Requirements for reliable metabolite profiling

Ivan Tkac

Center for Magnetic Resonance Research, University of Minnesota, Minneapolis, MN, USA

Email: ivan@cmrr.umn.edu

Reliable metabolite quantification is the major goal of magnetic resonance spectroscopy (MRS). I think that all of us agree that “spectral quality” and “appropriate” data processing determine the resulting reliability of metabolite quantification. However, these terms are not well established. In this article I will present my viewpoint on “spectral quality” assessment and discuss a variety of factors that influence the quality of spectra. Secondly, I will focus on data analysis and discuss pros and cons of different approaches of metabolite quantification. Finally, I will present some examples of metabolite profiling in human and animal brains.

I. Spectral quality

Multiple spectral parameters have to be taken into account when assessing the overall spectral quality. An overview of these parameters is presented in Figure 1.

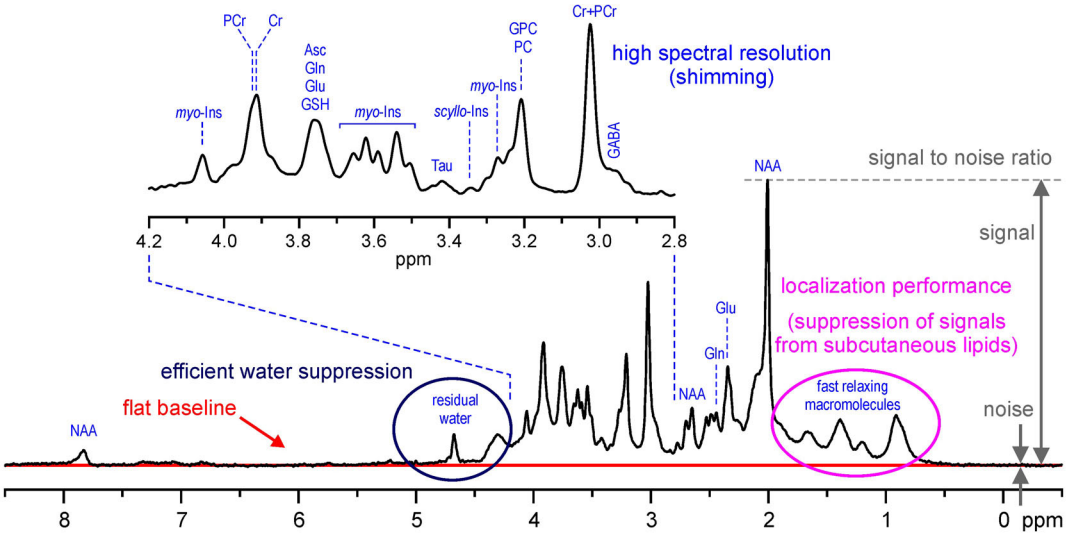

Fig. 1 Overview of factors determining the spectral quality. ^{1}H MR spectrum of the human brain (grey-matter-rich occipital lobe) acquired at 7T. STEAM (TE = 6 ms) with OVS and VAPOR water suppression, neither water signal removal nor baseline correction were applied.

The signal-to-noise ratio (SNR) and the spectral resolution are commonly considered as major factors determining the spectral quality. But other factors, such as localization performance, water suppression and baseline distortions (Fig. 1), are equally important. The sections below list these factors and discuss how they depend on hardware, software and scanning protocols.

Signal-to-noise ratio

The integral signal intensity increases with B_0 field, but despite optimal shimming the potential increase in SNR is partially offset by increased intrinsic signal linewidth [1-3]. The achieved SNR highly depends on the efficiency of B_0 shimming [4-6] and on the type and performance of RF coils and localization sequences used [2,3,7,8]. Moreover, SNR depends on the detection sensitivity of the MR scanner and the noise figure of the receiver. In addition, precise adjustment of rephasing gradients in the localization sequence is very important, as even a small imbalance can cause substantial signal attenuation. Last but not least, subject motion

during the scan can significantly reduce the SNR and deteriorate the overall spectral quality, including SNR.

Spectral resolution

MRS benefits from increased field strength due to increased chemical shift dispersion [9-11]. With higher field strengths, it becomes increasingly important that B_0 shimming is optimally performed in order to compensate field inhomogeneities induced by differences in magnetic susceptibility. Shimming is important at any B_0 field, but shim demands are increasing with the field strength, because B_0 inhomogeneities increase with field and spatially become highly non-linear. Successful shimming requires an accurate B_0 mapping method [4-6,12] and powerful higher order shim system [3,13]. Strong second-order shims are typically sufficient to minimize macroscopic B_0 field distortions in relatively small volumes selected for single voxel MRS. Resolved resonances of PCr (3.93 ppm) and Cr (3.91 ppm) in brain spectra of rodents are a good marker of successful shimming [7,9,11,14-16]. Efficient higher-order shimming not only reduces the spectral linewidth, but also has a substantial effect on correcting the signal lineshape, which has a direct effect on metabolite quantification. Optimal shimming is necessary but not sufficient requirement for producing high spectral resolution. Hardware instability and physiological motion result in frequency and phase fluctuations, which can significantly deteriorate the resulting spectral quality if remain uncorrected. Therefore, a single scan acquisition mode is preferable, because it allows correcting both frequency and phasing fluctuations before FID summation [17-20,37]. In addition, if some FIDs are not correctable, they can be easily eliminated from the final summation. If SNR of single scan data is not sufficient to perform these corrections, then data acquisition in arrays of small blocks, e.g. 4 or 8 scans per block, is recommended, because it allows to correct the frequency drift and fluctuations and to remove corrupted FIDs from the final summation.

