Astounding reproducibility of cerebral phenylalanine levels as determined by ^{1}H-MR spectroscopy

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Introduction

The reproducibility of metabolite content determined by ^{1}H MR spectroscopy (MRS) has been determined in a number of studies for the standard metabolites. Depending on the metabolite of interest and details of data acquisition, processing and fitting, it usually varies from a few to 20 and more percent, which usually translates to half a millimol/kg of proton density at best. When studying low concentration metabolites, like phenylalanine (Phe), where the tissue content of interest can be below 100 µmol/kg, All relevant factors in the MRS methods have to be optimized to reach the best possible reproducibility. The current work was directed at exploring the limits by determining scan by scan reproducibility for a single voxel MRS technique optimized for determination of cerebral Phe content at 1.5T.

Methods and Subjects

34 healthy controls (aged 6-48 years) and 20 patients with phenylketonuria (PKU) (11-33 y) were investigated using a protocol focused on the downfield part of the spectrum [1]. Patients were examined in 3 independent sessions, 2 of which performed back-to-back to prevent physiologic differences (levels of Phe and other large neutral amino acids (LNAA) co-transported by the same blood-brain-barrier carrier).

Spectra were recorded on a clinical 1.5T MR scanner (Signa, GE) using a quadrature head coil. Most data acquisition and processing steps were described in Ref. [1]. In brief: PRESS localization (20ms TE, 2.0s TR, 256 acquisitions/spectrum, 3 spectra per subject and session with manual reoptimization of shim and water suppression when needed, supraventricular ROI of ~70 cm^3) including non-water-suppressed scans for referencing (eddy current correction, compartmentation information, quantitation standard). Repositioning included angulation of the head and repositioning of the ROI using anatomical landmarks. Spectral fitting (Fig. 1) was performed with TDFDfit [2]. An averaged control spectrum from adult subjects was used to set up the fitting model: 10 Voigt lines for the normal downfield spectrum, the metabolite pattern for Phe, 1 line for residual water, a pattern for the upfield metabolites. In the final analysis to determine Phe content in all subjects, only few variables were fitted (amplitudes of Phe, the downfield baseline as a whole and the upfield pattern, as well as an overall frequency shift, and amplitude, phase and width of the water line). The phase was defined from the water scans, which were also used to define the line width and shape [2]. Reproducibility was judged from mean standard deviations (SD) between spectra and between mean results from different sessions. 2-way ANOVA tested interindividual differences.

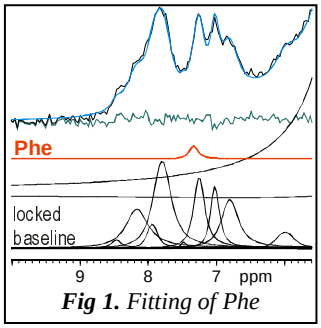

Fig 1. *Fitting of Phe*

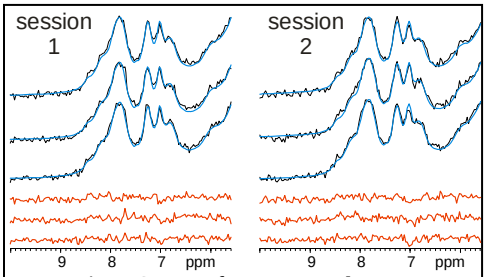

Fig 2. *Spectra from repeated exams*

Results

Fig 2. shows experimental and fitted spectra from a 15 y old PKU subject (0.24 mM blood Phe) from two back-to-back sessions. The average CRMVB obtained from all patients was 0.008 mmol/kg. The averaged SD for cerebral Phe from the 3 repeated spectra was 0.016 mmol/kg. The mean SD for Phe from independently repeated sessions was a mere 0.007 mmol/kg, which corresponds to the minimum expected from variance between spectra. This translates to 35 µmol/kg in proton density. The SD for Phe determined on different dates with different blood Phe levels is larger (0.029 mmol/kg). Fig 3 contains a plot of brain vs. blood Phe levels (data from identical subjects connected by a line; for some, the difference is too small to be noticed) providing an impression of reproducibility in the context of overall variations. ANOVA proves individuality of blood/brain Phe ratios ($p<<0.001$ for back-to-back repetition, $p=0.01$ for delayed rescan) while there is no significant difference between sessions.

Discussion & Conclusion

Brain Phe determined by ^{1}H MRS in independent sessions was found to vary by only 7 µmol/kg. Many optimized factors made such low variation possible: ROI size, repositioning accuracy, stable quantitation, use of maximal prior knowledge, incl. lineshape. Increased SD for later repetitions is likely due to physiologic reasons (varying blood Phe and LNAA content). The small size of the variation in brain/blood Phe ratios between different PKU patients and the confounding influence of LNAA levels does not seem to warrant recommendation of individual diets based on ^{1}H MRS.

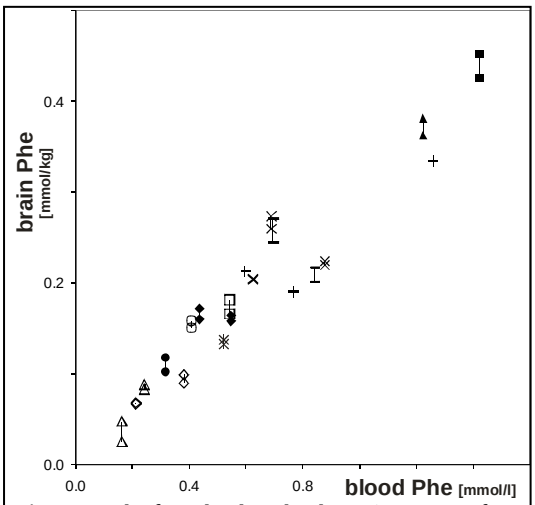

Fig 3. *Results from back-to-back sessions. Data from same subject connected by a line*

References

1. Pietz J et al. J Clin Invest. 1999;103:1169. **2.** Slotboom J et al. MRM 1998;39:899. **3.** Hofmann L et al. MRM 2002;48:440

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