

● *Original Contribution*

ANALYSIS OF 1.5 TESLA PROTON MR SPECTRA OF HUMAN BRAIN USING LCMODEL AND AN IMPORTED BASIS SET

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Automated analysis of 1.5 Tesla proton mass MR spectra using the LCModel program with basis sets obtained at other sites is expected to become more widespread, as such basis sets are now generally available. A calibration procedure to estimate absolute concentrations with such imported basis sets is suggested and the implications for differential T_2 attenuation are discussed. Based on STEAM localized spectra from parietal gray ($n = 51$) and white matter ($n = 43$), of which 28 (18 rsp) were quantified, the evaluation of 30 ms echo time (TE) spectra was validated against published results that were obtained at 2 Tesla and 20 ms TE. Good agreement for both absolute concentrations and metabolite ratios confirmed the usefulness of LCModel analysis with an imported basis set. However, in white matter, glutamine tended to be overestimated, and was assigned either to signal-to-noise depending baseline effects or the use of choline. Mutual interdependence of metabolites inherent to LCModel analysis is discussed in detail. © 1999 Elsevier Science Inc.

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INTRODUCTION

Fully automated methods for the analysis of localized proton magnetic resonance (MR) spectra have been developed in order to provide time efficient and operator independent routines for the evaluation of metabolite concentrations.^{1,2} The majority of analysis methods use line fitting methods^{2–5} and thus involve assumptions on the line shape. These methods may also suffer from systematic errors at short echo times (TE), where strong coupling, spectral overlap, and baseline contributions from unresolved resonances are most severe.

In contrast, the LCModel program¹ fits a linear combination of single metabolite signals with an arbitrary line shape under the constraints of a common baseline. By employing the whole spectral pattern of each metabolite over a specified spectral range, maximum information and uniqueness are incorporated into the analysis. Thus, spectral overlap, patterns of strong coupling, and fractional proton numbers are automatically accounted for. LCModel is especially useful for short TE and metabolites characterized by strongly coupled resonances,

namely *myo*-inositol, glutamate and glutamine. Flexibility of the analysis regarding number and type of metabolites, field strength, localization method, and sequence parameters can be achieved by acquiring a specific basis set under identical physical conditions. In this respect LCModel is relying on experimental data rather than on theoretical assumptions.

As the acquisition of a basis set is time consuming and costly, and may itself introduce experimental errors, a number of manufacturers are now providing ready-to-use basis sets for the standard MR spectroscopy protocols of their scanners. As the majority of clinical users will adhere to standard experimental paradigms, the use of LCModel with imported basis sets is expected to become widespread. This will support the exchange of results between different clinical sites.

So far, applications of LCModel have been reported for field strengths of 2.0 Tesla or higher using basis sets that were acquired in-house on the same scanner.^{1,5,6} In this case, basis set and in vivo data are both subject to the specific sensitivity and system units of the scanner. Using

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metabolite libraries imported from other sites, concentration ratios are readily available, but absolute quantitation requires a calibration on a standard of known concentration and a suitable correction for sensitivity changes.

The purpose of this work is, therefore, twofold: First, a calibration procedure is suggested, by which a common scaling factor is determined, and the systematic T_2 errors are described in contrast to the simpler case of using an in-house basis. Second, the imported basis set of localized STimulated Echo Acquisition Mode (STEAM)⁷ spectra at $TE = 30$ ms and 1.5 T is to be validated against results that have been acquired at the more favorable conditions of 2 Tesla and $TE 20$ ms. Practical aspects of reliability and mutual interdependence inherent to the analysis will be discussed in detail.

THEORY

Absolute Quantification

The signal S induced in the receive coil by an arbitrary transverse magnetization M_{tr} may vary with examination and location of the volume of interest (VOI) due to changing coil load and inhomogeneities of the radio frequency (rf) field. Changes of the sensitivity, i.e., the signal induced by unit magnetization, must be corrected before LCModel compares the in vivo spectrum to spectra of known concentration. The basis set provides not only the resonance patterns of the metabolite spectra, but serves also as a concentration standard. This, however, applies only for in-house acquired basis sets. Imported basis sets need an extra calibration.

