Dietary Treatment in PKU from experience to evidence
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Phenylalanine tolerance can already reliably be assessed at the age of 2 years in patients with PKU

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ABSTRACT

The clinical severity of phenylalanine hydroxylase deficiency is usually defined by pretreatment phenylalanine (Phe) concentration and Phe tolerance at 5 years of age. Little is known about the ability of these parameters to predict the tolerance at later age. The objective of this study was to assess the predictive value of Phe tolerance at 1 and 6 months, at 1, 2, 3 and 5 years of age as well as pre-treatment Phe concentration for Phe tolerance at 10 years of age. Pearson's r correlation was used to calculate the effect size. Data of 236 early and continuously treated Dutch PKU patients up to 10 years of age were used. Phe tolerance decreased logarithmically with age. Pearson's r correlation with tolerance at 10 years was highest at the ages of 2 (r=0.61, p<0.0005), 3 (r=0.73, p<0.0005) and 5 years (r=0.66, p<0.0005). Pearson's correlation of Phe tolerances before the age of 2 years and Phe tolerance at 10 years of age varied between 0.11 (1 month) and 0.39 (1 year). There was no significant correlation of the pre-treatment Phe concentration and Phe tolerance at 10 years of age (p=0.1). We conclude that already at an age of 2 years Phe tolerance can be reliably assessed.

INTRODUCTION

Phenylketonuria (PKU; McKusick/OMIM 261600) is an inborn error of metabolism caused by a deficiency of the enzyme phenylalanine hydroxylase (PAH; EC 1.14.16.1) due to mutations in the human PAH gene. The deficient activity of PAH results in a decreased conversion of the essential amino acid phenylalanine (Phe) into tyrosine. Consequently, blood concentrations of Phe are elevated. Left untreated this condition usually results in severe mental retardation. Neonates are screened for PKU by measuring Phe concentrations in blood a few days after birth. Treatment aims to reduce Phe concentrations by means of a life-long diet, low in natural protein, and supplemented with a Phe-free protein substitute. Early diagnosed and continuously treated individuals with PKU are of normal intelligence although some minor neuropsychological dysfunction remains.1-2

There is a large scale of severity of the PAH deficiency. The strongest severity (almost no residual PAH activity) results in lower amounts of Phe in the prescribed diet. This is necessary to meet treatment goals, expressed as target ranges for the blood Phe concentrations. One of the difficulties of PKU is how to define the clinical severity of PAH deficiency and the resulting Phe restriction in the diet. So far, various methods have been proposed including measurement of enzyme activity in a liver specimen, expression analysis of mutations within the PAH gene, in-vivo protein or Phe loading-tests with measurement of the Phe concentration after 72 hours, studies with radioactively labeled Phe, and continuous tracer or bolus tracer techniques with stable isotopes.3-8 None of these methods is satisfactory because they are rather invasive, do not have a clear relation with the clinical severity of the PAH deficiency, might be hazardous because of causing very high Phe concentrations for a period of time during and after the test, or because of radioactivity.

The parameters used to observe the clinical severity in day to day life include pre-treatment Phe concentration and Phe tolerance (i.e. the amount of Phe per kg body weight per day a patient can tolerate without blood concentrations above
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the highest target concentration). Güttler proposed a differentiation between 3 subgroups using the Phe tolerance at 5 years of age. However, although both the pre-treatment Phe concentration and the Phe tolerance at 5 years of age have been related to the genotype, intellectual outcome and long-term Phe concentrations, the clinical significance of the pre-treatment Phe concentration and Phe tolerance at early age to predict Phe tolerance at a later age has not been studied. Only one report studied the course of the dietary Phe tolerance till 8 years of age and only in a very small group of patients.

In the present study, the predictive value of pre-treatment Phe concentration and Phe tolerance up to 5 years of age for Phe tolerance at 10 years of age was investigated.

METHODS

From the start of the national screening program for PKU in the Netherlands in 1974, children with positive screening results have been referred to one of the eight Dutch pediatric university clinics. Data have been collected nationwide and registered at the central PKU-registry. The Dutch Steering Committee considered a patient to have PKU when pre-treatment blood Phe concentrations are (a) >500 μmol/L in the untreated newborn and (b) when the Phe tolerance is <50 mg/kg/day at 12 months of age.

Between 1974 and 1996, PKU was detected in 236 children by neonatal screening and treated ever since. These 236 patients were included in this study.

