

Brain Imaging and Proton Magnetic Resonance Spectroscopy in Patients With Phenylketonuria

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ABSTRACT. Magnetic resonance imaging studies in patients with phenylketonuria (PKU) revealed white matter alterations that correlated to most recent blood phenylalanine (Phe) concentrations as well as to brain Phe concentrations measured by magnetic resonance spectroscopy. The clinical significance of these changes is unknown. Magnetic resonance imaging data thus have no impact on therapeutic recommendations for adolescents and adults with PKU. Kinetic investigations of patients by magnetic resonance spectroscopy showed differences in brain Phe concentrations despite similar blood Phe levels. These were influenced by interindividual variations of blood-brain barrier Phe transport constants and by variations of the individual brain Phe consumption rate. Blood-brain barrier Phe transport characteristics as well as brain Phe consumption rates thus seem to be causative factors for the individual outcome in PKU. *Pediatrics* 2003;112:1580–1583; *phenylketonuria, brain imaging, proton magnetic resonance spectroscopy.*

ABBREVIATIONS. Phe, phenylalanine, PKU, phenylketonuria; MRI, magnetic resonance imaging; K_t , Michaelis-Menten transport constant; T_{max} , maximal transport velocity; V_{met} , consumption velocity.

With elevated phenylalanine (Phe) levels ($>600 \mu\text{mol/L}$), patients with phenylketonuria (PKU) generally demonstrate symmetric patchy and/or band-like areas of enhanced signal intensity on T_2 -weighted images. The changes predominantly affect the posterior/periventricular white matter, the area of latest myelination in humans. In more severely affected patients, the lesions extend to the frontal and subcortical white matter, including the corpus callosum and the area of the association fibers.^{1–3} In addition, discrete signal changes within the corticospinal tract, extending from the internal capsula to the cerebral peduncles, were described in some adolescent and adult patients.^{2,4} In one series, a high proportion of infratentorial white matter alterations, including the brain-

stem, was reported ($\sim 25\%$).⁵ The focal subcortical white matter changes were associated with a decrease of signal intensity in T_1 -weighted sequences.⁶

The regional distribution of the white matter changes does not seem to be age dependent as early, late, and untreated patients (infants and adults) show the same regional distribution pattern.^{4,7} Using standard T_2 -weighted images (1.5-T magnetic field), most patients with blood Phe levels $>10 \text{ mg/dL}$ demonstrate white matter changes, corroborating data in non-PKU hyperphenylalaninemic patients. White matter involvement was even described at blood Phe levels of $\sim 180 \mu\text{mol/L}$.^{4,8} Magnetic resonance imaging (MRI) abnormalities worsen after relaxation of diet and improve after several months after reintroduction of a strict therapy.^{2,9} Recovery from reduced myelogenesis was also described in hyperphenylalaninemic rats with change of diet.¹⁰

The severity of myelin alterations could be correlated to the actual blood Phe level, the mean Phe level during the last 1 to 5 years before the MRI examination, or the time when diet was stopped. In patients off diet, the changes generally plateau. It is not clear whether this plateau is a consequence of a nonprogressive myelin disorder or simply reflects that in adult patients, concurrent Phe concentrations correlate to long-term biochemical control.^{4,11} In a recent study, using proton spectroscopy, white matter alterations were correlated to the actual intracerebral Phe levels as well as the apparent Michaelis transport constant for Phe at the blood brain barrier.¹²

The clinical significance of white matter alterations in patients with PKU remains obscure as no correlation to neurologic deficits was found based on results of different neuropsychological and electrophysiologic studies, as well as IQ values. The last finding is not surprising as the MRI changes seem to depend on “most recent” Phe levels, and the IQ of the patients is predominantly influenced by Phe levels up to the age of 8 to 10 years.^{9,11} In our hands, cortical atrophy was a rare finding, especially in early-treated patients, but it was frequent in series with late-treated patients.^{13,14}

MRI results principally corroborate pathomorphologic examinations revealing “pallor of myelin,” frequently present within the parieto-occipital region, the corpus callosum, and the area of the association fibers. They do not confirm the high rate of histochemical changes within the optical tract and thus do

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not explain the prolonged latencies of visual evoked potentials described in many patients with PKU.^{14–18}

We even do not know on which morphologic alterations the white matter changes, as measured by MRI, are based. Furthermore, it is unknown whether Phe is primarily toxic to oligodendrocytes or to neurons/axons, leading to secondary changes in myelin formation as discussed in hyperphenylalaninemic rats.¹⁰

Results of T_2 relaxometry indicate a “dysmyelination” as originally described by Hommes¹⁹ in the hyperphenylalaninemic rat, namely that the decreased synthesis of sulfatides and other myelin compartments is associated with an increased myelin turnover leading to disruption/splaying of myelin lamellae associated with an increased water content.

