WM metabolite concentration ratio estimate of 1.4. This is similar to the results obtained in the simulated data analysis when the data were generated with TE=40 ms and TR=1.5 s and tissue-specific relaxation times were used rather than tissue-averaged times (bold line versus thin line for TE/TR = 0.04/1.5s in Fig. 1). The sum of the square residuals of the linear regression shown in Fig. 2A was within 0.1% of the sum of the square residuals of the linear regression shown in Fig. 2B. Hence, no improvement in the fit of the data to a straight line was observed when using tissue-specific relaxation factors to adjust the data in this case. To explore the possibility of converging toward a stable value for the metabolite ratio with an iterative application of Eq. [11], the analysis was repeated assuming a GM/WM metabolite ratio of 1.4, but without a substantial change (<0.1%) in the results.

## Discussion

The reliable estimation of neurometabolite concentrations by  $^1\text{H-MRS}$  is faced with numerous challenges (20), not the least of which is the heterogeneity of the tissue within the voxel. To simplify this problem, it is generally assumed that the sampled brain region is composed of only three compartments - GM, WM, and CSF - and, at times, a fourth compartment encompassing an MRI-detectable lesion. Each of these compartments is likely to have not only a different metabolite concentration and water density, but also different relaxation times associated with the signals. In a

previous report, we presented Eq. [2] for adjusting molal concentration estimates for partial volume and water signal relaxation effects (13). We also examined the impact of errors arising from estimates of the relative fractions of GM, WM, and CSF in the voxel (segmentation errors) as well as errors in the water signal relaxation times associated with those fractions. In the present report, we extend Eq. [2], along with a similar equation for estimating molarity, to account for tissue-specific metabolite signal relaxation. To our knowledge, this is the first valid expression presented for accomplishing this. In view of alternative methods that have been proposed for performing this correction (16,17), it is worth emphasizing that scaling only the GM and WM volume or water fractions by the distinct metabolite relaxation factors is not equivalent to scaling the GM and WM metabolite signal fractions by these factors. In order to calculate the size of the latter signal fractions, the relative metabolite concentrations in GM and WM must be known or assumed. Furthermore, any valid expression for correcting for metabolite signal relaxation differences must reduce to the simpler valid equations under simplifying conditions, such as when the observed signals are fully relaxed or when there is only a single tissue type in the voxel.

We analyzed the case of just one metabolite (NAA) at a field strength of 3T in this work, assuming relaxation times previously reported for that metabolite at 3T. These results, of course, can be extended to any metabolite under different sets of assumptions with respect to partial volume fractions, tissue concentrations, relaxation rates, and pulse parameters: the general trends in errors resulting from ignoring differences in tissue specific relaxation rates, or assuming inaccurate values, will be the same. Since the principle factor underlying these errors will be the difference in GM and WM metabolite relaxation times, knowledge of these times is essential to correcting for them accurately. Unfortunately, such information is lacking for most metabolites across the wide range of magnetic field strengths currently in use for brain studies, and few studies have attempted to measure the relaxation times of the various distinct proton signals that make up a metabolite spectrum. Adding to this uncertainty, metabolite relaxation times have been shown to vary with age, pathology, and brain region (21-24; and, given that metabolite  $T_2$  times appear to shorten with increasing field strength (25-29) while  $T_1$  times may lengthen (28,29), the sensitivity to TE and TR times is expected to increase at higher fields.

In the case of NAA at 3T, we estimate that when tissue-averaged relaxation times are used for GM and WM instead of tissue-specific relaxation times, errors of a few percent arise over the range of representative TE and TR values examined. These errors, of course, are maximal in pure

GM and pure WM voxels and could be predicted based on the differing GM and WM relaxation rates alone. However, in voxels with mixed tissue composition, the full expression (Eq. 11) is needed to estimate the effects of tissue-specific relaxation. This entails that the relative metabolite concentrations in each compartment are assumed since, when dealing with real data, they are not known *a priori*. Reasonable assumptions about GM and WM metabolite concentrations might be obtained from prior estimates, for example, from multi-voxel studies in which the fractional signals were assumed to relax with common  $T_1$  and  $T_2$  times. In the present report, we adjusted NAA spectroscopic imaging data with different GM and WM NAA signal relaxation times using Eq. [11] and a GM/WM NAA ratio obtained from a prior analysis in which equivalent tissue-averaged GM and WM relaxation times were assumed. Repeating the tissue-specific analysis with the concentration ratio obtained in the prior analysis did not substantially alter the results (<0.1%). We note that this approach would not be feasible in single voxel studies; and, hence, assumptions about the GM/WM concentration ratio for any metabolite would be an important caveat in the interpretation of the results, particularly in studies on pathological tissue or differing regions of the brain. Though the errors in GM or WM metabolite concentration estimates are small in the simulated and real data analyses of this report, they nonetheless introduced a measurable analysis bias in the results.