The sensitivity changes due to the reciprocity between excitation and reception in a transmit/receive coil have been comprehensively described for single volume MRS.⁸ In case the transmitter gain (TG) is available instead of the peak voltage of the rf pulses,⁸ TG may be used to correct for sensitivity changes after conversion from logarithmic (decibel) units to linear (amplitude) scale:

$$S_{corr} = S \cdot TG(x)/V \quad (1)$$

$TG(x)$ denotes a possible spatial dependence of the sensitivity and may be determined by finding the maximum of the localized signal.⁹ After the correction by Eq. (1) S_{corr} remains proportional to V_{coil} impedance Z , that is why a constant Z (i.e. tuning and matching of the rf coil) is implicitly assumed.⁸

The calibration phantom, however, may load the rf coil much less than a normal head and may change Z , if

the rf coil is not tuned properly. This may alter the sensitivity of the calibration experiment, and introduce systematic shifts of the estimated concentrations. Z may be monitored by the signal S_{ext} from a reference VOI V_{ext} in an external phantom. Because the external phantom must be placed next to the head (or the calibration phantom), the sensitivity at the fringes of the rf field may be considerably lower and should be corrected by Eq. (1):

$$S_{ext} \cdot TG_{ext}/V_{ext} \propto Z \quad (2)$$

At the same time other changes of the reciprocity between S and TG are monitored as well. These may arise from the wearing of the rf amplifier tube or the change of rf hardware components. Combining Eqs. (1) and (2) yields the corrected signal independent of the local rf field and the changes of the reciprocity:

$$S_{corr} = \frac{S \cdot TG(x)/V}{S_{ext} \cdot TG_{ext}/V_{ext}} \propto M_{tr} \propto \text{concentration} \quad (3)$$

Arbitrary system units of S cancel by division, as does the arbitrary factor introduced by the conversion of TG to linear scale. Because the volume V of the VOI is a product of three slice profiles, it may differ from the nominal value. If the reference VOI is selected with the same sequence, slice profile weighting can also be eliminated by applying Eq. (3).

T_2 Dependence of Estimated Metabolite Concentrations

The STEAM signal of time parameters TE , TM (middle interval), and TR (repetition time) is further weighted by the relaxation times T_1 and T_2 , where the term in square brackets describes the dynamic steady state. At short TM and a long recovery delay $TR - TM - TE/2$ the T_1 dependent terms in Eq. (4) impose negligible relaxation weighting. In this study the T_1 signal loss is about 1% during TM and 1–3% saturation due to almost full relaxation.¹⁰

Measurement of T_2 relaxation is time consuming and difficult for coupled resonances, where TE and TM determine also the evolution and transfer of magnetization. Individual T_2 determination is not regarded as practical in patient studies for reasons of measuring time, although pathologic changes may occur. Moreover, it ought to be considered that T_2 corrected concentrations involve an extrapolation to zero TE and will only be needed to

$$\left[1 - \exp\left(-\frac{TR - TM - TE/2}{T_1}\right) \right] \cdot \exp\left(-\frac{TM}{T_1}\right) \cdot \exp\left(-\frac{TE}{T_2}\right) \quad (4)$$

compare spin-echo based localization with other methods, i.e., high resolution MRS or biochemical methods. Minimizing (instead of correcting) systematic T_2 errors seems to be an acceptable strategy. LCModel compares the metabolite signal of the model solution to the *in vivo* experiment, both of which appear weighted by relaxation.

1. When using an in-house basis set, the concentration estimate $\text{conc}(A)$ of each metabolite A is weighted by the difference of the transverse relaxation rates *in vivo* and *in vitro*:¹⁰

$$\text{conc}(A) \propto \exp\left(-TE \cdot \left(\frac{1}{t_{2,\text{vivo}}(A)} - \frac{1}{T_{2,\text{mod}}(A)}\right)\right) \quad (5)$$

$$\frac{\text{conc}(A)}{\text{conc}(B)} \propto \exp\left(-TE \cdot \left(\frac{1}{t_{2,\text{vivo}}(A)} - \frac{1}{t_{2,\text{mod}}(A)} - \frac{1}{T_{2,\text{vivo}}(B)} + \frac{1}{T_{2,\text{mod}}(B)}\right)\right) \quad (6)$$

