Comparison of two programs for quantification of ^{1}H MR spectra of rat brain using a vascular dementia model

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Abstract: The quantification of *in vivo* ^{1}H magnetic resonance (MR) spectra measured from the rat brains provides important information about the brain metabolite concentrations and can help to understand the role of the metabolites under normal and pathological conditions.
The purpose of this study was to compare the most frequently used algorithms for quantification of ^{1}H spectra: LCModel (Linear Combination of Model spectra) and QUEST (QUantitation based on QUantum ESTimation) from jMRUI software (Java based Magnetic Resonance User Interface). The comparison was done on a rat model of vascular dementia (VD). The MR spectra were measured on 4.7T spectrometer with ultra-short echo time by sequence SPECIAL. For these types of spectra the contribution from the macromolecules and lipids is large.
Our analysis revealed that all values determined by QUEST, except for one value, were lower in comparison to values obtained by LCModel. The minimal differences were found in N-acetylaspartate/(phospho) creatine (–0.3 %) and maximal in inositol in both control and VD rats. This underestimation of a metabolite concentration in QUEST may be caused by an overestimation of baseline.
Although our study found the different values of metabolite concentrations by these two methods, the quantified metabolite changes in pathological brain were comparable in both analyses.

Keywords: brain, *in vivo* ^{1}H MRS, LCModel, QUEST

Introduction

The quantification of *in vivo* ^{1}H magnetic resonance (MR) spectra measured from the rat brains provides important information about the metabolite concentrations and can help to understand the role of the metabolites in normal and pathological conditions. Localized ^{1}H magnetic resonance spectroscopy (MRS) enables the detection of changes in metabolite concentrations such as N-acetylaspartate, (phospho)creatine, glutamate, glutamine, choline-containing compounds, inositol, taurine and others in specific region of the brain (Pfeuffer et al., 1999; Tkáč et al., 2003).
^{1}H MR brain spectrum obtained at short echo-time contains contributions from metabolites, water and a large baseline component — background. The background has an a priori unknown shape and intensity and consists of signals from unresolved proteins, polypeptides and subcutaneous lipids. Furthermore, in the case of brain pathologies the amount of macromolecules and lipids can vary. Therefore accurate and reliable quantification of ^{1}H spectra acquired with short echo from normal and pathological brain is not trivial (Cudalbu et al., 2008a; Seeger et al., 2003).

Several programs have been proposed for an accurate quantification of *in vivo* ^{1}H MR spectra. The programs are based on deconvolution of frequency or time domain spectra using metabolite basis sets. The two most frequently used algorithms for ^{1}H spectra quantification are: LCModel (Linear Combination of Model spectra) working in the frequency domain (Provencher, 1993) and QUEST (QUantitation based on QUantum ESTimation) (Ratiney et al., 2004, 2005) from jMRUI software (Java based Magnetic Resonance User Interface) working in the time domain (Naressi et al., 2001a, 2001b).
LCModel method analyses an *in vivo* spectrum as a linear combination of spectral signatures from individual components, which were obtained from *in vitro* metabolite solutions. Set of these spectra used for quantification is called "basis set" and involves also components for macromolecules and lipids. By using a model of complete spectra two metabolites with overlapping peaks in one spectral region can be resolved if their spectra are different at other chemical shifts. LCModel uses a constrained regularization method to find the best compromise between the smoothest lineshape and baseline consistent with the data. LCModel is a fully automatic program without subjective input (Provencher, 2001).

QUEST is a nonlinear least-squares algorithm which fits a time-domain model function (made up from a basis set) to *in vivo* data (Ratiney et al., 2004). The basis set can be simulated with the software package NMR-SCOPE (also included in jMRUI software) or can be obtained from *in vitro* measured metabolites. Contrary to LCModel, macromolecules and lipids are not included in the basis set but remain in the background (baseline). The background signal can be estimated by two semiparametric approaches: InBase and Subtract method. With InBase method, the estimated background signal is included in the basis set and plays the same role as a metabolite. Therefore it is strongly correlated with the metabolite signals. In Subtract method, the background signal is determined and subtracted from the measured signal before quantification of metabolites (Ratiney et al., 2005).

