Metabolite Quantification

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Localized proton magnetic resonance spectroscopy (^{1}H MRS) became an important tool for investigating brain metabolism non-invasively. In animal models, high field ^{1}H MRS allows detection of almost 20 metabolites [1-4], also called the neurochemical profile (taurine (Tau), creatine (Cr), phosphocreatine (PCr), phosphocholine (PCho), glycerophosphocholine (GPC), glutamate (Glu), glutamine (Gln), myo-inositol (Ins), ascorbate (Asc), alanine (Ala), aspartate (Asp), γ-aminobutyrate (GABA), glutathione (GSH), N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), glucose (Glc), lactate (Lac), phosphoethanolamine (PE) and glycine (Gly). Since brain metabolite concentrations can vary depending on the type of pathology, the usefulness of ^{1}H MRS has been demonstrated in many brain disorders, e.g., in hepatic encephalopathy, Alzheimer's, Huntington's, and Parkinson's diseases, acute traumatic brain injury, cancer, dementia, etc. [5-6]. The metabolite changes help to understand metabolic processes related to the studied pathologies and/or enable to monitor effect of treatment. At longer echo times (TE above 20 ms), the number of spectral lines, which can be used for quantification, is reduced. As a result, in most in vivo ^{1}H MRS studies only changes of NAA, Cr+PCr, Cho and Lac have been detected. Short-echo-time in vivo ^{1}H MRS spectra (TE=1-20 ms) contain more information due to minimal distortions of multiplets of coupled spin systems such as Glu, Gln, Ins, Glc, Asp, Ala, GABA, Asc, PE and Tau. Accurate and precise quantification of brain metabolites is challenging and depends on several parameters: hardware performance, pulse sequence design, adjustments of acquisition parameters, data processing and quantification strategies. The purpose of data processing is to estimate the signal amplitude or peak area of each metabolite in a given spectrum, which is proportional to the metabolite concentration. Then by using different quantification methods the signal amplitude or peak area is converted in tissue metabolite tissue concentration. Measurements at high magnetic field benefit from increased spectral dispersion and higher signal to noise ratio, which likely improve quantification precision and accuracy [1, 7-8]. However, due to overlap of proton signals from brain metabolites, sophisticated approaches for the separation of the metabolite signals are required. Consequently, metabolite concentrations are usually determined by fitting a measured in vivo ^{1}H MRS spectrum to a linear combination of spectra of individual metabolites (also called metabolite basis set). The metabolite basis set can be obtained either by measuring aqueous solutions of pure metabolites or by quantum-mechanical simulations using known spectral parameters [9].

In the present course an overview of the factors that should be considered when performing and evaluating metabolite quantification is given. The course will be structured in 6 sections:

1. Short description of the time domain and frequency domain algorithms.
2. Factors affecting the quality and reliability of in vivo spectra
3. Key points to consider during the quantification (lineshape correction, macromolecule contribution)
4. Quantification techniques