3. If an imported basis set is calibrated by measuring a metabolite C in a phantom with known concentration and $T_{2,\text{cali}}(C)$, the T_2 weighting takes a similar form:

$$\text{conc}(A) \propto \exp\left(-TE \cdot \left(\frac{1}{t_{2,\text{vivo}}(A)} - \frac{1}{t_{2,\text{mod}}(A)} - \frac{1}{T_{2,\text{cali}}(C)} + \frac{1}{T_{2,\text{mod}}(C)}\right)\right) \quad (7)$$

As discussed with regard to Eq. (6), the T_2 of the calibration phantom should be chosen close to the *in vivo* value to partially cancel the unavoidable T_2 attenuation. Of note, the T_2 error can be made smaller than in Eq. (5), if $1/T_{2,\text{vivo}}(A)$ and $1/T_{2,\text{cali}}(C)$, as well as $1/T_{2,\text{mod}}(A)$ and $1/T_{2,\text{mod}}(C)$ cancel each other well enough.

MATERIALS AND METHODS

Experimental Protocol

Healthy adult subjects of an age range between 20 and 42 years were examined on a 1.5 Tesla clinical MR system (Signa Advantage, General Electric Medical Systems, Milwaukee, WI) using the standard quadrature transmit/receive birdcage head coil.

T_1 -weighted 3D SPOiled GRAdient echo (SPGR) images ($TR/TE/\alpha = 15 \text{ ms}/4.8 \text{ ms}/20^\circ$) in coronal view were used to adjust the VOIs to anatomy to minimize partial volume contributions. The MR signal was selected with the standard STEAM spectroscopy sequence that has been described in detail.² Sequence time parameters were $TR/TE/TM = 6000/30/13.7 \text{ ms}$. A single scan of the unsuppressed water signal was acquired for eddy current correction¹¹ of the 64 averaged water suppressed scans. The bandwidth of the water suppression pulses has been reduced to 40 Hz to avoid distortions of the 3.96 ppm resonance of creatine. Shimming on the localized water signal and adjustment of the water suppression was done manually. Inversion of the residual water was

The error imposed by “differential T_2 attenuation” will be smaller than $\exp(-TE/T_{2,\text{vivo}}(A))$, that is the error of the uncorrected *in vivo* spectrum analyzed with a model spectrum corrected for its T_2 attenuation $\exp(-TE/T_{2,\text{mod}}(A))$.

2. Concentration ratios of metabolites A and B are characterized by differences of differences of relaxation rates, so that the shorter *in vivo* T_2 times of metabolites A and B may mainly cancel each other as do the longer T_2 of the model solutions:

avoided, since the resulting bend of the baseline is difficult to control and, thus, adds a moment of inaccuracy to the results.

The quantitation protocol⁹ included two series of unsuppressed water scans: First TG was varied through six values between 40 and 115 to determine $TG(x)$. Then, a series of 8 scans was acquired at echo times between 30 and 1500 ms for T_2 based VOI segmentation into tissue and cerebrospinal fluid (CSF). The amplitude of the long T_2 component s_{CSF} was expressed relative to 100% water and used as an estimate for partial CSF volume.^{3,4,12} The reference was acquired as a TG series from a cubic VOI of 17 mm side in a water-filled glass sphere mounted on the coil axis behind the subject’s head. The external phantom was moved to the isocenter of the B_0 field and specific shim values were used to achieve reproducible RF and B_0 conditions. The repetition time between two single scans was at least 15 s, satisfying full relaxation *in vivo* and in the phantoms.

LCModel Analysis

The water suppressed time domain data were analyzed in the ppm region between 1.0 ppm and 4.0 ppm without further T_2 correction on a Sparc Server 1000 (Sun Microsystems) using the `ge_steam30_95a.basis` (supplied by S. W. Provencher). The basis set was acquired according to the recommendations in the LCModel User’s Manual and then scaled to a consistent TG.

The same type of 400 mL plastic spheres and an excess of potassium phosphate buffer was used (R. E. Hurd, personal communication). Consistent sensitivity of the basis set can be assumed due to identical coil loading.