The purpose of this study was to compare LCmodel and QUEST in quantification of the MR spectra measured on 4.7T spectrometer with ultra-short echo time by the sequence SPECIAL (Mlynárik et al., 2006) in a rat model of vascular dementia (VD) where contributions from macromolecules and lipids are large. In this study we have compared concentrations of following metabolites: N-acetylaspartate + N-acetylaspartylglutamate (tNAA), (phospho)creatine (tCr), choline compounds (tCho), glutamate + glutamine (Glx), inositol (Ins), Taurine (Tau) and their ratio to tCr in the healthy and pathological rat brains. To the best of our knowledge, no such comparative study has been performed.

Materials and methods

Animals and measurements

In the experiment aged male rats (15-months old Wistar rats, from Velaz, Czech Republic) — 10 control rats and 7 rats with vascular dementia (VD) — were used. Pathophysiological VD model was induced by severe hypoperfusion — permanent four vessel occlusion according to Ferreira (Ferreira et al., 2011).

During measurement animals were anesthetized by inhalation of 1.5—2.0 % isoflurane in a gas mixture of oxygen and nitrous oxide. Body temperature was maintained at 37 °C by warm air. Monitoring of respiration and body temperature was carried out by the device SAII (SA Instruments, Inc., Stony Brook, NY, USA). Experiments were conducted with the approval of the Local Animal Ethical Committee according the Guidelines of European Convention for the Protection of Vertebrate Animals Used for Experimental Purposes.

Measurements were performed on a 4.7 T Agilent spectrometer equipped with a 400 mT/m gradient insert using dual-tuned $^{1}H/^{31}P$ surface coil (Rapid Biomedical, Germany). For spectra localization, FSEMS (fast spin echo multi-slice) images ($TR/TE_{effective}$ = 2650/80 ms) were used. Single voxel localized spectroscopy with ultra-short echo time (SPECIAL) (Mlynárik et al., 2006) was used for spectra acquisition from selected voxel of the brain (Fig. 1) with following parameters: TR = 4000 ms, TE = 2.8 ms, voxel size: 3 × 4 × 5 mm, number of array × number of averages: 8 × 64, 9 × 64 or 6 × 96. The water signal was suppressed by VAPOR method (Tkac et al., 1999). The homogeneity of the magnetic field was adjusted automatically using FASTMAP (Gruetter, 1993). Shimming resulted in an unsuppressed water spectral linewidth of 9—11 Hz. For absolute quantification of metabolites additional spectra without water suppression (2 averages) with the same parameters were acquired.

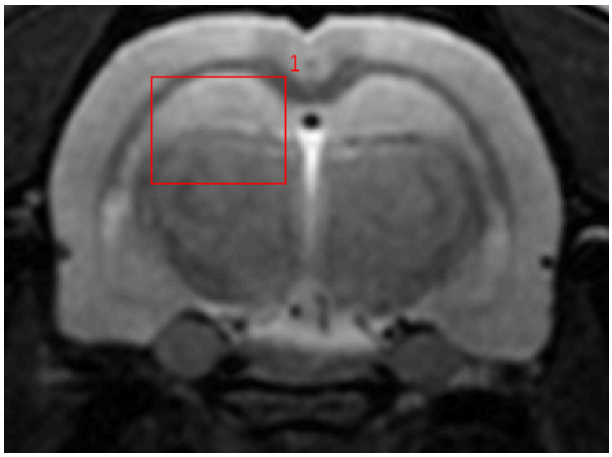

Fig. 1. The position of the voxel (size: 3 × 4 × 5 mm).

Metabolite Quantification

Spectra were analyzed using LCModel (Version 6.3-1) (Provencher, 1993) and QUEST (jMRUI Version Number: 5.0) (Ratiney et al., 2004). For all used spectra average value of signal to noise was 8.9 ± 2.2.