To address the issue of tissue-specific water signal relaxation, a method was proposed to measure brain water signal relaxation and density differences on a voxel-by-voxel basis by acquiring *in situ* water relaxation and density maps along with  $^1\text{H}$ -MRS imaging data (31). However, while reliably measuring the relaxation rates of the large water signal can be accomplished with straightforward imaging techniques, *in situ* GM and WM metabolite signal relaxation measurements are prohibitively time-consuming, technically challenging and, hence, impractical in a clinical setting. Given this impracticality, the most straightforward approach for improving the accuracy of absolute concentration estimates is simply to use acquisition parameters that minimize the impact of signal relaxation as much as possible, a remedy that has long been recommended to improve the accuracy of MRS concentration estimates. Minimizing  $T_2$  effects has become feasible with the advent of ultra-short-TE sequences (24,32,33), while acquiring the water signal with a small pulse angle (24) reduces the impact of water signal  $T_1$  differences. Small pulse angles, unfortunately, are not practical for the acquisition of metabolite signals, and lengthening TR comes at a cost increasing the total acquisition time. For the example of the NAA signal and assumed relaxation times at  $3T$  used in this report, we estimate the errors to be as large as 8%

when TE= 144 ms and TR=1.5 s but less than 1% when tissue-averaged rather than tissue-specific relaxations are used for partial volume correction of data acquired with TE= 6 ms and TR=6 s.

In summary, the brain tissue milieu sampled by a typical MRS voxel is complex, conferring different MR properties on the detected signals depending on the cellular or extracellular compartment of origin. In order to interpret these signals in a practical manner, simplifying assumptions need to be made. In this report we present an extension of our earlier expression for correcting for partial volume effects in <sup>1</sup>H-MRS data from the brain assuming three compartments: GM, WM, and CSF. The fuller expression accounts for tissue-specific metabolite signal relaxation and requires assumptions not only with respect to the relaxation times, but with respect to the relative metabolite concentrations in GM and WM. With simulated data, we demonstrate the magnitude of the errors that arise when there are differences in GM and WM metabolite signal relaxation times that are not taken into account or when incorrect assumptions about the GM/WM concentration ratio are made. Given the substantial technical challenges of reliably measuring metabolite signal relaxation *in situ*, the simplest approach to reducing these errors is to acquire the data with pulse sequence parameters that minimize the effect of relaxation on the signal.

## References

1. Hetherington HP, Pan JW, Mason GF, Adams D, Vaughn MJ, Twieg DB, Pohost GM. Quantitative H-1 spectroscopic imaging of human brain at 4.1 T using image segmentation. *Magn Reson Med* 1996;36(1):21-29.
2. Tal A, Kirov, II, Grossman RI, Gonen O. The role of gray and white matter segmentation in quantitative proton MR spectroscopic imaging. *NMR Biomed* 2012;25(12):1392-1400.
3. Mandl RC, van den Heuvel MP, Klomp DW, Boer VO, Siero JC, Luijten PR, Hulshoff Pol HE. Tract-based magnetic resonance spectroscopy of the cingulum bundles at 7 T. *Hum Brain Mapp* 2012;33(7):1503-1511.
4. Nuzillard D, Bourg S, Nuzillard J. Model-free analysis of mixtures by NMR using blind source separation. *J Magn Reson* 1998;133(2):358-363.
5. Ladroue C, Howe FA, Griffiths JR, Tate AR. Independent component analysis for automated decomposition of in vivo magnetic resonance spectra. *Magn Reson Med* 2003;50(4):697-703.
6. Sajda P, Du S, Brown TR, Stoyanova R, Shungu DC, Mao X, Parra LC. Nonnegative matrix factorization for rapid recovery of constituent spectra in magnetic resonance chemical shift imaging of the brain. *IEEE Trans Med Imaging* 2004;23(12):1453-1465.
7. Lee P, Adany P, Choi IY. Imaging based magnetic resonance spectroscopy (MRS) localization for quantitative neurochemical analysis and cerebral metabolism studies. *Anal Biochem.* 2017 Jul 15;529:40-47.

