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Evaluation of nuclear magnetic resonance spectroscopy variability

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Abstract **Introduction**: The intrinsically high sensitivity of Magnetic Resonance Spectroscopy (MRS) causes considerable variability in metabolite quantification. In this study, we evaluated the variability of MRS in two research centers using the same model of magnetic resonance image scanner. **Methods**: Two metabolic phantoms were created to simulate magnetic resonance spectra from in vivo hippocampus. The phantoms were filled with the same basic solution containing the following metabolites: N-acetyl-aspartate, creatine, choline, glutamate, glutamine and inositol. Spectra were acquired over 15 months on 26 acquisition dates, resulting in a total of 130 spectra per center. **Results**: The phantoms did not undergo any physical changes during the 15-month period. Temporal analysis from both centers showed mean metabolic variations of 3.7% in acquisitions on the same day and of 8.7% over the 15-month period. **Conclusion**: The low deviations demonstrated here, combined with the high specificity of Magnetic Resonance Spectroscopy, confirm that it is feasible to use this technique in multicenter studies in neuroscience research.

Keywords Magnetic resonance spectroscopy, Quality assurance, Brain metabolites.

Introduction

Localized Magnetic Resonance Spectroscopy (MRS) is a nuclear magnetic resonance-based technique that allows *in vivo* non-invasive measurements of biomolecules. The feasibility of acquiring quantitative biochemical information from a certain region of interest makes this technique widely used in magnetic resonance (MR) centers, especially in neuroscience. Multicenter studies have a major role in increasing the power of neuroscience studies because they allow the acquisition of large data sets in short periods of time and can also show that a method is robust and has clinical potential. Several studies have analyzed MRS variability considering different aspects, such as voxel localization (Bovée et al., 1998; Burtscher et al., 1999), quantification techniques (De Beer et al., 1998), spectral quality and specific artifacts (Kreis, 2004). However, few studies have evaluated the spectral stability over time over a period of months or years. Despite the good specificity of MRS, its intrinsically high sensitivity causes considerable variability, which can affect data quality. The aim of this study was to evaluate the reproducibility of MRS and the feasibility of using data from different facilities in a multicenter study. To avoid the inherent variability of brain metabolism, phantoms were created to simulate MR spectra from *in vivo* human hippocampus.

Methods

Two spherical glass phantoms of approximately 300 mL (Figure 1) and 8.5 cm in external diameter were created and fully filled with a base solution containing the metabolite concentrations described in Table 1.

Sodium salts (Na_2HPO_4 and NaH_2PO_4) were used to create a buffer solution with a pH of 7.2, and sodium azide (NaN_3) was used to prevent microorganism proliferation. The metabolic concentrations of the phantom solution reproduced the basic neurochemical profile from a healthy human brain hippocampus (Govindaraju et al., 2000).

Data acquisition was performed in two identical 3T MR scanners (Philips, Achieva) from two different centers, named here as center A and center B. The phantoms were placed inside a thermal container filled with water during spectra acquisition to reduce the chemical shifts created by temperature changes and susceptibility effects. Water suppression was achieved using a CHESS scheme. A PRESS sequence with TE/TR=35/1500 ms, a bandwidth of 1725 Hz, 2048 points, 128 averages, second order shimming and a volume of interest (VOI) of 40x15x10 mm^3 placed in the center of the phantom was used for spectra acquisition. One spectrum without water suppression was acquired for absolute concentration quantification. The motivation for testing the protocol described above was its common use in clinical routines. Spectra were acquired over 15 months in both centers, comprising 26 acquisition dates for a total of 130 spectra (5 spectra from each acquisition date without removing the phantom from the scanner). The signal-to-noise ratio (SNR) and full-width at half maximum (FWHM) were estimated in LCModel (Provencher, 2014).