5. Quantification improvements at high magnetic fields
6. Pitfalls during the data processing

1. <u>Short description of the time domain and frequency domain algorithms</u>

Well-known time and frequency–domain algorithms [10-21] based on extensive prior knowledge are currently used for accurate quantification. The jMRUI software [22] working in the time domain which contains the AMARES [17] and QUEST [12] algorithms will be described and also some advises for fine tuning will be given. In addition, the AQSES algorithm [13] will be shortly presented. Then the frequency domain algorithm LCModel [10-11] and the main functionally will be described. The last algorithm which will be described is TDFDfit [15], an iterative nonlinear least-squares fitting algorithm in the frequency domain using time domain models for quantification of complex frequency domain MR spectra. The error estimates obtained from the model fitting (Cramer Rao lower bounds) will also be discussed [23-24]. Examples of short and long echo-time ^{1}H and ^{13}C NMR spectra [25] acquired at high magnetic field and processed using the above described algorithms will be given (Fig 1).
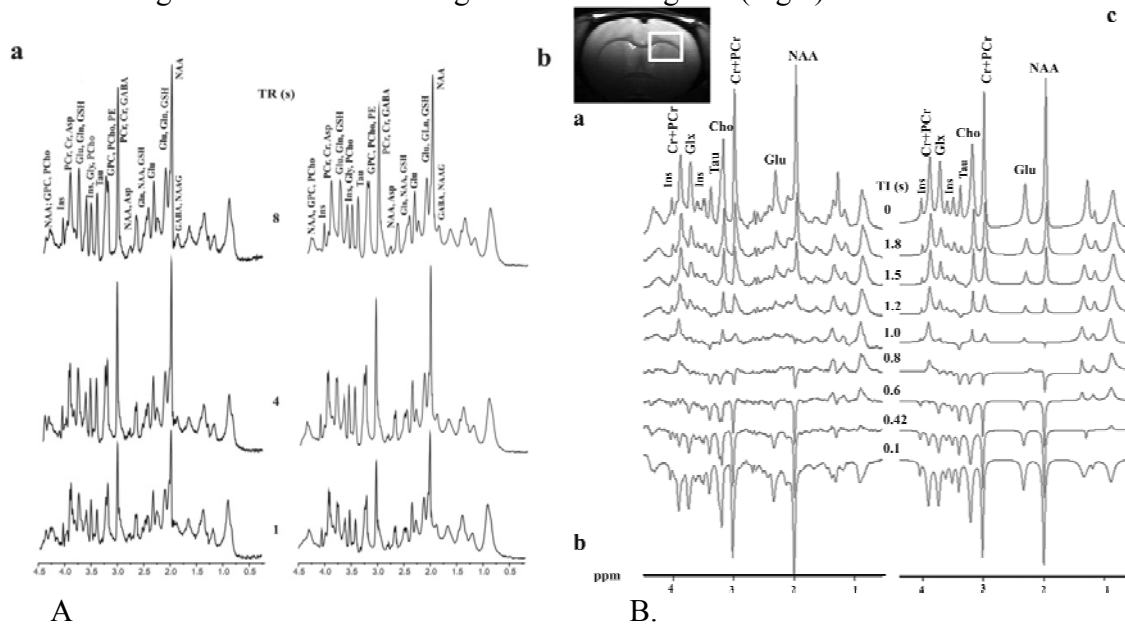

Fig. 1: A. One series of in vivo spectra acquired at 14.1T in the rat brain with different repetition times (TR) and a TE=2.8 ms (a) and the corresponding estimates using LCModel (b). A shifted Gaussian function (gf = 0.08, gfs = 0.04) was used for modest resolution enhancement. No baseline correction or preprocessing for water signal removal was applied; B. Coronal view of a rat brain showing a typical position of a volume of interest (a). One series of in vivo spectra acquired at 14.1T in the rat brain with different inversion times (TI), ranging from 0 to 1.8 s and a TE=20 ms (b) and the corresponding estimates using AMARES (c). For quantification purpose the in vivo data were Lorentzian line broadened with 20 Hz. No preprocessing for water signal removal was applied.

2. <u>Factors affecting the quality and reliability of in vivo spectra</u>

This part of the course will briefly present in some examples the main factors that can lead to poor spectral quality, artifacts and misinterpretation of spectra (i.e outer volume contamination, poor water suppression, eddy currents, etc) [26].

3. Key points to consider during the quantification (lineshape correction, macromolecule contribution)

Lineshape correction

Short echo-time ^{1}H MRS data often contain lineshape distortions due to residual eddy currents and magnetic field inhomogeneities [27-34]. In single voxel spectroscopy, the signal from the whole shimmed volume contributes to one spectrum. The lineshape is mostly given by signal coming from the well-shimmed part of VOI, but the poorly-shimmed (broad) contributions affect (broaden) the bottom part of the line. These lineshape distortions, if left uncorrected, lead to errors in the quantification when using methods that incorporate model function with specific lineshapes [35].

Macromolecule contribution

Spectra measured at short echo-times are further complicated by the presence of macromolecules (broad signals ascribed mainly to cytosolic proteins [36]), which further complicates the quantification. Reliable quantification of the macromolecule signals in short echo-time ^{1}H MRS spectra is particularly important at high magnetic fields for an accurate quantification of metabolite concentrations due to effectively increased spectral resolution of the macromolecule components. Consequently, an error in the macromolecule estimation can lead to substantial errors in the obtained metabolite concentrations [4, 37-38]. In addition, it is well known that changes in the macromolecule concentrations can be considered as disease markers [39-40].