8. Harris AD, Saleh MG, Edden RA. Edited 1 H magnetic resonance spectroscopy in vivo: Methods and metabolites. *Magn Reson Med*. 2017 Apr;77(4):1377-1389.
9. Ernst T, Kreis R, Ross BD. Absolute quantitation of water and metabolites in the human brain. I. compartments and water. *J Magn Reson* 1993;102:1-8.
10. Barker PB, Soher BJ, Blackband SJ, Chatham JC, Mathews VP, Bryan RN. Quantitation of proton NMR spectra of the human brain using tissue water as an internal concentration reference. *NMR Biomed* 1993;6(1):89-94.
11. Michaelis T, Merboldt KD, Bruhn H, Hanicke W, Frahm J. Absolute concentrations of metabolites in the adult human brain in vivo: quantification of localized proton MR spectra. *Radiology* 1993;187(1):219-227.
12. Christiansen P, Henriksen O, Stubgaard M, Gideon P, Larsson HB. In vivo quantification of brain metabolites by 1H-MRS using water as an internal standard. *Magnetic Resonance Imaging* 1993;11(1):107-118.
13. Gasparovic C, Song T, Devier D, Bockholt HJ, Caprihan A, Mullins PG, Posse S, Jung RE, Morrison LA. Use of tissue water as a concentration reference for proton spectroscopic imaging. *Magn Reson Med* 2006;55(6):1219-1226.
14. Knight-Scott J, Haley AP, Rossmiller SR, Farace E, Mai VM, Christopher JM, Manning CA, Simnad VI, Siragy HM. Molality as a unit of measure for expressing 1H MRS brain metabolite concentrations in vivo. *Magnetic Resonance Imaging* 2003;21(7):787-797.
15. Di Costanzo A, Trojsi F, Tosetti M, Schirmer T, Lechner SM, Papolizio T, Scarabino T. Proton MR spectroscopy of the brain at 3 T: an update. *Eur Radiol* 2007;17(7):1651-1662.
16. Gussew A, Erdtel M, Hiepe P, Rzanny R, Reichenbach JR. Absolute quantitation of brain metabolites with respect to heterogeneous tissue compositions in (1)H-MR spectroscopic volumes. *Magma* 2012;25(5):321-333.
17. Rupsingh R, Borrie M, Smith M, Wells JL, Bartha R. Reduced hippocampal glutamate in Alzheimer disease. *Neurobiol Aging* 2011;32(5):802-810.
18. Gasparovic C, Bedrick EJ, Mayer AR, Yeo RA, Chen H, Damaraju E, Calhoun VD, Jung RE. Test-retest reliability and reproducibility of short-echo-time spectroscopic imaging of human brain at 3T. *Magn Reson Med* 2011;66(2):324-332.
19. Mlynarik V, Gruber S, Moser E. Proton T (1) and T (2) relaxation times of human brain metabolites at 3 Tesla. *NMR Biomed* 2001;14(5):325-331.
20. Kreis R. Issues of spectral quality in clinical 1H-magnetic resonance spectroscopy and a gallery of artifacts. *NMR Biomed* 2004;17(6):361-381.
21. Kirov, II, Fleysher L, Fleysher R, Patil V, Liu S, Gonen O. Age dependence of regional proton metabolites T2 relaxation times in the human brain at 3 T. *Magn Reson Med* 2008;60(4):790-795.
22. Traber F, Block W, Lamerichs R, Gieseke J, Schild HH. 1H metabolite relaxation times at 3.0 tesla: Measurements of T1 and T2 values in normal brain and determination of regional differences in transverse relaxation. *Journal of Magnetic Resonance Imaging : JMRI* 2004;19(5):537-545.
23. Marjanska M, Emir UE, Deelchand DK, Terpstra M. Faster metabolite (1)H transverse relaxation in the elder human brain. *PLoS One* 2013;8(10):e77572.
24. Ding XQ, Maudsley AA, Sabati M, Sheriff S, Dellani PR, Lanfermann H. Reproducibility and reliability of short-TE whole-brain MR spectroscopic imaging of human brain at 3T. *Magn Reson Med* 2015;73(3):921-928.
25. Michaeli S, Garwood M, Zhu XH, DelaBarre L, Andersen P, Adriany G, Merkle H, Ugurbil K, Chen W. Proton T2 relaxation study of water, N-acetylaspartate, and creatine in human brain using Hahn and Carr-Purcell spin echoes at 4T and 7T. *Magn Reson Med* 2002;47:629-633.
26. Otazo R, Mueller B, Ugurbil K, Wald L, Posse S. Signal-to-noise ratio and spectral linewidth