LCModel basis set included 17 metabolites alanine, aspartate, creatine, phosphocreatine, gamma-aminobutyric acid, glucose, glutamate, glutamine, glycerophosphorylcholine, phosphorylcholine, glutathione, inositol, lactate, N-acetylaspartate, N-acetylaspartylglutamate, scyllo-inositol, taurine and negative creatine CH_2 singlet (—$CrCH_2$), as well as simulated signals of lipids and macromolecules.

QUEST metabolite basis set was quantum-mechanically simulated at 4.7 T for the *in vivo* experimental protocol (sequence with two 90 rectangular pulses, TE = 2.8 ms, 4096 data points) with NMR Scope-B. The spectra of 16 metabolites alanine, aspartate, glycerophosphorylcholine, creatine, gamma-ami-

nobutyric acid, glucose, glutamate, glutamine, glutathione, lactate, inositol, N-acetylaspartate, phosphocreatine, phosphorylcholine, scyllo-inositol, taurine were simulated using the spin Hamiltonian parameters (number of spins, chemical shifts, J-couplings) given in literature (Govindaraju et al., 2000). The macromolecule and lipid signals were included in the background signal which was determined from the first data-points (17—22 truncate points) of the MRS signal using Subtract-QUEST method. This method utilized the fact that signals from the macromolecules and lipids decay rapidly in the time-domain. Therefore a strategic truncation of these initial points can separate the metabolite signals from the background signal (Ratiney et al., 2004, 2005). Spectra pre-processing in jMRUI involved phasing and removal of residual water components using HLSVD (Hankel—Laclosz single value decomposition) filter (Pijnappel et al., 1992).

In both programs no corrections (i.e. relaxation time, tissue segmentation) were used for data evaluations.

Signals of tNAA (i.e. N-acetylaspartate + N-acetylaspartylglutamate in LCModel and only N-acetylaspartate in QUEST), tCr, tCho, Glx, Ins and Tau were quantified. The concentration of N-acetylaspartylglutamate is small in comparison to the concentration of N-acetylaspartate and thus can be neglected in this case. Unsuppressed water signal was used as an internal reference for the metabolite quantification. For all evaluated metabolites (tNAA, tCr, tCho, Glx, Ins and Taurine) in all used spectra Cramér—Rao lower bounds reported by LCModel were below 15 %.

Number of arrayed spectra and their averages were taken into account. Absolute concentrations of the metabolites were expressed as mmol/kg tissue. Various concentrations of water in the brain and different number of array and averages can also cause deviations in quantification of absolute concentrations. Therefore the ratios of metabolites to tCr were also determined and compared.

The data for each metabolite in LCmodel and in QUEST was tested for normal distribution (Shapiro — Wilk test) and homogeneity of variance (Levene's tests — absolute and squared deviations) resulting in the confirmation of null hypothesis. The differences between control and VD rats were evaluated using two-tailed Student's t-test and $p < 0.05$ was considered as a statistically significant difference.

Differences between LCModel and QUEST were determined in percentages relative to concentration values estimated by LCModel.

Result and discussion

The representative spectra from an analysis by LCModel and QUEST are shown in Fig. 2 and Fig. 3, respectively. As shown in a residual spectrum in Fig. 3, the peak at 0.9 ppm was not ideally estimated by the QUEST quantification. Nevertheless, this region contains extensive signals from macromolecules and lipids and their comparison was not the goal of this study. However, evaluated signals (tNAA, tCr, tCho, Glx, Ins,Tau) were clearly discernible by using these two methods.