Water suppression

A strong residual water signal adversely affects the spectral baseline, which complicates metabolite quantification. The residual water signal can be efficiently removed from spectra using HSVD approach [20], however, sidebands of the water signal that overlap with metabolite resonances can interfere with metabolite quantification. Therefore, effective water suppression is the most reliable solution. The VAPOR method [8,11] has been extensively used due to its robustness and decreased sensitivity on B_1 adjustment. Fully automatic setting of the VAPOR parameters using a local B_1 calibration is sufficient for highly efficient water suppression [3,10,13,18,21] (Fig. 1).

Localization performance

Low sensitivity is a general problem of MRS, therefore, pulse sequences using the full M_z magnetization from the voxel, such as PRESS, LASER [22] or SPECIAL [7] are preferable. However, signal intensity provided by the localization sequence in not the only important parameter for metabolite quantification. The STEAM sequence can provide signal intensity proportional for just one half of the available M_z. However, the advantage of STEAM is the possibility to use an ultra-short TE [8,11], minimizing the T_2 decay and J-evolution, which substantially simplifies absolute metabolite quantification [3,9]. In addition, the STEAM sequence may be the method of choice in human MRS at ultra-high fields, to overcome problems with the chemical shift displacement error, when the available B_1^+(max) is not high enough [3,8,10]. The PRESS sequence is practically unusable for human applications at ultra high fields due to insufficient bandwidths of rephasing RF pulses. This drawback was overcome by using pairs of broadband adiabatic RF pulses in the LASER sequence [22], but the penalty was increased duration of TE. This problem was partially resolved by a compromise solution in semi-LASER [18,23], where one pair of adiabatic RF pulses was eliminated due to slice selective excitation. This modification of the LASER sequence allows decreasing the minimum

TE. Localization performance of the sequences, i.e. the ability to provide the maximum signal from a selected volume of interest (VOI) with the minimum contamination of signals arising from outside of VOI, is essential for reliable metabolite quantification. The chemical shift region 0.5 – 2.0 ppm is the most sensitive spectral region to assess the quality of the localization performance (Fig. 1). Desirable localization performance should result in a spectral pattern with four broad resonances of fast relaxing macromolecules, which must be discernible in all short TE spectra [2,3,7-11,13-15]. Insufficient localization performance results in unwanted spectral contamination by signals of subcutaneous lipids. These lipid signals appear in the spectrum around 1.5 ppm, typically with the wrong phase. The profile of a single RF pulse is typically not good enough for an efficient localization. This limitation in properties of commonly used RF pulses can be solved by a "double localization", e.g. using pairs of RF pulses for each slice selection as in LASER [22] or combining the localization sequence, such as STEAM, with the outer volume suppression (OVS) [8,10,11].

Baseline

A flat baseline is critical for a reliable quantification of weakly represented metabolites. As already was mentioned, baseline distortions are typically caused by bad water suppression and by low-quality localization performance, which lead to spectral contamination by unwanted signals arising from outside of VOI. Baseline distortions can result from extensive use of first-order phase correction to compensate an improper timing of the beginning of data acquisition. This problem can be easily solved by appropriate timing of the first sampling point of the FID [8].

Chemical shift displacement error

Chemical shift displacement error (CSDE) is a general problem of all MRS techniques based on voxel selection using slice selective pulses. CSDE basically means that volumes selected for off-resonance signals are spatially displaced from the nominal VOI. This becomes a significant problem at high magnetic fields due to increased chemical shift dispersion. Although this problem is typically not visible in a spectrum, it may lead to a significant misinterpretation of measured data. CSDE is proportional to the ratio of RF pulse bandwidth to the chemical shift range of interest expressed in Hz. Therefore, the higher the magnetic field, the broader bandwidth of slice selective pulses needed. The B_1^+(max) of RF coils used in small animal MRS is typically high enough for very short RF pulses to minimize CSDE [11]. As mentioned above, limited B_1^+(max) and the resulting large CSDE is currently one of the most challenging factors for pulse sequence design for human application at ultra-high fields. Due to limitations of B_1^+(max), the STEAM sequence is preferable to the PRESS sequence for reducing the CSDE. When B_1^+(max) is not sufficient to decrease CSDE to a reasonable value using amplitude modulated RF pulses, then pulse sequences using full passage adiabatic (AFP) pulses, such as LASER [22] or semi-LASER [18,23], become the methods of choice. Bandwidths of AFP pulses are determined by the frequency sweep widths during the pulse, thus, broadband pulses requiring less B_1 can be created [22]. However, the penalty for creating broadband AFP pulses using low B_1 is an increase in their duration, which consequently increases the minimum TE of the localization sequence. When multi-channel RF coils are used, the efficiency of RF transmission can be improved by B_1 shimming, i.e. by optimizing the phase and amplitude of RF field transmitted by individual coil elements [24].