The basis set consisted of 16 metabolites: *N*-acetyl-aspartate (NAA), creatine (Cre), choline (Cho), *myo*-inositol (*myo*-Ins), lactate (Lac), alanine (Ala), glutamate (Glu), glutamine (Gln), NAA-glutamate (NAAG), aspartate (Asp), glucose (Glc), taurine (Tau), citrate (Cit). The singlets of *scy*-inositol (*scy*-Ins) and glycine (Gly) were mimicked by shifting the acetate resonance (¹³ and LC-Model User's Manual). All model spectra were essentially free of baseline distortions in the chemical shift range between 0.1 ppm and 4.5 ppm.

Eq. (3) was used to correct the individual sensitivity of both the calibration (denoted by the superscript "cali") and the in vivo experiment. The common factor C was calibrated on a manufacturer supplied brain MRS phantom. It contained 12.5 millimoles/liter (mM) NAA, 10 mM Cre, 3 mM Cho, 7.5 mM *myo*-Ins, 5 mM Lac, and 12.5 mM Glu, besides 20 mM trimethyl-phosphoric acid (TMPA) for technical purposes. Coil loading, concentrations and relaxation times roughly match in vivo conditions. All corrections (except the VOI sizes) were combined in the TRansmitter AMPLitude (*TRAMP*) control parameter of the LCModel raw files (cf. LCModel User's Manual, LCModel parameters are given in italics):

$$TRAMP = C \cdot \frac{TG(x)/(1 - s_{CSF})}{\frac{S_{ext} \cdot TG_{ext}/V_{ext}}{TG^{cali}(x)/V^{cali}}} \quad (8)$$

$$\frac{S_{ext}^{cali} \cdot TG_{ext}^{cali}/V_{ext}^{cali}}$$

Because VOI sizes are represented by the *VOLUME* control parameter, only the subtraction of CSF partial volume ($1 - s_{CSF}$) appears in Eq. (8). All TGs appear converted from GE system units of tenth of decibel to linear scale:

$$TG \rightarrow 10 \wedge (TG/200) \quad (9)$$

All signals appear corrected for the linearized TG, that was actually used, to 90° flip angle by the formula

$$S \rightarrow S/\sin^3(90^\circ \cdot TG(x)/TG) \quad (10)$$

RESULTS AND DISCUSSION

Calibration Experiments

Special care is necessary when calibrating a common scaling factor all the metabolites. Absence of baseline artifacts, like tilting and residual water, was found to be essential for reliability of the in vitro spectra. After

incorporating TMPA and its hydrolyzation products into the basis set a complete, and almost artifact free, analysis of the phantom spectra became possible. The *myo*-inositol triplet was found to be attenuated in the basis spectrum and, therefore, the upper ppm limit was set at 4.0 ppm. With an unaffected basis spectrum the additional information in the triplet could be utilized to increase both the reliability of *myo*-Ins and the baseline on the left side of the proton spectrum.

Transverse relaxation times were determined from 13 measurements at TE between 30 and 1400 ms using the TE 30 basis set and yielded T₂ values of 460 ms for NAA, 270 ms for Cre, 180 ms for Cho, and an apparent T₂ of 120 ms for the pseudo singlet of *myo*-Ins. These values conform very well to the values reported for a predominantly white matter VOI in parieto-occipital regions in human brain (see⁵ for a synopsis), except for Cho, that may have reacted chemically with TMPA. The concentration ratios of the TE = 30 ms spectrum differed according to the differences of the respective T₂ times. After extrapolation to zero TE all metabolites (except Cho) including the metabolites that are subject to (strong) coupling and less reliably determined were within ± 1.5% of the expected Cre ratios.

For the final calibration a high quality spectrum was measured at TE = 30 (as used in vivo), analyzed by LCModel without further correction (*TRAMP* = 1), and the scaling factor C in Eq. (8) derived as the true Cre concentration divided by the LCModel Cre estimate. The choice of creatine was motivated by practical reasons: The determination of T₂ was not hampered by coupled resonances and the result was quite close to the in vivo value of 220 ms, that is an estimated average from several studies.⁶ By referring to Cre, differential T₂ attenuation of absolute concentrations is minimized with regard to other metabolites: Identifying both A and C in Eq. (7) with Cre, the difference between 1/T_{2,vivo} and 1/T_{2,cali} yields a 2.5% underestimation of Cre. If finally B is identified with Cre, Eq. (6) becomes almost equivalent with Eq. (7). In other words, systematic T₂ errors imposed onto absolute concentrations and creatine ratios are about the same.