The results of difference between values estimated using LCModel and QUEST are presented in Table 1 for metabolite concentrations and in Table 2 for metabolite to tCr ratios. Our analysis revealed that all values determined by QUEST with the exception of Glx/tCr ratio in VD rats were lower than in LCModel. The minimal differences between metabolite

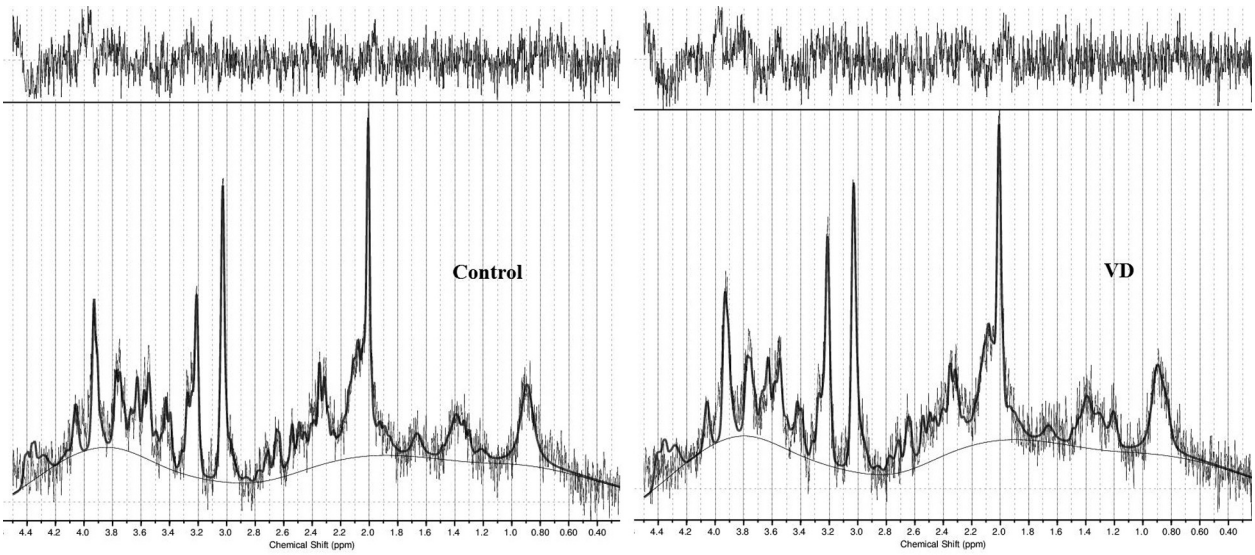

Fig. 2. Analysis of a representative spectrum by LCModel. No filtering or line broadening was used.

