Necessity of tissue volume composition correction for internal referencing

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Introduction: In order to compare obtained metabolite signals from MRS measurements with other MRS studies and in the best case with other biochemical measurements, it is beneficial to determine absolute concentrations. In most investigations metabolite intensities are referenced to assumed amounts of internal water (IWR)[1] or total Creatine (tCr), resulting in concentrations expressed in mMol per liter [mM/l] of brain volume. However, since the water and tCr concentrations as well as the relaxation properties differ between grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF), these calibration methods have to be used with care and rely on several necessary corrections, when used in heterogeneously composed voxels as previously proposed for IWR[2,3]. ***In this study*** an adopted method for heterogeneous voxel composition correction for the internal creatine reference standard is proposed and concentrations obtained in heterogeneous voxels with IWR and tCr as reference were compared, to see whether the corrections lead to similar metabolite concentrations for both references.

Methods: PRESS spectra were measured in 18 healthy controls (HC). The voxel with a size of 16 ml was placed in the right dorsolateral prefrontal cortex (rDLPFC). The settings for the measurement were as follows: 3T Achieva (Philips Healthcare, Best), TE/TR = 25/1600 ms, 32 averages, VAPOR water and interleaved inner volume suppression was used. For IWR additional 8 interleaved scans were acquired without water suppression. The obtained spectra were fitted using LC Model using simulated basis sets. Tissue segmentation was performed on a T_1-weigthed 3D image using SPM8, resulting in volume fractions for the three different tissue types (f_{GM},f_{WM},f_{CSF}), which were also used to calculate the molal fractions *fm.* For the calculation of moles of metabolite m per volume of brain tissue c_m [mM/l], the fitted resonance areas S_m were referenced to tCr ($c_{m,tCr}$) or to internal water (c_{m,H_2O}). The formulas used for both references are shown below [Eq. 1 and 2] and were slightly adapted from Ref.[2]. Only the fitted resonance area of tCr and the internal water in these ratios were corrected with the relaxation attenuation factors R, using relaxation times summarized in Table 1 and taken from Ref.[3,4,5]. The relaxation attenuation of the metabolites was omitted, leading to slightly overestimated concentration values. The ratio to internal water was corrected for partial volume effects due to CSF [second factor in Eq. 1]. Relative water densities of (α_{GM}=0.78, α_{WM}=0.65, α_{CSF}=0.97)[1] were used, whereas c_{H_2O} was set to 55'126 [mM/l], the pure water concentration at 37°C. The last factor in Eq. 2 is an estimation of the tCr concentration based on the voxel composition in each subject, with $c_{tCr,GM}$= 9.59 [mM/l] and $c_{tCr,WM}$ = 4.83 [mM/l] taken from Ref.[2]. Concentrations were compared for the metabolites tNAA, mI, Glu+Gln and tCho. The concentration of tCr was determined using IWR only, here relaxation attenuation was taken into account for both metabolite and reference standard. In addition the correlation of the obtained $c_{m,tCr}$ with the c_{m,H_2O} values was investigated and compared to the correlation of the simple, uncorrected ratios S_m/S_{tCr} and S_m/S_{H_2O}.

[Eq. 1] $$c_{m,H_2O} = \frac{S_m}{S_{H_2O}} \cdot \frac{1}{(1-f_{csf})} \cdot \frac{n_{H_2O}}{n_m} \cdot \left(f_{GM}\alpha_{GM}R_{H_2O,GM} + f_{WM}\alpha_{WM}R_{H_2O,WM} + f_{CSF}\alpha_{CSF}R_{H_2O,CSF}\right) \cdot c_{H_2O}$$

[Eq. 2] $$c_{m,tCr} = \frac{S_m}{S_{tCr}} \cdot \frac{n_{tCr}}{n_m} \cdot \left(fm_{tCr,GM}R_{tCr,GM} + fm_{tCr,WM}R_{tCr,WM}\right) \cdot \left(\frac{f_{GM}c_{tCr,GM}+f_{WM}c_{tCr,WM}}{f_{GM}+f_{WM}}\right)$$

