Brain Phenylalanine Concentrations in Phenylketonuria: Research and Treatment of Adults

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ABSTRACT. *Objective.* **To assess the effects of 2 pharmacologic interventions (amino acid supplements) on the brain levels of phenylalanine (Phe) in adults with phenylketonuria (PKU).**

Methods. **A prospective study was conducted in an outpatient treatment and follow-up setting. The volunteers who were recruited for the first intervention included 4 subjects with classic PKU. The second intervention included 3 adults with classic PKU. The first intervention consisted of dietary supplementation during 1 day with Phlexy 10. Two individuals were given a dose of 0.5 g/kg/d, and 2 were given 1.0 g/kg/d. The second intervention consisted of dietary supplementation with PreKUnil at 0.4 g kg/d over a period of 6 months. Brain Phe was measured by magnetic resonance spectroscopy. The number of the patients involved precluded analysis for significance.**

Results. **The first, shorter intervention resulted in a decrease in brain Phe. The second intervention resulted in a 20% decrease in brain Phe, which was maintained after 6 months of treatment.**

Conclusion. **Dietary supplementation of large neutral amino acids seems to lower the brain Phe in adults who have PKU and have difficulty following their diet.** ***Pediatrics*** **2003;112:1575–1579;** ***magnetic resonance spectroscopy, blood/brain barrier, large neutral amino acids.***

ABBREVIATIONS. PKU, phenylketonuria; Phe, phenylalanine; MRS, magnetic resonance spectroscopy; LNAA, large neutral amino acid; MRI, magnetic resonance imaging.

For >45 years, the treatment of individuals with phenylketonuria (PKU) with the phenylalanine (Phe) restricted diet has avoided the devastating neurologic sequelae caused by high blood Phe levels. The results of dietary treatment early in life have been well demonstrated. Now that many of the individuals have reached adulthood with normal intelligence, several new areas have come to the forefront. One covers the problem related to maternal PKU, a topic that is well addressed in this supplement. Another deals with issues related to the currently incompletely answered question to what degree adults should continue to be treated with the standard treatment regimen originally implemented for the developing brain during infancy and childhood. Although it is our opinion that "diet for life" makes the most sense, unfortunately, this objective is proving to be unrealistic for many adolescents and adults. Briefly, 1 reason is demonstrated by an informal survey of the success of treatment in adolescents and adults that suggests that 40% to 60% of this population are off diet, and a published report[1] indicated that as much as 80% do not adhere to recommendations. This less-than-ideal compliance has recently forced us to review our treatment regimens for adults.[2] Recently, as our clinic has struggled with treatment protocols for adults, one focus has been to study the brain Phe concentrations by magnetic resonance spectroscopy (MRS) and the possible influence of the medical products. Our identification of exceptional classical individuals (those who have high IQ with no sign of deterioration despite being off diet for >10 years with high blood Phe concentrations) encouraged us to continue our investigations.[3,4]

Here we report the response to dietary supplements of large neutral amino acids (LNAAs) in 2 studies by measuring blood and brain concentrations in 7 individuals with PKU in 2 separate studies. The Phe concentration in the brain is a direct result of transport of Phe across the blood-brain barrier. This transport is competitively inhibited by the presence of other LNAA in the blood. Thus, the dynamic equilibrium of brain levels measured in this study could be influenced by the concentration of other LNAAs in the blood, which, in turn, could be influenced by an individual's diet. During the past 10 years, several different groups besides ours have studied this relationship.[5–9] Although it is generally agreed that the level of brain Phe can be measured, its clinical significance remains to be proved.

METHODS

MRS methods were as previously reported,[3,4] except that control values based on the literature have been reevaluated. Data for Fig 1 are a compilation of 2 previous reports[3,4] plus additional patients, bringing the totals to 6 control subjects, 8 carriers, 4 individuals with mild PKU and hyperphenylalaninemia, 22 individuals with typical classical PKU, and 6 exceptional classical individuals (1 repeat examination). Blood and brain measurements were done simultaneously. Informed consent for all individuals was obtained. Plasma amino acids were analyzed by a certified metabolic laboratory at Childrens Hospital Los Angeles using a Beckman 7300 amino acid analyzer.