Studies of the PAH^{enu2} PKU mouse indicate a regional Phe sensitivity of oligodendrocytes as a result of a variable inhibition of the key regulatory enzyme in cholesterol biosynthesis, 3-hydroxy-3-methyl-glutaryl-CoA-reductase.²⁰ The regional distribution pattern of white matter changes might be additionally explained by different Phe-uptake constants of different brain areas as suggested by positron emission tomography studies in men.²¹

In summary:

- The MRI studies indicate that Phe is a life-long “toxin” for myelogenesis.
- The white matter changes are reversible, are independent of the patient’s age, and affect brain areas of short as well as prolonged cycles of myelination.
- The clinical significance of white matter alterations in patients with PKU remains obscure. The published data do not provide unequivocal information for the question of whether adolescent and adult patients should stay on a strict diet.

PROTON MAGNETIC RESONANCE SPECTROSCOPY

Quantification of brain Phe concentrations in vivo was first described by Avison et al²² using hyperphenylalaninemic rabbits. Intracerebral Phe concentrations correlated well with measurements of brain Phe levels made on post mortem samples by amino

acid analyzer. Under conditions of these experiments, Phe did not achieve equal concentrations on both sides of the blood-brain barrier. These findings initiated additional studies in men as variations in individual ratios of blood to brain concentrations might explain the different cognitive outcome as well as white matter changes detected by MRI despite a comparable dietary control.

In our experiments in patients with PKU, brain Phe peak areas were determined from different spectra versus volunteer spectra recorded in identical brain regions. The absolute brain Phe concentration was calculated from ratios of peak areas of Phe/creatinine using creatinine as an internal reference.^{23,24}

Studies in >30 patients with PKU revealed Phe concentrations that corresponded to those obtained biochemically by McKean²⁵ and Adriaenssens et al²⁶ in untreated patients with PKU. No statistical difference of Phe concentrations in different brain areas was obtained. We found a wide interindividual variation of brain to blood Phe concentrations (0.2–0.7), strengthening the hypothesis that differences in transport kinetics of Phe might influence the clinical outcome of the patients. Similar results were obtained by Moats et al²⁷ and Koch et al.²⁸ In contrast, others described a more constant relationship between brain and blood Phe concentrations with a ratio of ~0.2 to 0.3.^{24,29}

Assuming Michaelis-Menten transport kinetics, one can analyze the relation between blood and brain Phe levels by a simplified 2-compartment model describing Phe uptake quantitatively by an apparent Michaelis-Menten transport constant (K_t) and the ratio of the maximum Phe transport rate and the metabolic Phe consumption rate (T_{\max}/V_{met} ; for details see ^{12,30}).

Phe brain-to-blood concentrations of different patients during “steady-state conditions of diet” demonstrated a linear correlation up to blood concentrations of ~1.5 mmol/L. Increasing deviations from linearity were observed for higher blood levels. On the basis of the Michaelis-Menten model, the combined data from the patients yielded a $K_t = 0.16 \pm 0.1$ mmol/L and $T_{\max}/V_{\text{met}} = 9.0 \pm 4.1$ (Fig 1).¹²

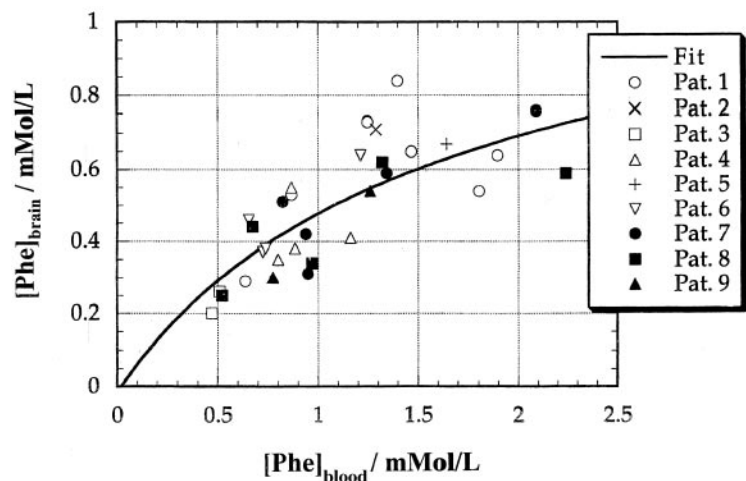


Fig 1. Plot of parieto-occipital brain Phe concentrations versus corresponding blood levels assuming saturable Phe transport described by an apparent Michaelis constant.