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Briefly, the SNR from each spectrum was calculated by the software as the ratio of the difference between the maximum from the analysis window and the adjusted baseline and two times the square root of the residue from the analysis (for more information on the SNR and FWHM calculations, see Provencher, 2014). The standard parameters from LCModel were used for data processing in one of the centers, while the other center optimized data processing for *in vitro* spectra. Spectra quantification and the calculation of absolute concentrations were performed automatically using the software LCModel. Briefly, the software estimates the absolute concentrations using the unsuppressed water signal as an internal reference (Barreto and Salmon, 2010; Graaf, 2007). The absolute concentrations were not corrected for relaxation effects because the main goal of this study was not to evaluate variability from the quantification process. The estimated concentrations were defined as the mean concentrations from all acquisitions from each center. The intra coefficient (Intra Coef., Table 2) was defined as the mean coefficient of variation (CV) calculated from the CVs of each acquisition date and demonstrates the variability on each acquisition date. The inter coefficient (Inter Coef., Table 2) was defined as the CV calculated from all the acquisitions and demonstrates the technique's variability over the entire 15-month period. The maximum deviation (Max. deviation, Table 2) was defined as the maximum variation for each metabolite with respect to its mean value over the entire analysis period.

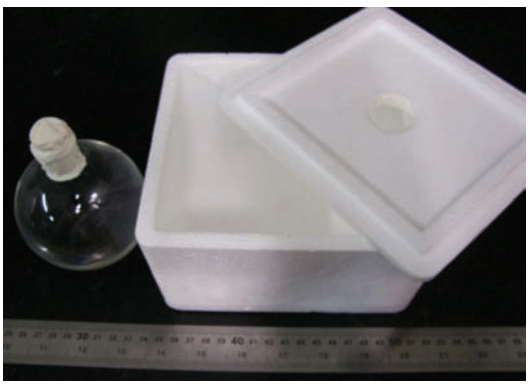

Figure 1. Image of the multimetabolic phantom.

Table 1. Metabolic concentrations present in the phantoms.

	Chemical component	Concentrations (mM)
Metabolites	N-Acetylaspartate (NAA)	10
	Creatine (Cr)	8
	Choline (Cho)	2.5
	Inositol (Ins)	8
	Glutamate (Glu)	10
	Glutamine (Gln)	5
Base solution components	Na_2HPO	72
	NaH_2PO_4	28
	NaN_3	15

Results

No metabolic changes were detected over the 15 months of analysis, and the phantom did not suffer any physical changes, such as color changes or salt precipitation, that would indicate deterioration of the solution. This result was confirmed by the quality of the spectra acquired, which did not change over time (Figure 2). The VOI positioning variability was 0.97, 0.28, and 0.68 cm in the anteroposterior, right-left, and head-feet directions, respectively.

The mean SNR and FWHM for the spectra acquired in one of the centers were 16.3±2.1 and 0.025±0.011 ppm, respectively. No relationship between the SNR and FWHM was observed, suggesting that water suppression was possibly the main source of SNR variability (Figure 3). These findings were very similar between centers. The maximum deviation in the

Table 2. Coefficient of variation (Inter Coef.) and maximum variation with respect to the mean value (Max. Deviation) from all measurements, the mean coefficient of variation from each acquisition date (Intra Coef.), the mean estimated concentrations and the phantom concentrations.

	NAA	Cho	Cr	Glu+Gln	Glu	Ins
Phantom conc. (mM)	10	2.5	6	15	10	8
			Center A			
Intra Coef. (%)	2.2	2.4	3.3	3.7	4.2	3.9
Inter Coef. (%)	6.5	9.8	10.9	8.1	7.8	12.4
Max. Deviation (%)	15.6	20.4	20.6	23.2	22.9	31.5
Estimated conc. (mM)	10.6	2.1	6.3	13.7	10.4	8.7
			Center B			
Intra Coef. (%)	2.4	2.7	4.3	7.1	4.6	3.7
Inter Coef. (%)	4.9	6	7.8	8.1	14	8.3
Max. Deviation (%)	11.8	12.7	19.1	40.1	29	21
Estimated conc. (mM)	10.3	2.6	6.9	10.5	8.8	7

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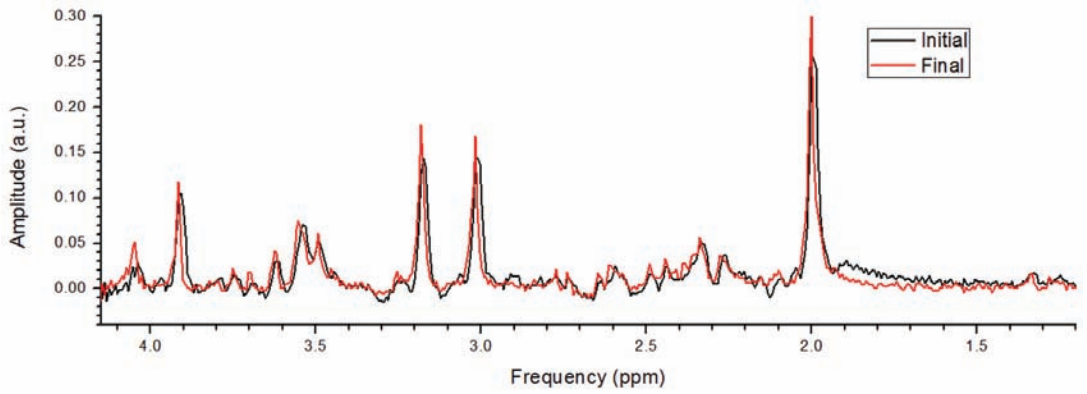