In Vivo Spectroscopy

Included in this study were the analyses of 51 spectra of predominantly gray matter (GM) from VOIs in parame-dian position in the parietal cortex, and of 43 spectra of predominantly white matter (WM) in subparietal position (Table 1). Of these, 28 or resp. 18 have been acquired with the quantitation protocol, and were used to estimate absolute metabolite concentrations (Table 2). In order to monitor biologic variation rather than experimental failure, spectra with obvious motion artifacts, suppressed methylene resonance of Cre, inhomogeneous

Table 1. Estimated metabolic concentration ratios* to total Cre in human brain†

	pa GM	pa WM
Number of Spectra	51	43
Volume (mL)‡	14.9 (5.26–24.36)	9.5 (4.01–18.60)
SNR‡	11.6 (6–18)	10.9 (6–20)
FWHM‡	0.051 (0.038–0.065)	0.046 (0.039–0.052)
Class A		
(NAA + NAAG)/Cre	1.49 ± 9%	1.88 ± 10%
NAA/Cre	1.38 ± 12%	1.53 ± 12%
Cho/Cre	0.17 ± 11%	0.28 ± 12%
Class B		
myo-Ins/Cre	0.63 ± 17%	0.64 ± 21%
Glu/Cre	1.23 ± 21%	1.23 ± 19%
(Glu + Gln)/Cre	1.81 ± 22%	1.89 ± 20%
scy-Ins/Cre	0.04 ± 60%	0.04 ± 76%
Class C		
Gln/Cre	0.57 ± 47%	0.66 ± 43%
Gln/Glu	0.46 ± 45%	0.59 ± 51%
NAAG/Cre	0.11 ± 118%	0.34 ± 44%

* Values given as mean ± %SD, where %SD = 100 SD/mean. No corrections were made for finite TR = 6000 ms and TE = 30 ms. See text for metabolite abbreviations.

† Paramedian parietal gray matter (pa GM) and subparietal white matter (pa WM) examined in a cohort of healthy adult subjects.

‡ Values given as mean and range (in brackets).

noise and heavily distorted baseline were excluded, as were singular spectra with linewidths (*FWHM* as determined by LCModel) exceeding 0.070 ppm. Typical spectra of GM and WM as fitted with the basis set are shown in Fig. 1.

The mean and range of parameters related to spectral quality (*VOLUME*, *FWHM* and signal-to-noise ratio

(*SNR*)) are appended to Tables 1 and 2. Linewidths were typically smaller in WM than in GM, as the white matter VOIs were smaller and anatomically less structured. Less than 0.050 ppm were routinely (i.e., in 90% of spectra) achieved in WM (0.060 ppm in GM). *SNR* adds an element of inaccuracy to the analyses, that is characterized by average VOI size. Although the average *SNR*

Table 2. Estimated absolute metabolic concentrations* in human brain

	pa GM	pa WM
Number of Spectra	28	18
Volume [ml]‡	13.1 (5.26–17.1)	9.9 (4.01–17.4)
SNR‡	12.9 (8–18)	10.1 (6–18)
FWHM‡	0.049 (0.038–0.063)	0.046 (0.038–0.062)
Class A		
NAA + NAAG	10.62 ± 5%	10.06 ± 4%
NAA	10.07 ± 8%	8.53 ± 11%
Cre	7.00 ± 9%	5.46 ± 13%
Cho	1.22 ± 16%	1.61 ± 8%
Class B		
myo-Ins	4.31 ± 16%	3.33 ± 26%
Glu + Gln	12.87 ± 17%	9.85 ± 21%
Glu	8.70 ± 20%	6.56 ± 20%
scy-Ins	0.30 ± 60%	0.23 ± 84%
Class C		
Gln	4.17 ± 31%	3.29 ± 39%
NAAG	0.55 ± 126%	1.54 ± 51%

* Values given as mean ± %SD, where %SD = 100 SD/mean, and means are in millimoles/liter. See legend of Table 1 for further details.