Positron emission tomography studies in men as well as studies by the double-indicator method also revealed a saturable brain Phe uptake in men.^{21,31} Saturable Phe transport was also obtained by Avison et al²² in hyperphenylalaninemic rabbits.

During the course of a Maternal PKU Study, we found 3 untreated women with classic PKU but normal or nearly normal intelligence. Their brain Phe levels were below the detection limit of proton spectroscopy (<0.15 mmol/L).³² Similar exceptional patients have additionally been reported by others.²⁷ We therefore started a series of dynamic magnetic resonance spectroscopy experiments to examine the Phe transport kinetics at the blood-brain barrier.

After a Phe loading test (100 mg/kg body weight), wide interindividual variations of both the apparent K_t (0.1–1.0 mmol/L) and the ratio of T_{max}/V_{met} (14.0–4.3) were found (Table 1). The atypical, untreated patients with PKU presented a high K_t , $K_{t, app}$ leading to a low brain uptake during the loading test, as well as a low ratio of T_{max}/V_{met} , indicating a high intracerebral Phe consumption rate.^{12,30} Wide interindividual variations for K_t , $K_{t, app}$ and T_{max} (factors 15 and 7) were also described by Knudsen et al³¹ in healthy volunteers using H³-L-phenylalanine in a double-indicator study. The results show that interindividual differences in brain Phe uptake and consumption are not limited to a few exceptional patients but seem to be “more common.” Mutations that affect the function of different amino acid transport systems will influence the K_t , $K_{t, app}$ value. The consumption rate will be influenced by different rates of protein synthesis and Phe degradation, for example, by the activity of different hydroxylases, including tyrosine hydroxylase.

In addition, most severe white matter abnormalities were observed in patients with small values for K_t , $K_{t, app}$ and high ratios of T_{max}/V_{met} .¹² The brain concentrations of the classical metabolites measured by proton spectroscopy, namely N-acetylaspartate, inositol, lactate, and creatinine, were found to be normal. Even the concentration of choline, described to be elevated in acute demyelinating disorders with enhanced membrane lipid turnover, was in the normal range.^{23,28}

TABLE 1. Phe Loading Test: Kinetic Data Obtained by Magnetic Resonance Spectroscopy

Patient	K_t , $K_{t, app}$ ($\mu\text{mol/L}$)	T_{max}/V_{met}
1	0.10 \pm 0.03	14.00 \pm 3.40
2	0.10 \pm 0.04	7.80 \pm 2.20
3	0.24 \pm 0.27	4.40 \pm 2.50
4	0.28 \pm 0.25	3.70 \pm 1.60
5	0.35 \pm 0.22	3.12 \pm 0.66
6	0.36 \pm 0.22	3.22 \pm 0.55
7	0.38 \pm 0.13	3.46 \pm 0.43
8	0.44 \pm 0.52	3.30 \pm 1.60
9	0.45 \pm 0.42	3.57 \pm 0.66
10	0.63 \pm 0.45	4.60 \pm 1.10
11	0.63 \pm 0.45	3.01 \pm 0.23
12	0.63 \pm 0.49	2.74 \pm 0.28
13	0.75 \pm 0.34	2.62 \pm 0.14
14	0.84 \pm 0.33	2.61 \pm 0.14
15	1.03 \pm 0.78	4.32 \pm 0.53

CONCLUSION

Our data give evidence that interindividual differences in brain Phe uptake and consumption influence the neurologic/cognitive outcome of patients with PKU. They confirm the low correlation between mutations at the Phe hydroxylase gene and clinical outcome of untreated patients with PKU, as well as the surprisingly high rate of untreated patients with PKU with normal intellectual development.³³

So far, it is not known whether our measurements of kinetic parameters in adulthood provide an appropriate characterization of the situation during childhood. Including the limitation of the method so far, no critical brain concentration was found that could justify a relaxation of diet.

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