Study 1

The Phlexy-10 study consisted of 4 subjects, 1 man and 3 women with classical PKU. The product was supplied by SHS

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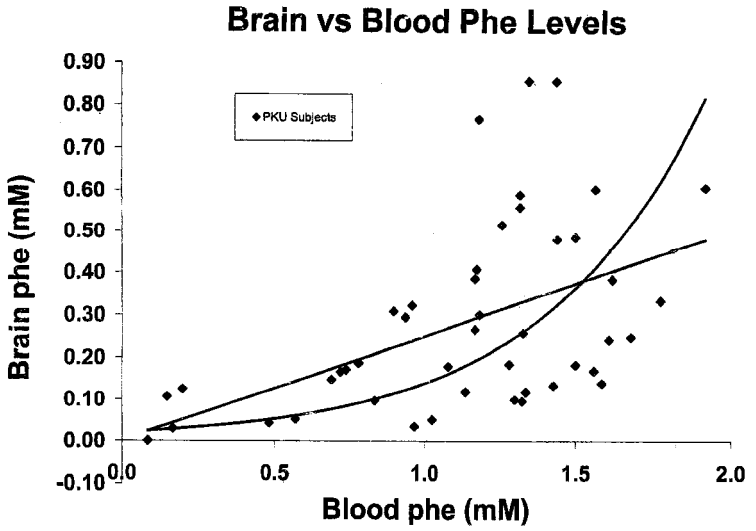

Fig 1. Summary of brain Phe concentrations versus blood Phe concentrations in an individual with PKU.

North America. Three of the subjects had never discontinued their medical product, and 1 had discontinued the diet at the age of 6 years. Two subjects maintained blood Phe concentration between 600 and 900 μmol/L, and 2 were above 1200 μmol/L or 1.2 mmol/L. Each subject was studied for 2 consecutive days and given the Phlexy-10 sachets. Two subjects were given 0.5 g/kg, and 2 were given 1 g/kg of the supplement (Fig 2).

Study 2

Three adults with classical PKU, 1 man and 2 women, volunteered for this study. Two of the subjects had never discontinued their medical product, and 1 had been off diet for >20 years. All 3 subjects maintained blood Phe concentrations above 1.2 mmol/L. Each subject was studied for 3 consecutive days, then 1 month later, and 6 months later for MRS and analysis of plasma amino acids. After initial baseline examinations, each subject was given 0.4 g/kg of PreKUnil (amino acid supplement supplied by NiLab, Middlefart, Denmark). They were instructed to implement a relaxed diet, which included approximately 50 to 60 g of natural protein per day.

RESULTS

Figure 1 shows the result of previous brain MRS examinations in individuals with PKU examined is subjective. The absolute values differ slightly from our past results because control values have been recalibrated slightly downward (based on reanalysis of the literature), which in turn lowers patients' values, and several new PKU data points have been added.

Individual variability across the group, in the clinically relevant blood concentrations between (600–1500 μmol/L), showed a 3-fold variation in brain Phe concentration. We believe that this is a true difference as previously proposed.[3,4]

If we focus on our exceptional individuals (blood, brain), we see that their brain Phe concentrations range from 0.1 to 0.2 mM. Their brain concentrations differ from typical PKU individuals as they fall below 0.25 mM. The frequency of these exceptional individuals is essentially unknown and may be as high as 10%. The brain Phe concentrations of the individuals with mild PKU also had values <0.25 mM.

The data suggest that the brain Phe concentrations are higher in carriers than those seen in control subjects, but because of the small numbers of subjects, the difference between carriers and control subjects as groups did not reach significance. Four individu-

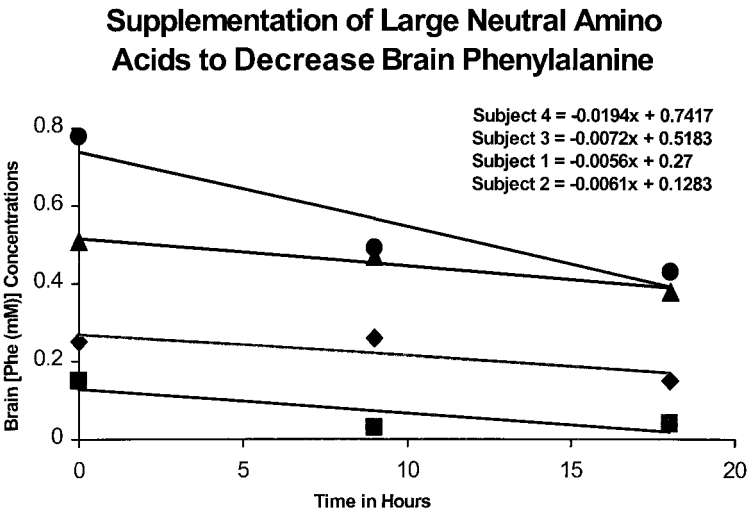

Fig 2. Study 1: brain Phe concentrations versus blood Phe concentrations in individuals with PKU over 2 days.