‡ Values given as mean and range (in brackets).

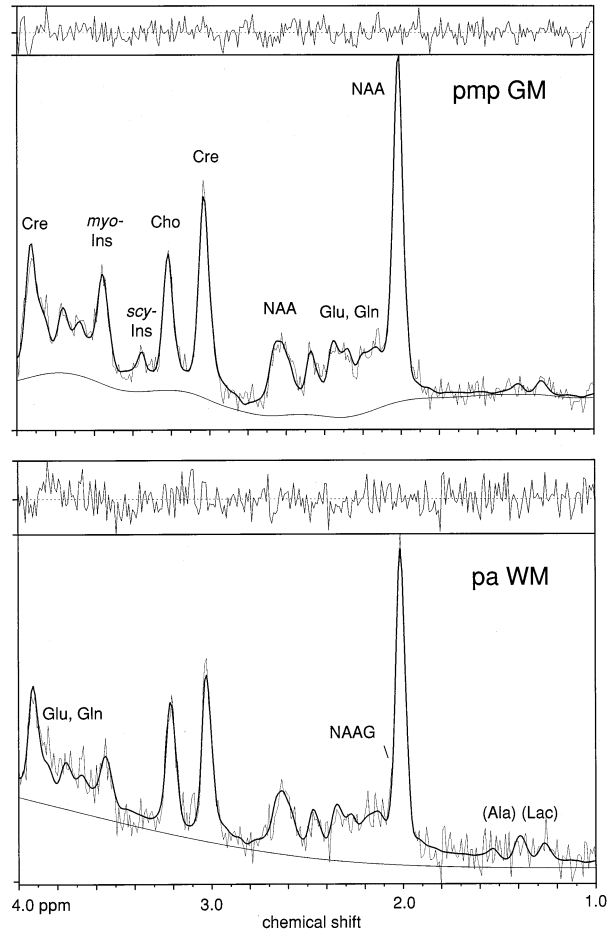


Fig. 1. Typical STEAM localized proton MR spectra from the same subject as analyzed by LCModel. (LCModel fit: bold line Spectrum, baseline, residuals: thin line). Not to scale. Resonances of typical metabolites are labeled. Top: 17.1 mL VOI of paramedian-parietal gray matter (pmp GM). $FWHM = 0.060$ ppm. Bottom: 8.4 mL VOI of parietal white matter (pa WM). $FWHM = 0.055$ ppm. Note the difference of SNR and baselines and the partial leveling of the trough next to *myo*-inositol.

was higher in GM than in WM, SNR per volume was lower in GM due to increased partial volume of CSF.

Metabolite Concentration Ratios

Metabolic ratios provide an immediate basis for comparison of results, as they are readily available for the LCModel user even if quantitation is lacking or insufficient. The concentration ratios relative to total creatine are listed in Table 1 for the classes A, B and C.¹⁴ Class D metabolites have not been included, as even at 2.0 Tesla they cannot be reliably estimated from individual spectra.¹⁵ From the individual concentrations of Glu and Gln the sum (Glu + Gln, often referred to as Glx) and the ratio (Gln/Glu) were determined (Table 1). The relative

standard deviation (SD) of Glu + Gln is about the same as for Glu, whereas the relative SD of Gln/Glu is dominated by the much larger SD of Gln.

If the Cre ratios obtained in this study are compared to those of the original publication,¹ (obtained from spectra at 2 Tesla with $TE = 20$ ms and an in-house basis set), NAAG and *scyllo*-Ins ratios in the original study should be multiplied by 1.23, since these spectra were acquired later, and a long term change in sensitivity was not recognized until after publication. (S. W. Provencher, personal communication). The numbers of the studies are comparable and should warrant a reliable averaging of intersubject variation.

