Localized ^{1}H-MRS of brain phenylalanine in adults with phenylketonuria

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Target audience - This work is relevant to clinicians and researchers interested in inborn errors in metabolism in general, and to researchers with an interest in brain ^{1}H-MRS and/or phenylketonuria (PKU) in particular.

Purpose - PKU is an inborn error in hepatic phenylalanine hydroxylase, an enzyme that catalyzes the conversion of phenylalanine (Phe) into tyrosine (Tyr). This amino acid metabolism deficiency results in elevated plasma Phe concentrations. Untreated PKU can lead to intellectual disability. Restriction of dietary Phe intake very soon after birth until adulthood prevents most of the neuropsychological complications, but early-treated subjects may still experience significant neurocognitive sequelae. The consequences of elevated plasma Phe concentrations for cerebral metabolism are not fully understood. A noninvasive assessment of brain Phe levels is instrumental for investigations of the pathophysiologic mechanisms that lead to impaired cognitive development. ^{1}H-MRS has been used to detect and measure Phe in the human brain,[1-3] although its application is limited because in the normal brain, concentrations of Phe are usually below the detection limit for clinical ^{1}H-MRS.[4] The purpose of this work is to provide quantitative measures of brain Phe levels with localized ^{1}H-MRS at 3 Tesla as part of a multimodal study that includes brain imaging, cognitive testing, and metabolic profiling of PKU patients.

Methods - The study protocol was approved by the institutional review board, and all participants gave their written informed consent. Fourteen PKU patients (age 18-42 y; female/male 8/6) were examined on a Philips Ingenia 3.0 Tesla MR system (Philips Medical Systems, Best, The Netherlands) equipped with a 16-channel head coil (Philips). Patients presented between 6-8 am. Venous blood samples were collected after an overnight fast, immediately followed by MR examination. A standard 3D turbo gradient-echo protocol was performed to acquire images of the brain for anatomic reference. Next, the voxel of interest (~20 × 20 × 20 mm) was carefully positioned in the parietal white matter, aiming to minimize the inclusion of CSF or grey matter. After volume-selective shimming of the voxel of interest, ^{1}H MR spectra were acquired with a point resolved spectroscopy (PRESS) sequence: water presaturation bandwidth 140 Hz; TE = 36 ms; TR = 2000 ms; number of averages = 128; 2048 data points; phenyl ring protons of Phe at 7.37 ppm on-resonance. In the normal cerebral ^{1}H-MR spectrum, multiple signals downfield of water overlap with the phenyl ring protons of Phe at 7.37 ppm. For an unambiguous detection and quantification of Phe, we therefore calculated the difference spectra by subtracting a baseline control spectrum from the PKU patient spectra.[2] A control spectrum was constructed by averaging the spectra recorded in five healthy volunteers (age 22-31 y; female/male 1/4) using the settings described above. Blinded to patient characteristics or other readouts of this study, patient spectrum and control spectrum were manually phased and referenced to the creatine-methyl resonance at 3.03 ppm in jMRUI. Next, the control spectrum was subtracted from the patient spectrum (Fig. 1), and the area under the peak was determined for the signal at 7.37 ppm in the difference spectrum. The same procedure was followed for estimating the brain Phe levels in the individual healthy volunteers. This integral served as a quantitative measure of brain Phe levels relative to the baseline control level as determined in healthy volunteers (ΔPhe).

Results - Subtraction of the averaged control spectrum from the PKU patient spectra typically resulted in a prominent excess peak at 7.37 ppm (Fig. 1), attributable to elevated levels of Phe in the brain of PKU patients. Brain Phe levels were higher in PKU patients compared to healthy controls (P = 0.00015; Fig. 2). As expected, ΔPhe levels in healthy volunteers relative to the averaged control spectrum were essentially zero. Mean plasma Phe concentration was 654 ± 263 µmol/L in PKU patients. Notably, brain Phe levels (ΔPhe) correlated with plasma Phe concentrations (r = 0.74, P = 0.003; Fig. 3).

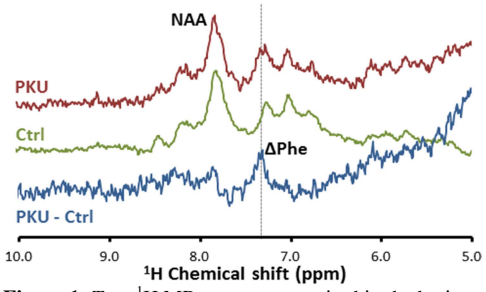

Figure 1. *Top:* ^{1}H MR spectrum acquired in the brain of a PKU patient. *Middle:* Averaged baseline spectrum of controls (n = 5). *Bottom:* Difference spectrum revealing ΔPhe at 7.37 ppm.

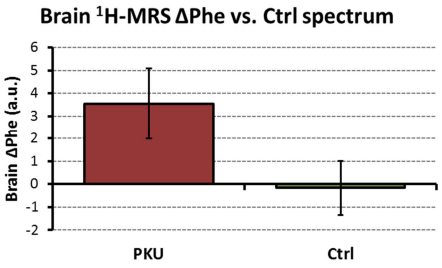

Figure 2. Brain Phe levels relative to the averaged baseline of healthy volunteers. ΔPhe was higher in PKU patients (n = 14) compared to controls (n = 5; P = 0.00015). Error bars indicate SD.

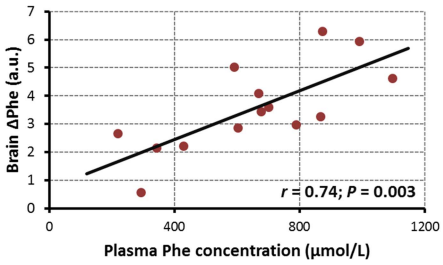

Figure 3. Correlation between brain ΔPhe levels quantified with ^{1}H-MRS vs. plasma Phe concentrations (n = 14).

Discussion - Based on difference spectra of individual PKU patients relative to an averaged control spectrum obtained from healthy volunteers, we were able to detect and quantify elevated brain Phe levels using ^{1}H-MRS. We did not derive absolute brain Phe concentrations (e.g., in mmol/kg wet weight), which could in principle be achieved via additional processing that incorporates corrections for tissue water content, metabolite T_1 and T_2 relaxation time constants, and partial volume effects.[5] Nonetheless, we demonstrated that relative brain Phe levels can be quantitatively assessed and correlated with plasma Phe concentrations. Within the framework of the current study, relations between brain Phe levels and assessments of cognitive function will be investigated to achieve a better understanding of the pathophysiologic mechanisms that could lead to cognitive impairments in patients suffering from PKU. As such, ^{1}H-MRS of the brain offers an important, feasible, and noninvasive readout of cerebral metabolism in PKU.

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References - [1] Novotny EJ, *et al*. *Pediatr Res* **1995**;37:244-9. [2] Kreis R, *et al*. *J Magn Reson B* **1995**;107:242-51. [3] Sijens PE, *et al*. *Eur Radiol* **2004**;14:1895-1900. [4] Pietz J, *et al*. *J Inherit Metab Dis* **2003**;26:683-92. [5] Jansen JFA, *et al*. *Radiology* **2006**;240:318-32.