Figure 2. Spectra obtained during the first (black line) and last (red line) acquisition from center A.

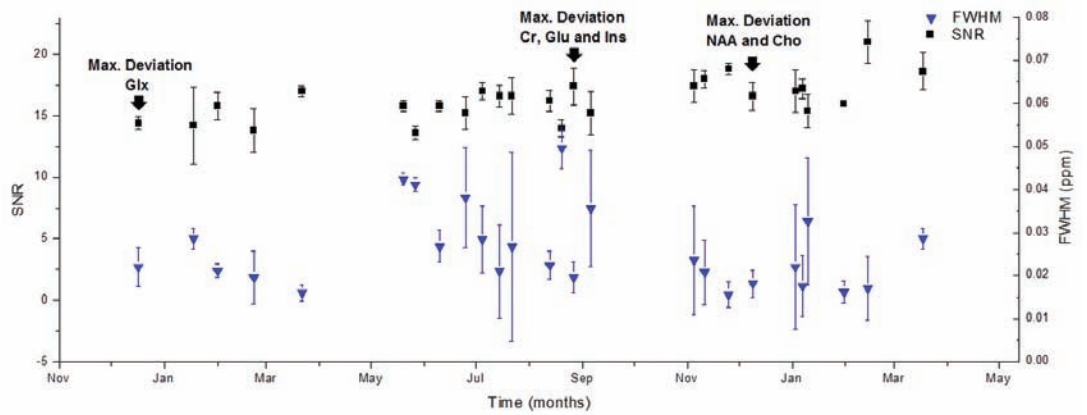

Figure 3. Temporal evolution of the SNR and FWHM for the spectra acquired in center A. Arrows indicate when the maximum deviation occurred.

metabolites occurred on three different dates, which shows that statistical fluctuations were responsible for the variability, not deterministic fluctuations related to the scanner functioning.

Spectra acquired on the same date resulted in quantifications that showed Intra coefficients below 4.6%, except for the sum (Glu+Gln), which was 7.1% in one of the centers. Over 15 months, the quantification of all spectra returned estimated concentrations with a maximum Inter coefficient of 14% for all metabolites. A detailed description is presented in Table 2.

The time series for the most relevant metabolites for the clinical routine (NAA, Cho and Cr) were stable and showed no changes over time. Small fluctuations in all three metabolites were visible on different dates (Figure 4), possibly caused by factors affecting the entire spectrum. Thus, the use of internal references, i.e., normalization to Cr or the water signal, allowed comparison between different subjects and also removed the effect of small longitudinal fluctuations.

Discussion

The intra coefficient was smaller than the Inter coefficient for all metabolites because acquisitions from the same date were not affected by the variability due to different positionings and were less sensitive to instabilities related to scanner functioning. Therefore, a comparative study between patients and healthy subjects would benefit from smaller variability if the acquisitions from both groups were performed on the same date (due to the low Intra Coef.) and over the shortest period possible (in order to avoid a high Inter Coef.).

NAA was the most stable metabolite (Inter coefficients of 4.9% and 6.5%), and its estimated concentration was the closest to the real value (3 and 6% higher than the phantom's concentration). The least accurate estimated concentration was Glu+Gln, with a mean value approximately 30% lower than the phantom's concentration. Ins and Glu were the most variable, as shown by the Inter Coef. from centers

A and B, possibly due to intense J coupling, which makes quantification less accurate.

Considerable differences for all metabolites (ranging from 2 to 25%) were observed between the absolute concentrations estimated in both centers. These differences in calculated concentrations were higher than the Inter coefficient for certain metabolites (Glu, Glu+Gln, Ins and Cho) and were most likely caused by different post-processing approaches in LCModel (example: to include or not the metabolites absent from the phantom solution in the LCModel spectral dataset). Thus, additional precautions should be taken not only during acquisition but also during data processing in multicenter studies.