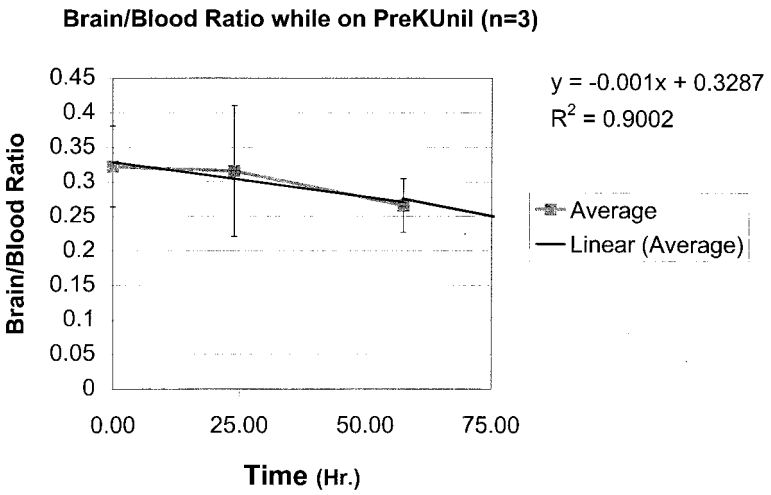

Fig 3. Study 2: brain Phe concentrations versus blood Phe concentrations in individuals with PKU over 3 days.

als identified with mild PKU (residual enzyme activity in 1 allele) exhibited similar brain Phe concentrations, in the range of the exceptional subjects.

The overall distribution of the data (Fig 1) as a whole provided an interesting observation. In determining the direction of the range of blood Phe concentrations, it seems to be exponential rather than linear. It is expected that at higher blood Phe concentrations, brain Phe concentrations would begin to reach equilibrium as a result of saturation of the carrier, thus making the shape of the curve sigmoidal.

It would be important to investigate the ability of the LNAAs in the medical products to compete for transport into the brain, thus lowering the brain Phe concentrations. Therefore, brain Phe levels were measured before and after the supplementation with 2 products: study 1 (n = 4) using the Phlexy-10 sachets and study 2 (n = 3) using the PreKUnil tablets. In this study, a very small correction to brain Phe levels as a result of tyrosine has been made based on the tyrosine levels in the blood.

Study 1 was performed over 2 consecutive days, and 3 cerebral MRS measurements were obtained. The LNAAs were supplied by the Phlexy-10 sachets at 0.5 g/kg body weight for 2 subjects and at 1 g/kg body weight for the other 2. Study 2 was performed over a period of 6 months, and the LNAAs were supplied by the PreKUnil tablets at a dose of 0.4 g/kg body weight.

In study 1 (Fig 2), all 4 subjects demonstrated a decrease in brain Phe concentration. Daily diet records indicate that natural protein intake ranged from 10 to 40 g. However, a brain/blood ratio comparison could not be completed because of a lack of blood data at several points. Because the study population was so small, no measure of significance was attempted.

In study 2, an observable decrease in brain Phe concentrations of approximately 20% was observed over a period of 3 days (Fig 3). This difference did not reach significance. However, the decrease became more apparent at the 6-month interval (Fig 4) despite that all of the subjects were on a normal diet consisting of 40 to 80 g of natural protein daily. Because of such a small population, it is premature to say whether the effect is significant. Should this declining trend in brain Phe concentrations be shown in a larger study population, it would be of clinical relevance. A total amino acid profile on these subjects was also obtained at the study intervals. Both the blood tyrosine and tryptophan increased from below normal to normal levels (Fig 5).

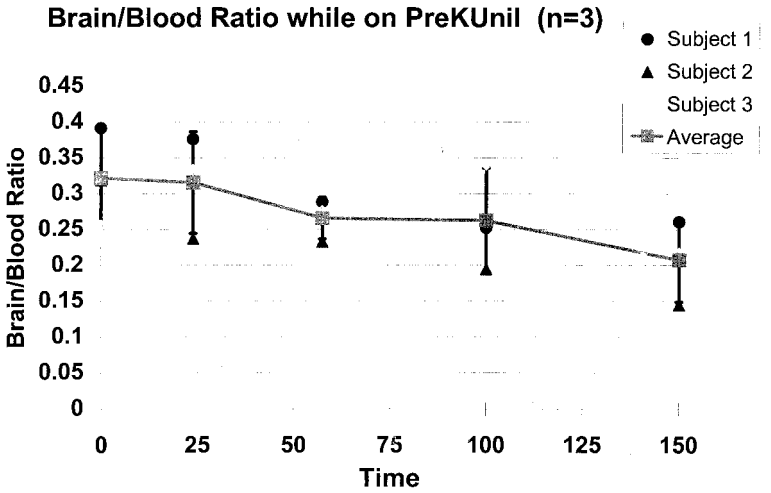

Fig 4. Study 2: brain Phe concentrations versus blood Phe concentrations in individuals with PKU over 6 months.