In GM, the relative SDs were generally bigger than in the 2 Tesla study. This was potentially due to the lower SNR caused by the lower field strength and longer TE, since the VOIs and the linewidths were comparable. The singlet dominated class A metabolites were less affected by this, but for strongly coupled metabolites the relative SD was about twice as big, that may be attributed to additional losses due to incomplete coherence transfer and diminished uniqueness with the increase of strong coupling at lower field strength. A slight overestimation of NAA+NAAG ratios and an underestimation of *myo*-Ins/Cre can be attributed to the increasing of TE by 10 ms in this study. The difference in NAAG/Cre may be a secondary effect caused by interaction with glutamate and glutamine. The other metabolite ratios differed by the second decimal digit, that is well within the experimental range. This confirms the consistency of the basis sets.

In WM the strongly coupled metabolites appeared to be overestimated, most severely glutamine. Probably due to smaller VOI size and lower concentrations, the WM spectra tended to lose the characteristic baseline hump in the α -CH region [cf. Fig. 1 of (1)]. This may have resulted in higher estimates, especially for Glu and Gln ratios. In consequence, NAAG was underestimated. The baselines of the GM spectra were similar to the GM spectrum shown in the original publication (cf. Fig. 2 of (1)). Severe underestimation of Glu or Gln due to a bent baseline, occurred more often in gray than in white matter and resulted in an overestimation of NAAG in GM.

Absolute Concentrations

The mean values and SDs of estimated tissue concentrations are given in Table 2. Although they are subject to similar T_2 errors, the SDs of NAA+NAAG concentrations were only half as big as the respective creatine ratios. Absolute concentrations are subject to errors of the quantitation scheme, while NAA/Cre ratios, on the other hand, are affected by the uncertainties of determining creatine from the spectrum (SD between 6% to 9%).

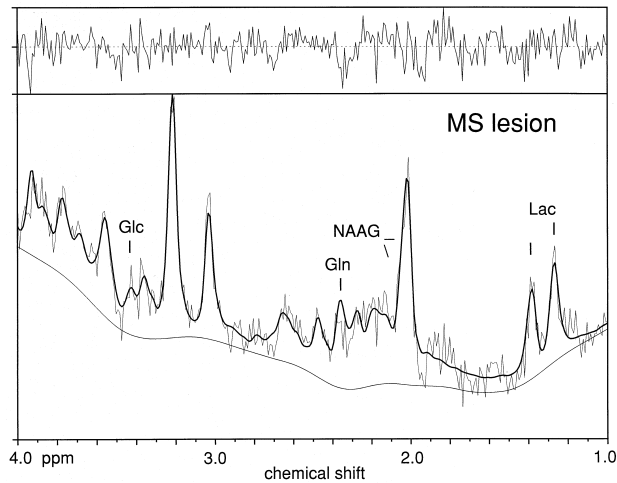


Fig. 2. STEAM localized proton MR spectra from 10.4 mL VOI of a contrast enhancing ring lesion in a patient with multiple sclerosis as analyzed by LCModel. Note the high choline, glucose and the distortions of the baseline and residues at 2.46 ppm due to overestimation of Gln. Elevated lactate of 4.5 mM was reliably detected (SD = 15%) even at TE 30 ms, and in the presence of broad macromolecular resonances. In spectra of normal brain, lactate and alanine show a tendency to compensate for artifacts or residual macromolecular resonances around 1.3 ppm, as the signal rarely matched both peaks of their doublets (cf. Fig. 1).

At least for NAA, that is the most reliably determined metabolite in the proton spectrum, a higher accuracy was reached by quantitation scheme than for metabolite ratios. A detailed description of the sensitivity correction [Eqs. 1–3] will be published elsewhere.

The concentrations of class A metabolites correspond almost exactly to the uncorrected VOI concentrations published by Michealis et al.,¹⁰ if the tissue concentrations in Table 2 are converted to VOI concentrations by the average CSF fraction of 9% for GM, and 2% for WM. Deviations in the strongly coupled metabolites may be due to differences between manual integration and LCModel evaluation. Particularly the lower LCModel estimates for *myo*-inositol may be due to leveling of the characteristic “trough” in the baseline between *myo*-Ins and Cho (cf. Fig. 1) in addition to relaxation effects.