The maximum deviations of the metabolites were due to sporadic events on specific dates; these high deviations were up to three times larger than the Inter coefficients. Larger maximum deviations were observed in Ins (31.5%) from center A and in the sum Glu+Gln (40.1%) from center B. The deviations from center A were larger than the deviations from center B, except for Glu and the sum Glu+Gln.

Two types of MRS artifacts were observed in a small number of spectra, as shown in Figure 5.

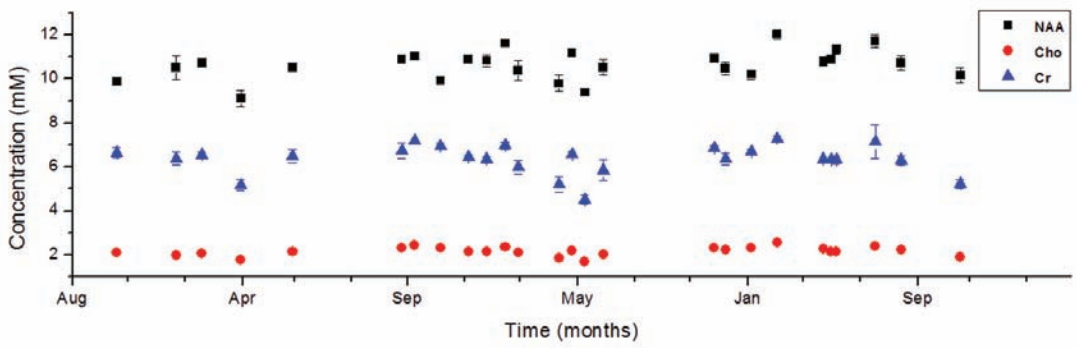

Figure 4. Time series from NAA, Cho and Cr over 15 months from center A.

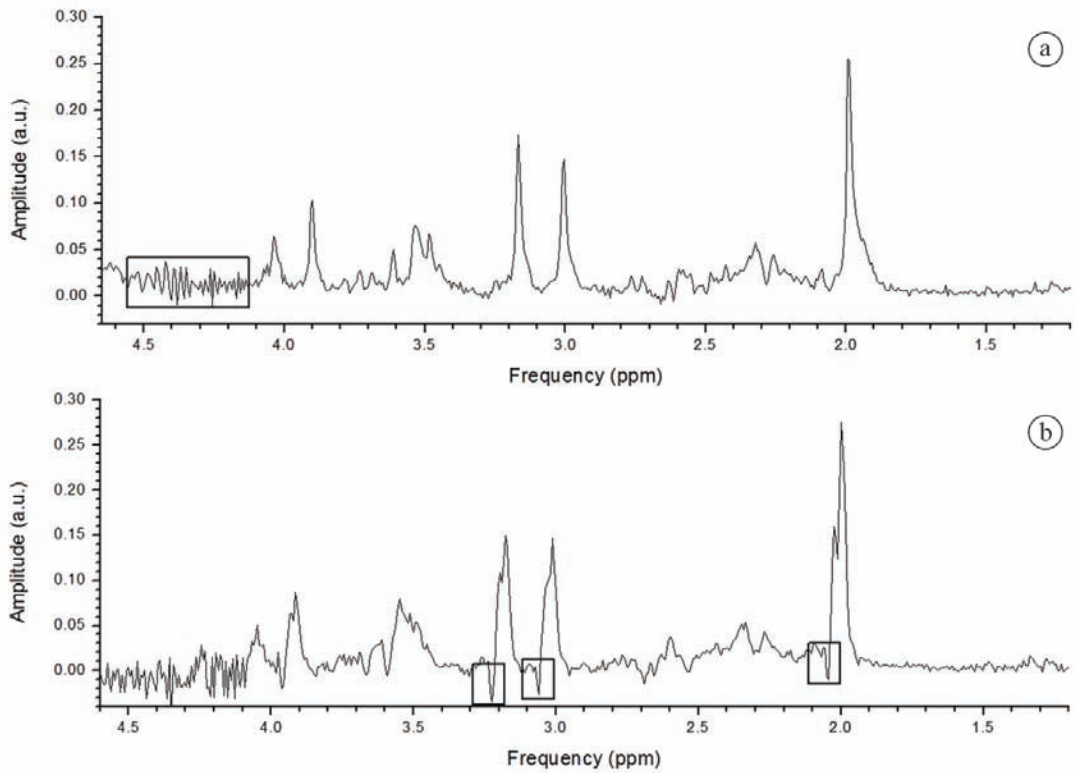

Figure 5. Observed artifacts: a) Spurious echoes created by the insufficient amplitude of spoilers and local inhomogeneities from the static magnetic field. b) The unbalanced amplitude of the spoiler gradients creates small phase distortions in the base of resonances.