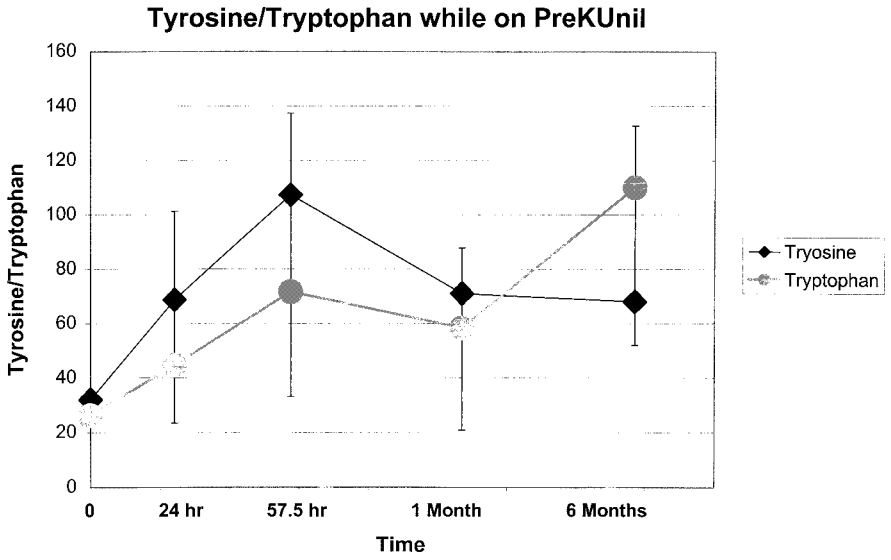

Fig 5. Study 2: blood tyrosine and tryptophan concentrations in individuals with PKU during the course of the study.

DISCUSSION

In our studies, only 1 of the 10 of our subjects with mild PKU ($n = 4$) and our exceptional PKU individuals ($n = 6$) reached brain Phe concentrations >0.25 mM. These people are cognitively normal. In the original collaborative study on children with PKU at 12 years of age, IQ decreases were noted in subjects only when blood levels of Phe were >900 μmol/L. Clinically, it seems that adults are less sensitive to Phe toxicity than children. Previously, we had suggested that brain Phe concentrations up to 0.25 mM were reasonable to accept in a clinical setting, and the additional data included here strengthen our opinion. However, we do not exclude the possibility that some individuals may not be hypersensitive to higher concentrations of Phe.

A recent analysis of a subset of these data viewed strictly from a clinical interpretation suggested that brain Phe levels up to 0.35 mM/L may even be largely benign in adults.[3] As a group (Fig 1), these brain Phe concentrations are reached at blood Phe concentrations of 1000 μmol/L, a level compatible with the recommendations of the United States, British, and German consensus recommendations.[2] Medical product supplementation at the doses given in our study lowers brain Phe concentrations; however, confirmation from a larger study is needed.

The question of the nature of the increase in brain Phe concentrations with increasing blood Phe is a relevant point for discussion. The apparent "better fit" of exponential data to the overall data is not surprising and should be expected. This was not apparent early in the study. For example, if the concentrations of all other amino acids are held constant but the Phe is increased, then the Phe will occupy an increasing number of sites on the LNAA transporter. This will have the effect seen here of making the curve seem to be more exponential. Regulatory mechanisms would tend to upregulate the number of transporters, thus increasing the overall transport of LNAAs. These factors may be subject to individual variability. Thus, the time-dependent blood-brain equilibrium may be more complex than can be modeled with a simple linear equilibrium model.

The use of MRS in a clinical setting is best illustrated by these examples. A 15-year-old subject presented to the clinic expressing a wish to go off diet. His parents supported his assessment that the diet is an excess burden to place on an adolescent. A magnetic resonance imaging (MRI)/MRS examination was obtained. The MRI images showed a large amount of white matter changes, and MRS indicated a relatively high brain/blood Phe ratio. This concrete example of the effect of the high Phe concentrations provided support for the physician to persuade parents and the PKU subject to accept that diet discontinuation was unacceptable.

The second case involved 1 of the exceptional individuals. The subject presented to the clinic, and a blood Phe specimen was obtained. The blood Phe result was found to be near 1386 μmol/L. An MRI/MRS examination was performed and demonstrated a normal-appearing brain with a brain Phe concentration in the 0.2 to 0.25 mM range. For this reason, we did not work aggressively to reverse this subject's decision to relax dietary control.

CONCLUSION

MRS of the brain is an excellent diagnostic modality for determining brain Phe concentrations and has been shown to be useful in the management of PKU. Despite intensive counseling, many adults and adolescents find it difficult or impossible to follow a Phe-restricted diet closely. In addition, there are certain individuals who are exceptional and may not need to be as strict in their dietary adherence as those with the more common classical PKU. Dietary supplementation with LNAAs has been shown to lower brain Phe concentrations in adults with PKU. However, the most important intervention in young children with PKU remains the Phe-restricted diet, which must be followed closely by infants and children to avoid adverse future neurologic sequelae or fetal complications during pregnancy.

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