Eddy current correction

The lineshape of localized short-TE spectra is affected to some degree by residual eddy currents despite all efforts for the hardware eddy current compensation. These lineshape distortions may cause major problems in accuracy of metabolite quantification. Effects of residual eddy currents can be easily removed from metabolite spectra using an unsuppressed water signal [8,25].

II. Metabolite quantification

Fitting methods and the prior knowledge

Despite increased chemical shift dispersion at ultra-high magnetic fields, spectra of individual metabolites are highly overlapped. Therefore, MRS quantification methods require extensive prior knowledge for meaningful spectra analysis. Both of the most commonly used fitting programs, jMRUI [26,27], working in the time domain, and LCModel [28], working in the frequency domain, require metabolite spectra databases which can be experimentally measured or simulated based on published information about metabolite chemical shifts and J-couplings [29]. If the same type of the prior knowledge is used, both methods should provide very similar results. Quantification errors are estimated by Cramér-Rao lower bounds (CRLB). It should be emphasized that CRLB are estimated on the basis of assumption that the model (spectral basis set) is correct and complete. Obviously, this is not possible and always simplifying assumptions must be made. But if a detectable metabolite is missing in the basis set then the quantification of other metabolites may be systematically under or overestimated. This is nicely demonstrated in the case of ascorbate in analysis of spectra acquired from the developing brains [30]. Independent of the type of basis set, measured or simulated, inaccuracies in spectral pattern cause a bias and result in underestimated CRLB. In general, differences in estimated metabolite concentrations using experimentally measured or simulated basis sets are small, thus a simulated basis set can be used in place of a measured one [31].

Macromolecule background

Short TE spectra have a significant signal contribution from fast relaxing macromolecules, which are dominantly proteins in healthy brain. These broad signals must be taken into account in metabolite quantification. Including the whole macromolecule spectrum in the basis set is a very robust approach, and data analyses using this approach provide neurochemically reasonable values for weakly represented metabolites, such as GABA [2,3,8,9,14,15]. There are other options for accommodating background signals [32,33], but too much flexibility in modeling of the macromolecule signal might reduce the robustness of fitting. Macromolecule spectra can be experimentally measured using an inversion-recovery experiment [9]. The small residual metabolite signals can be suppressed using diffusion-weighted approach [34], or they can be eliminated from the spectra by the post-processing [3].

Referencing

Neurochemical profiling requires appropriate referencing. The signal of total creatine (tCr) has been use widely as an internal reference, but because of differences in tCr content between brain regions [13,18], variations in tCr content due to brain development [15,35] and due to neurodegenerative processes [21], using tCr as an internal reference is far from being optimal. Using unsuppressed water signal as a reference is very useful approximation and works extremely well in multiple MRS applications [1-3,9,13-15,21,35-38]. Recently a new referencing approach using the Electric REference To access In vivo Concentrations (ERETIC) method was described [39].

Relaxation

An ultra-short TE and long TR data acquisition approach substantially minimizes relaxation effects and simplifies the “absolute” metabolite quantification. If TE is longer or TR is shorter, correction for T_2 and T_1 relaxation have to be used. In general, each single proton in a molecule has its own relaxation properties, which makes assessment of all T_1 and T_2 values extremely difficult from in vivo spectra. But using a number of simplified assumptions assessment of metabolite T_1 and T_2 can be accomplished [40-42].

III. Examples of neurochemical profiling

The primary goal of neurochemical profiling is to extend the range of quantifiable metabolites and to improve the reliability, i.e., the precision as well as the accuracy of their quantification. Using advances in high-field MR technology and data processing, reliable quantification up to

twenty metabolites is feasible in animal and human brains [1-3,7-9,13-15,18,19,21,36-38]. Quantification of weakly represented metabolites, such as ascorbate [30,43] and glycine [44,45], has been described. Neurochemical profiling was applied in studies of the brain development [15,35], in transgenic mouse models [21,38], in animal models of hypoglycemia [46] and deep anesthesia [47]. The feasibility of extended neurochemical profiling in human brain and the beneficial effect of increased magnetic field was demonstrated in these papers [1-3].

In conclusion, a reliable quantification of extended range of brain metabolites is feasible using MRS methodology. However, the reliability of quantification requires high-quality MR spectra and sophisticated processing tools with optimized prior knowledge.

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