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The artifact from Figure 5a was caused by local inhomogeneities in the static magnetic field and insufficient amplitude of the crusher gradients, which combined can lead to the refocusing of spurious echoes (Kreis, 2004). This artifact can be easily removed during post-processing by using apodization with an exponential function. Figure 5b shows an artifact caused by an insufficient amplitude of crusher gradients, which shifts the position of the maximum amplitude and creates observable negative areas in the bases of large resonances. Despite the presence of these artifacts in the *in vitro* environment, these most likely would not be visible in *in vivo* spectra due to smaller signal amplitudes and shorter T_2.

NAA is an abundant amino acid in the nervous system, and it can be used as a neuronal density indicator in the brain. During reversible incomplete ischemia (Brulatout et al., 1996), brain damage (De Stefano et al., 1995) and multiple sclerosis (Tsai and Coyle, 1995), it is frequently observed that the degree of neuronal loss correlates with the NAA decrease. Cho is an essential nutrient for the synthesis of acetylcholine and the formation of cellular membranes. Cho concentration changes in the human brain can reveal abnormalities in membrane metabolism during ischemia, Alzheimer's disease and multiple sclerosis. In addition, increased Cho levels are an excellent indicator of demyelination. Tumors also show elevated Cho caused by the high rates of cell division, which increase the fractional volume of membranes and phospholipids in a tissue. Cr is an essential component for energy metabolism in muscle tissue, but it is also present in smaller concentrations in the brain. It is produced from phosphocreatine after the transfer of an inorganic phosphate ion for ATP synthesis, which plays an important role as a fast metabolic pathway for ATP synthesis during the first seconds of increased energetic demand. The Cr concentration in the brain remains highly stable at different ages and in many diseases (Saunders et al., 1999), which makes it an excellent internal reference for longitudinal studies (Govindaraju et al., 2000).

It is worth mentioning that the metabolic changes caused by disease can be highly variable and depend on the nature of the disease. In epilepsy, Colon et al. (2010) observed a 15% decrease in the NAA/Cr ratio in the affected region in comparison to the contralateral hemisphere. In tumors, Dowling et al. (2001) reported that Cho can increase by up to 60%, and NAA can be reduced by 80%. Despite these high metabolic changes in epilepsy and tumors, other diseases cause much milder changes that are on the edge of the detection threshold using MRS. Chang et al. (2003) reported a 5% decrease in the NAA/Cr ratio in the dorsolateral prefrontal cortexes of children with a family history of bipolar disorder. Such metabolic changes are close to the intrinsic *in vitro* variability measured in this study. A large sample size is required in order to accurately measure equivalent or inferior metabolic changes in comparison to the intrinsic variability reported here.

Artifacts were identified on a few acquisition dates; however, their low occurrence and low amplitudes did not affect spectral quantification. The *in vitro* temporal stability of the metabolites decreased in the order: NAA, Glu, Glu+Gln, Cho, Cr, Ins and NAA, Cho, Cr, Glu+Gln, Ins, Glu in centers A and B, respectively. The estimated NAA concentration was the most accurate, reliable and reproducible over the experiments. Temporal analysis demonstrated the feasibility of comparing results from short- or long-term multicenter studies, which benefit from the low deviations of MRS.

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References

Barreto F, Salmon C. Quantificação metabólica absoluta in vitro por espectroscopia de ressonância magnética. Revista Brasileira de Física Médica. 2010; 4(1):83-8.