Modelling the macromolecule contributions in an in vivo spectrum can be done using two main approaches. The macromolecule signal is approximated by a mathematical function and then included in the fit [10-11, 41-43], or is separately estimated in a pre-processing step [12-13, 23, 41, 44-47]. It is well known that these procedures provide only a mathematical approximation of the real in vivo macromolecule spectra [37-38, 48]. This mathematical approach can be satisfactory at low magnetic fields. However, more prior knowledge is needed to obtain an accurate estimation of the metabolite concentrations at higher magnetic fields, where the macromolecule spectrum is better resolved. In addition to the mathematical approximation of the macromolecules, a second approach was proposed, which provides the necessary prior knowledge. It is based on a separate acquisition of the in vivo macromolecule spectra using an inversion recovery method (a metabolite-nulled spectrum). Then, during the quantification step the in vivo acquired spectrum of macromolecules is either added to the basis-set [4, 37-38, 49-53] or subtracted from the in vivo signal [48, 54-55]. Small residuals attributed to metabolites are still observed in the metabolite-nulled spectra due to variability in longitudinal relaxation times of metabolites (T_1). Thus, knowledge of the T_1 relaxation times of metabolites is required for identification of these residual metabolite signals and subsequent removal by postprocessing.

Several studies compared the simulated and experimentally obtained spectra of macromolecules in the quantification of in vivo proton spectra acquired at different magnetic fields and quantified using different algorithms [37-38, 48]. They showed that an experimental estimation of the macromolecule spectrum can improve the quality of the fit. The absence of the measured macromolecule spectrum in the database can led to a large and unpredictable bias in concentrations of many metabolites. Even at the highest magnetic fields available for in vivo experiments, spectral overlap of proton signals from brain metabolites and macromolecules still

require an experimental assessment of macromolecular contribution to the proton spectrum for accurate metabolite quantification (Fig 2 and 3). The main reason of the poor mathematical estimation of macromolecules at high magnetic fields is due to the fact that the largely field-independent linewidth of the signals of macromolecules increasingly approaches that of metabolites. As can also be seen from references [1, 37-38, 56], an effectively increased spectral resolution of the macromolecule components can be observed at higher magnetic field strengths, making the estimation of the macromolecules by the means of mathematical approximations even more difficult.
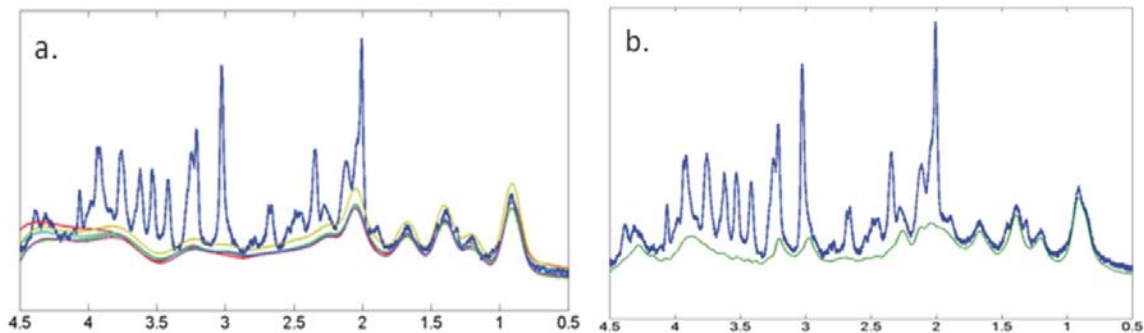

Fig. 2: A representative 14.1T spectrum (320 averages) of rat brain measured from a VOI=3x4x4mm³ (blue line) combined with a) the built-in LCModel spline baselines obtained from fitting spectra of five rats, which are plotted in different colors; and with b) the measured in vivo macromolecule spectrum (green line). Note the difference in the macromolecules estimation around 2 ppm, 3.0 ppm, 3.2 ppm and 4 ppm.

4. Quantification techniques

We saw until now how to estimate the signal amplitude or peak areas of each metabolite in a given spectrum. Since the signal amplitude or peak area is proportional to the metabolite concentration, by using different quantification methods we can convert it in tissue metabolite concentration ($mmol/kg_{ww}$). Two main approaches will be discussed for the absolute quantification: the external concentration reference used mainly for X nuclei and the internal concentration reference used mainly for proton data [57-65]. Corrections to be done in case of different pathologies will also be discussed.