- improvements between 1.5 and 7 Tesla in proton echo-planar spectroscopic imaging. *Magn Reson Med* 2006;56:1200–1210.
27. Jiru F, Skoch A, Wagnerova D, Dezortova M, Viskova J, Profant O, Syka J, Hajek M. The age dependence of T2 relaxation times of N-acetyl aspartate, creatine and choline in the human brain at 3 and 4T. *NMR Biomed.* 2016 Mar;29(3):284-92.
  28. 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 Aug;56(2):386-94.
  29. Lopez-Kolkovskiy 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 Feb;75(2):503-14.
  30. 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 Apr;69(4):931-6.
  31. Gasparovic C, Neeb H, Feis DL, Damaraju E, Chen H, Doty MJ, South DM, Mullins PG, Bockholt HJ, Shah NJ. Quantitative spectroscopic imaging with in situ measurements of tissue water T1, T2, and density. *Magn Reson Med* 2009;62(3):583-590.
  32. Seeger U, Klose U, Seitz D, Nagele T, Lutz O, Grodd W. Proton spectroscopy of human brain with very short echo time using high gradient amplitudes. *Magnetic Resonance Imaging* 1998;16(1):55-62.
  33. Knight-Scott J, Shanbhag DD, Dunham SA. A phase rotation scheme for achieving very short echo times with localized stimulated echo spectroscopy. *Magnetic Resonance Imaging* 2005;23(8):871-876.

## Figure Captions

**Figure 1.** Errors due to assuming average  $T_1$  and  $T_2$  values for the metabolite averaged across GM and WM (thin solid lines) rather than using distinct tissue specific relaxation values (thick line). Metabolite axis ( $[M]$ ) units are moles/Kg water and x-axis units are normalized gray matter water mole fractions ( $f_{GM}/f_{GM}+f_{WM}$ ). We assume that the water signal is characterized by the following parameters: CSF  $T_1/T_2 = 4 \text{ s}/2.55 \text{ s}$ ; GM  $T_1/T_2 = 1.304 \text{ s}/0.11 \text{ s}$ ; WM  $T_1/T_2 = 0.830/0.080 \text{ s}$  (specific sources cited in reference 18); CSF/GM/WM water density = 0.97/0.78/0.65 (9). Raw data were simulated with the metabolite GM  $T_1/T_2 = 1.47 \text{ s}/0.247 \text{ s}$  and the metabolite WM  $T_1/T_2 = 1.35 \text{ s}/0.295 \text{ s}$ , reported for the N-acetyl protons of NAA (19). Eq. [11] was used to adjust the raw data, assuming either the latter tissue-specific  $T_1$  and  $T_2$  values or GM-WM averaged  $T_1$  and  $T_2$  values ( $T_1 = 1.41 \text{ s}$  and  $T_2 = 271 \text{ ms}$  for both GM and WM). This was done for a range of TEs and TRs: 0.04 s/1.5 s, 0.02 s/1.5 s, 0.006 s/1.5 s, 0.006 s/2.0 s, 0.006 s/3.0 s. The thick line terminating at 15

moles/Kg tissue water and 18 moles/Kg tissue water is the plot of the adjusted data when the tissue-specific values are used in Eq. [11] for any of the tested TE and TR combinations (i.e., lines for all 5 sets overlap). The thinner lines show increasing deviations from the thick (correct) line with increasing TE or decreasing TR (i.e., maximum deviation with TE/TR= 0.04 s/1.5 s, minimum deviation for with TE/TR= 0.006 s/3.0 s).

**Figure 2.** Raw Data simulated with GM/WM metabolite ratio equal to 1.2 and adjusted assuming either a ratio 1.2 (solid line) or a incorrect ratio of 1.5 (dotted line).

**Figure 3. A.** 1H-MRS imaging NAA data from a supraventricular slab in the brain of a healthy subject. Circles: Data before adjustment for partial volume and relaxation effects. Diamonds: Molal concentrations (moles/Kg tissue water) adjusted with Eq. [2]. See text for details. **B.** Molal concentrations based on data displayed in Fig 2A. adjusted with Eq. [11] for distinct GM and WM relaxation times. See text for details.