Bovée W, Canese R, Decorps, M, Forssell-Aronsson E, Le Fur Y, Howe F, Karlsen O, Knun A, Kontaxis G, Kügel H, McLean M, Podo, F, Slotboom J, Vikhoff B, Ziegler A. Absolute metabolite quantification by in vivo NMR spectroscopy: IV. Multicentre trial on MRSI localization tests. Magnetic Resonance Imaging. 1998; 16(9):1113-25. http://dx.doi.org/10.1016/S0730-725X(98)00120-9

Brulatout S, Méric P, Loubinoux I, Borredon J, Corrèze J, Roucher P, Gillet B, Bérenger G, Beloeil J, Tiffon B, Mispelter J, Seylaz J. A one-dimensional (proton and phosphorus) and two-dimensional (proton) in vivo NMR spectroscopic study of reversible global cerebral ischemia. Journal of Neurochemistry. 1996; 66(6):2491-9. PMid:8632174. http://dx.doi.org/10.1046/j.1471-4159.1996.66062491.x

Burtscher I, Johasson E, Holtas S, Stahlberg F. Quality assessment of localization technique performance in small volume in vivo 1H MR spectroscopy. Magnetic Resonance Imaging. 1999; 17(10):1511-9. http://dx.doi.org/10.1016/S0730-725X(99)00076-4

Chang K, Adleman N, Dienes K, Barnea-Goraly N, Reiss A, Ketter T. Decreased N-acetylaspartate in children with familial bipolar disorder. Biological Psychiatry. 2003; 53(11):1059-65. http://dx.doi.org/10.1016/S0006-3223(02)01744-4

Colon A, Hofman P, Ossenblok P, Jansen J, Ter Beek L, Berting R, Stam C, Boon P. MRS-lateralisation index in patients with epilepsy and focal cortical dysplasia

or a MEG-focus using bilateral single voxels. Epilepsy Research. 2010; 89(1):148-53. PMid:20022471. http://dx.doi.org/10.1016/j.eplepsyres.2009.11.007

De Beer R, Van den Boogaart A, Cady E, Graveron-Demilly D, Knin A, Langenberger K, Lindon J, Ohlhoff A, Serrai H, Wylezinska-Arridge M. Absolute metabolite quantification by in vivo NMR spectroscopy: V. Multicentre quantitative data analysis trial on the overlapping background problem. Magnetic Resonance Imaging. 1998; 16(9):1127-37. http://dx.doi.org/10.1016/S0730-725X(98)00121-0

De Stefano, N, Matthews P, Arnold D. Reversible decreases in N-acetylaspartate after acute brain injury. Magnetic Resonance in Medicine. 1995; 34(5):721-7. PMid:8544693. http://dx.doi.org/10.1002/mrm.1910340511

Dowling C, Bollen A, Noworolski S, McDermott M, Barbaro N, Day M, Henry R, Chang S, Dillon W, Nelson S, Vigneron D. Preoperative Proton MR Spectroscopy Imaging of brain tumors: correlation with histopathologic analysis of resection specimens. American Journal of Neuroradiology. 2001; 22:604-12. PMid:11290466.

Graaf, R. In: Vivo NMR. Spectroscopy: principles and techniques. 2nd ed. Chichester: John Wiley & Sons; 2007.

Govindaraju V, Young K, Maudsley A. Proton NMR chemical shifts and coupling constants for brain metabolites. NMR in Biomedicine. 2000; 13(3):129-53. http://dx.doi.org/10.1002/1099-1492(200005)13:3<129::AID-NBM619>3.0.CO;2-V

Kreis R. Issues of spectral quality in clinical 1H-magnetic resonance spectroscopy and a gallery of artifacts. NMR in Biomedicine. 2004; 17(6):361-81. PMid:15468083. http://dx.doi.org/10.1002/nbm.891

Provencher S. LCModel & LCMgui user's manual. 2014. [acesso em 2014 jun 06]. Disponível em: http://s-provencher.com/pub/LCModel/manual/manual.pdf.

Saunders D, Howe F, Van den Boogaart A, Griffiths J, Brown M. Aging of the adult human brain: in vivo quantification of metabolite content with proton magnetic resonance spectroscopy. Journal of Magnetic Resonance Imaging. 1999; 9(5):711-6. http://dx.doi.org/10.1002/(SICI)1522-2586(199905)9:5<711::AID-JMRI14>3.0.CO;2-3

Tsai G, Coyle JT. *N*-Acetylaspartate in neuropsychiatric disorders. Progress in Neurobiology. 1995; 46(5):531-40. http://dx.doi.org/10.1016/0301-0082(95)00014-M

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