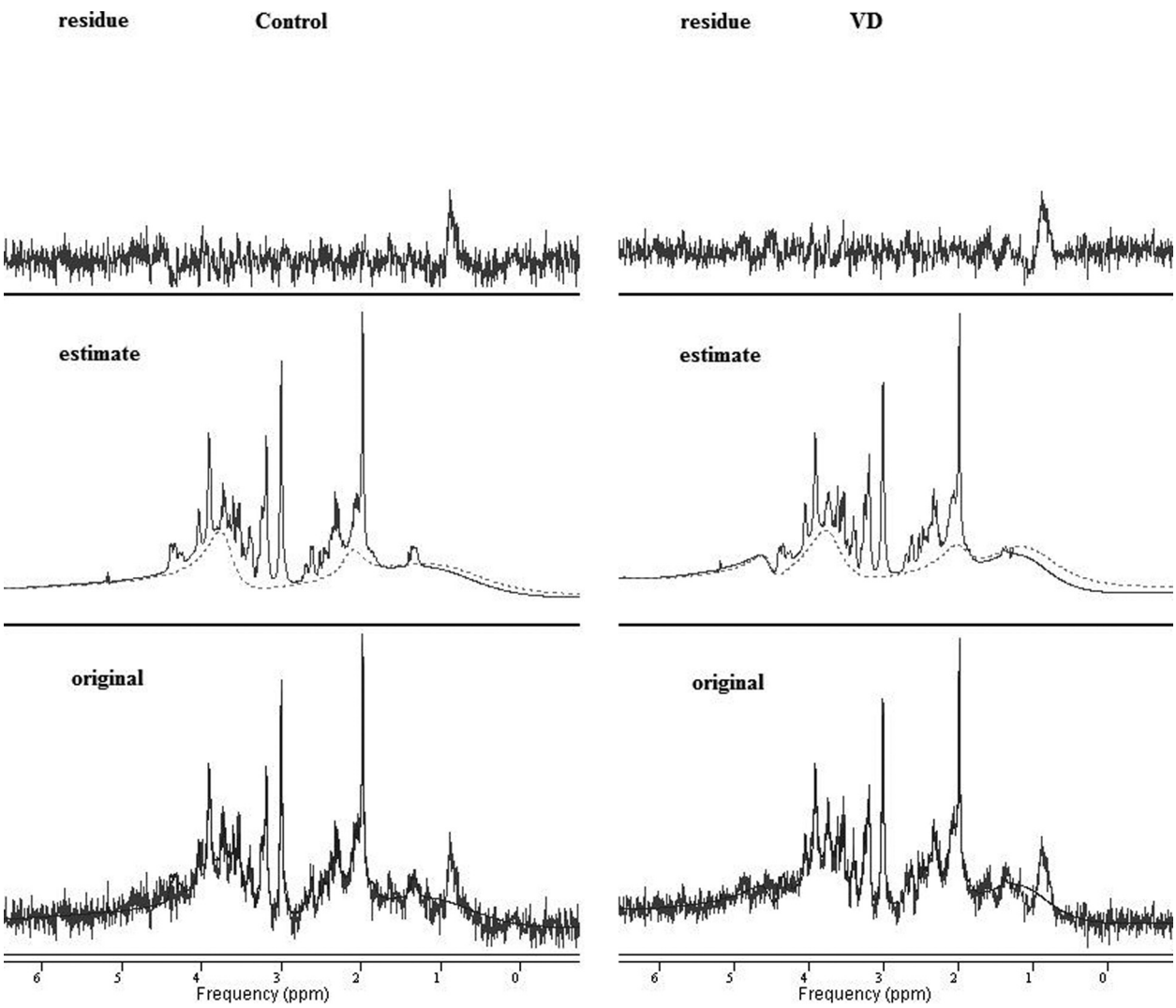

Fig. 3. Analysis of the representative spectra by QUEST. No filtering (except HLSVD filter to remove residual water) or line broadening was used.

concentrations were found in tCr (–4.5 %) of the control and in Glx (–5.2 %) of VD rats. The maximal difference was found in Ins of both, control and VD, rats. Differences of all ratios to tCr, except for tCho/tCr ratio, were lower in comparison with absolute metabolite concentrations. This result might be explained by not using spectra containing water for absolute quantification. Greater difference in tCho/tCr ratio could be caused by the influence of tCr.

In terms of the relative quantification, the minimal difference (0.3 %) in tNAA/tCr and maximal (20.7 %) in Ins/tCr was found.

Data from LCModel show significant differences in T-test of control and VD rats in the concentrations of tNAA, tCr, Tau and Ins/tCr ratio, respectively. However, by using QUEST only changes in tNAA and Tau were significant.

LCModel is a commercial software and therefore we assume that results from this program are more reliable than the results from QUEST. This fact is in agreement with smaller values of standard deviations in values determined by LCModel.

The quantification of the rat brain metabolites by QUEST has been used in several studies (Cudalbu et al., 2005, 2007, 2008a, 2008b). Some of them (Cudalbu et al., 2005, 2008b) show that there is no statistically significant difference between metabolite concentration estimated with simulated basis set and with basis set obtained from *in vitro* measurement.

Our findings in QUEST showed an underestimation of the metabolite concentrations which might be caused by several factors. We assume that the most crucial factor is a correctly determined baseline, which was in this study obtained with Subtract method. Another study shows that Subtract-QUEST led to an overestimation of the metabolite concentrations due to underestimation of the background contribution (Cudalbu et al., 2007). However, this study compares the quantification with background estimated by Subtract method and measured background.

Tab. 1. Metabolite concentrations determined by LCModel and QUEST (average values ± standard deviation) and their difference in percentage.