5. Quantification improvements at high magnetic fields

Several studies have investigated the increased spectral dispersion, the improvement in signal to noise ratio and the improved quantification precision and accuracy with increasing Bo [1, 7-8, 56, 66-70]. The improvements are important for low concentrated metabolites with overlapping spectral lines and for compounds with complex J-coupled spectral patterns. In this part of the course we will briefly discuss the improvements in human brain metabolite quantification at 7T and also those obtained at 14T in rat brain.
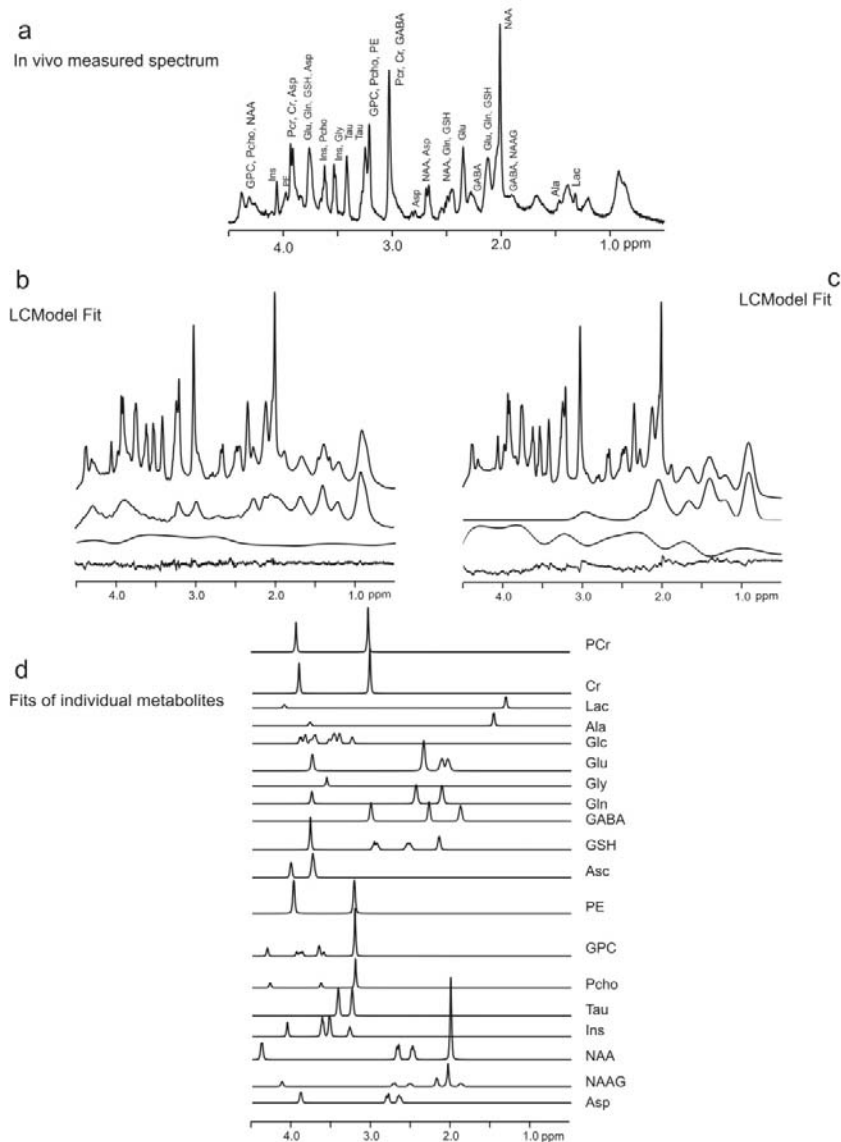
Fig 3: The LCModel analysis of a representative 14.1 T spectrum. The measured in vivo spectrum in the rat brain at 14.1T is shown in (a). The corresponding LCModel fits using the measured macromolecule spectrum and the built-in LCModel spline baseline are also displayed in (b) and (c), respectively. The traces below represent from top to bottom, measured (b) or modeled (c) macromolecules, residual baseline and the difference between the measured and fitted data (also called residue of the quantification). The fits of the individual metabolites are plotted in (d).

6. Pitfalls during the data processing

At the end of the course I will briefly present in examples some general pitfalls during data processing for each quantification algorithm: bad estimation of the macromolecules, bad assignment of the metabolites, etc.

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