Results and Discussion: The spectral quality was good [Fig. 1] and the metabolites could be fitted with low mean Cramér-Rao lower bounds over all subjects (tCr: 3.5, tNAA: 3.5, mI: 5, Glu+Gln: 7, tCho: 6.5). With the described corrections, similar mean concentrations for all metabolites were obtained, with no significant differences between the two reference standards as shown in [Fig. 2, A+B]. Without the partial volume correction for the IWR [C in Fig.2], and obviously by just assuming a pure GM tCr concentration [D in Fig.2], this could not have been realized, underlining the necessity of these corrections. Using the IWR for the estimation of the tCr concentration we obtained a mean concentration of 7.8 ± 0.6 [mM/l] over all subjects. This value is in good agreement with a tCr concentration of 7.7 [mM/l] predicted by literature values for $c_{tCr,GM}$ and $c_{tCr,WM}$ given above and using the average voxel composition ($f_{GM,mean}$=0.54, $f_{WM,mean}$=0.36). However the correlations of the metabolite ratios to tCr with the ratios to water [Fig. 3] are rather small and did surprisingly further decrease with the applied corrections from Eq. 1 and 2. This decrease in correlation was found for all metabolites, indicating that in this particular study the corrections did decrease the agreement between two reference standards within the same subjects. This is potentially explained by movement leading to inaccurately determined voxel compositions or potential individual deviations from the assumed relaxation properties and reference concentrations in the different tissue types. ***In conclusion***, the mean metabolite concentrations are only in agreement for both reference standards IWR and tCr, when the tissue composition corrections are applied.

Table 1	y	T_1[ms]	T_2[ms]
H_20	GM	1331	80
$R_{H_2O,y} = \exp\left(-\frac{TE}{T_{2,y}}\right) \cdot \left(1 - \exp\left(-\frac{TR}{T_{1,y}}\right)\right)$	WM	832	110
	CSF	4160	500
tCr	GM	1460	152
$R_{tCr,y} = \exp\left(-\frac{TE}{T_{2,y}}\right) \cdot \left(1 - \exp\left(-\frac{TR}{T_{1,y}}\right)\right)$	WM	1240	156

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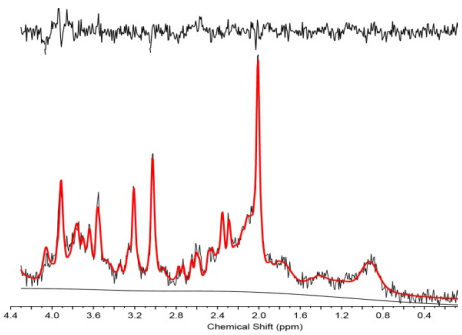
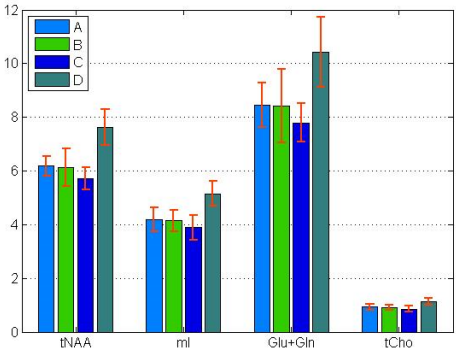
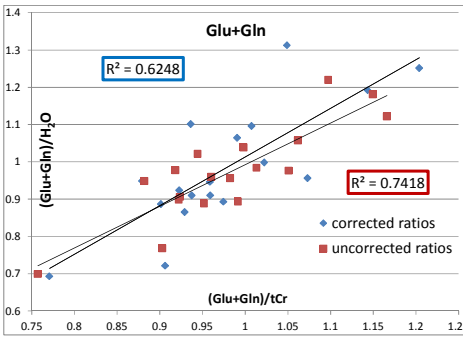


[Figure 1] *Example spectrum acquired in this study. The red line indicates the LCModel fit, the underlying black line indicates the measured signal. Above the residuum is shown.*



[Figure 2] *Mean metabolite concentrations averaged over all subjects in [mM/l] obtained with IWR c_{m,H_2O}(A) or internal tCr $c_{m,tCr}$(B). For C the partial volume correction in Eq. 1 was omitted. For D a pure GM tCr concentration was assumed instead of using the last factor in Eq. 2.*



[Figure 3] *The red squares represent the Glu+Gln level measured in a subject referenced to tCr on the x-axe and to water on the y-axe. The blue squares indicate the corrected ratios according to Eq. 1+2. All ratios were scaled with the mean value, in order to plot them in one graph. The linear correlation between these ratios is indicated by the black line and measured by R^2. It is obvious that the obtained values without correction stronger correlate.*