There is very good agreement for parietal WM with a more recent study from the same group, where LCModel analysis has been applied.⁷ Although information on average VOI size and SNR is lacking, from the depicted VOI may be judged that the congruence of *myo*-Ins and Glu concentrations is a result of similar VOI size. As for the ratios, Gln concentration in WM was overestimated in this study, while in GM there was excellent agreement. Hence, Gln appears to be more sensitive to low SNR than Glu or *myo*-Ins. The use of gaining SNR by choosing bigger VOIs is of course limited by increasing

partial volume effects. However, from singular well-shimmed GM spectra from 15 mL VOIs, Gln was estimated with a SD as low as 23%. In this study the GM VOIs were partly shifted toward the occipital position, that may explain the 10% higher average concentration of NAA.

Correlation between Metabolites

Spectral overlap of two metabolites is expected to result in an inverse correlation,¹ whereas neighboring resonances may be positively coupled by the baseline. Sensitivity changes affect all metabolites and, thus, will blur mutual interdependencies, that are inherent to the LCModel analysis. Because the quantification involves sensitivity correction, absolute concentrations may reveal LCModel specific correlations between metabolites, including Cre.

LCModel is expected to give independent estimates of NAA and Cre, because their resonances are well separated from each other. Lack of correlation between NAA and Cre concentrations in both gray and white matter confirmed that the quantification scheme reduces the effect of altered sensitivity or CSF inclusion below biological variation even for the most reliable metabolites. There was, however, a significant positive correlation between the neighboring Cho and Cre ($p < 0.01$ in GM, $p < 0.05$ in WM). The Glu and Gln correlated positively only in GM ($p < 0.01$ for both relative and absolute concentrations). This explains, why the Gln/Glu ratio had a smaller SD than Gln/Cre in GM, but a bigger one in WM. In gray matter Gln was correlated to total NAA ($p < 0.01$) indicating coupling through the baseline. However, the spectral overlap at 2.01 ppm destroyed the correlation between Glu and total NAA. A statistical correlation between Gln, Glu and NAAG was not found, but their interdependence can be demonstrated under pathologic conditions.

The spectrum shown in Fig. 2 was obtained from a multiple sclerosis lesion with a ring like contrast enhancement. LCModel analysis yielded a high level of glucose and an overestimation of glutamine, that can be identified by distortions in the residues and a sagging baseline at 2.46 ppm. These were caused by increased signal in the α -CH region probably due to an increase of glycerophosphocholine, that was not included in the basis set. Although the total concentration of choline containing compounds could be reliably estimated by the dominating *N*-trimethyl resonance at 3.24 ppm, the concomitant resonances of the glycerol protons¹⁵ are not accounted for by the choline spectrum, and are compensated by glutamine, glucose and possibly glutamate. Consequently, this lead to a severe underestimation of NAA-glutamate, as compared to excluding the α -CH region from the LCModel analysis. Thus, the reliability

of NAAG appears to be coupled to glutamate and glutamine. The inclusion of phospho-choline and glycerophospho-choline⁶ may improve the LCModel analysis in general and not only between 3.5 and 4.0 ppm.

Concluding Remarks

LCModel analysis of STEAM localized *in vivo* spectra is reliable also at 1.5 Tesla and TE 30 ms. A manufacturer supplied basis set of single metabolite spectra yielded relative concentrations in good agreement with known concentrations *in vitro* and with literature values, that were obtained at 2 Tesla and shorter TE of 20 ms using in-house basis sets. After calibration on a T₂ matched phantom and using a suitable sensitivity correction, even absolute metabolite concentrations could be reproduced with the imported LCModel basis set. The differential T₂ errors are expected to be smaller than with an in-house basis set. Singlet dominated metabolites (class A) are robustly determined, as well as metabolites of class B, namely *myo*-Ins, Glu and Glu + Gln for standard VOI sizes. Class C metabolites seem to require higher SNR, that in particular may hamper the reliable detection of glutamine in smaller VOIs, i.e., in white matter. Therefore information on VOI size and SNR should be provided to allow a critical comparison of LCModel evaluations. In pathologies the lack of glycerophospho-choline in the basis set may systematically affect the estimates of other metabolites. Hence, the decrease of spectral information should not be interpreted as higher tolerance to imperfection. On the contrary, as the conditions at 1.5 Tesla and TE 30 ms are more difficult for LCModel in terms of SNR and spectral uniqueness, the demands regarding spectral quality and the comprehensiveness of the basis set seem to be higher than at higher field strength.

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