Control	**tNAA**	**tCr**	**Glx**	**tCho**	**Ins**	**Tau**
LCModel	8.8 ± 0.6	8.1 ± 0.5	10.6 ± 0.8	1.53 ± 0.15	4.8 ± 0.4	5.3 ± 0.5
QUEST	8.4 ± 1.2	7.7 ± 0.9	9.1 ± 1.6	1.38 ± 0.11	3.6 ± 1.4	4.7 ± 1.3
percentage difference	–5.5	–4.5	–14.4	–10.1	–24.0	–11.8
VD	**tNAA**	**tCr**	**Glx**	**tCho**	**Ins**	**Tau**
LCModel	8.1 ± 0.5	7.5 ± 0.4	10.0 ± 0.8	1.46 ± 0.20	5.1 ± 0.2	4.6 ± 0.7
QUEST	7.1 ± 0.9	6.8 ± 1.0	9.5 ± 1.3	1.26 ± 0.13	3.6 ± 1.4	3.3 ± 0.8
percentage difference	–12.2	–8.8	–5.2	–13.5	–28.1	–27.3
T-test (p-value) Control-VD	**tNAA**	**tCr**	**Glx**	**tCho**	**Ins**	**Tau**
LCModel	0.01	0.02	0.12	0.45	0.08	0.02
QUEST	0.03	0.08	0.59	0.07	0.99	0.02

Tab. 2. Metabolite ratios to tCr determined by LCModel and QUEST (average values ± standard deviation) and their difference in percentage.

Control	**tNAA/tCr**	**Glx/tCr**	**tCho/tCr**	**Ins/tCr**	**Tau/tCr**
LCModel	1.10 ± 0.05	1.32 ± 0.08	0.19 ± 0.01	0.59 ± 0.06	0.66 ± 0.04
QUEST	1.09 ± 0.17	1.19 ± 0.24	0.17 ± 0.03	0.47 ± 0.17	0.61 ± 0.15
percentage difference	–0.30	–9.35	–11.77	–20.70	–7.90
VD	**tNAA/tCr**	**Glx/tCr**	**tCho/tCr**	**Ins/tCr**	**Tau/tCr**
LCModel	1.08 ± 0.04	1.34 ± 0.14	0.20 ± 0.02	0.68 ± 0.05	0.61 ± 0.06
QUEST	1.06 ± 0.19	1.42 ± 0.35	0.18 ± 0.02	0.55 ± 0.26	0.50 ± 0.14
percentage difference	–2.3	6.2	–8.2	–19.2	–18.6
T-test (p-value) Control-VD	**tNAA/tCr**	**Glx/tCr**	**tCho/tCr**	**Ins/tCr**	**Tau/tCr**
LCModel	0.60	0.71	0.59	0.007	0.10
QUEST	0.71	0.16	0.33	0.51	0.13

On the other hand underestimation in our study compare to others (Cudalbu et al., 2005, 2008b) might also be caused by not correcting T_2 effects in simulated basis set.

Ultrahigh fields can be used to obtain high quality spectra which increases the accuracy of quantification. Advantages of measurement in ultrahigh fields (e.g. over 7 T) are that the SNR of metabolites increases almost linearly with B_0 and strong coupling effects decrease. The main advantage is the increased spectral dispersion and spectral resolution. Due to reduced spectral overlap and simplified spectral patterns the quantification accuracy of metabolites is higher (especially those with lower concentrations and the scalar-coupled metabolites).

Disadvantage is that magnetic field inhomogeneity increases linearly with magnetic field strength due to susceptibility differences between tissues (blood, bone and air). This leads to shorter apparent T_2 relaxation time and broadening of spectral lines (de Graaf et al., 2006; Mlynárik, 2010).

Conclusions

Although our study found different values of metabolite concentrations by using LCModel and QUEST methods, metabolite changes in pathological brain were comparable in both analyses. Deviations from the correct baseline can significantly influence the obtained values of metabolite concentrations. A possible way around this could be the incorporation of the measured background signal in the metabolite basis set.

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