Actual weight, dietary Phe intake at 1 and 6 months and at 1, 2, 3, 5, and 10 years of age (all with a margin of ±1 month) were obtained as well as all blood Phe concentrations of the patients, including the pre-treatment Phe concentration. The Phe tolerance (mg/kg/day) was calculated from the prescribed intake for each individual patient at the seven time points described above.

The target range of blood Phe concentrations was 200 - 500 μmol/L for all ages according to Dutch recommendations at that time. The mean of Phe concentrations of blood samples collected at the seven time points were subsequently used to decide on metabolic control and to include or exclude data on tolerance of the individual patient at that moment.

To establish the mean course of the Phe tolerance with age for the total population, we calculated the mean Phe tolerance at the seven times points, making use of the blood Phe concentrations that fell within the target range of 200-500 μmol/L.

Statistics

Correlations between the individual pre-treatment Phe concentration and the individual Phe tolerance at 1 and 6 months, and at 1, 2, 3, and 5 years of age on the one hand, and the tolerance at 10 years of age on the other hand were computed using Pearson’s r correlation coefficient (in version 10 of the statistical program SPSS, Inc., Chicago, IL). To predict the Phe tolerance at 10 years of age from the tolerance at an earlier age, linear regression analysis was performed to construct a least square
regression equation of ages with a correlation >0.5. In all analyses a p-value <0.05 was considered to be statistically significant.

RESULTS

Data of 74 patients on the pre-treatment concentration could be included to compare with the Phe tolerance at 10 years. Data of the remaining 162 patients could not be used because of lack of tolerance figures at 10 years of age (n=141), or blood Phe concentrations not within the target range at 10 years of age (n=21). A total of 992 values of Phe tolerance could be included. These 992 values of Phe tolerance concerned 213 patients. Data of the remaining 23 patients could not be used due to incomplete data or values out of target range. The mean number of values of Phe tolerance per patient that could be used for the study was 5 (range 1-7) for the 7 time points.

The pre-treatment Phe concentration varied between 240-6000 μmol/L. The correlations between pre-treatment blood concentrations and Phe tolerance at 10 years of age was -0.192 (p=0.11) (Table 1).

The course of the mean Phe tolerance is shown in Figure 1. At 1 month of age

| Table 1. Pearson r correlations between pre-treatment Phenylalanine concentration and Phe tolerance values at six different ages with Phe tolerance at 10 years of age |
|------------------|------------------|
|                  | Phe tolerance at 10 years of age |
| Pre-treatment Phe concentration | r | -.181 |
|                        | p | .125 |
|                        | N | 73   |
| Phe tolerance at 1 month of age | r | .112 |
|                        | p | .390 |
|                        | N | 61   |
| Phe tolerance at 6 months of age | r | .276 |
|                        | p | .036 |
|                        | N | 58   |
| Phe tolerance at 1 year of age | r | .387 |
|                        | p | .002 |
|                        | N | 59   |
| Phe tolerance at 2 years of age | r | .608 |
|                        | p | .000 |
|                        | N | 57   |
| Phe tolerance at 3 years of age | r | .725 |
|                        | p | .000 |
|                        | N | 56   |
| Phe tolerance at 5 years of age | r | .661 |
|                        | p | .000 |
|                        | N | 59   |
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the mean Phe tolerance was 45 mg/kg/day, decreasing to 26 mg/kg/day at 2 years of age. The mean Phe tolerance further decreased to 21 mg/kg/day at 5 years of age and 14 mg/kg/day at 10 years of age. Correlations between the individual Phe tolerance at 1 and 6 months, and at 1, 2, 3, and 5 years of age on the one hand, and the tolerance at 10 years of age on the other hand were highest after the age of 2 years (Table 1).

Linear regression analysis between the tolerance up to 5 years and 10 years of age was performed with the correlations >0.5, i.e. at 2, 3, and 5 years of age, to calculate the least square regression equation. By this, the Phe tolerance at 10 years of age could be predicted as follows:

Phe tolerance [10 years] = 2.2 + (0.5 x tolerance [3 years])
Phe tolerance [10 years] = 5.4 + (0.3 x tolerance [2 years])
Phe tolerance [10 years] = 4.9 + (0.4 x tolerance [5 years])

DISCUSSION

Most important finding of our study is that from the age of two years on the Phe tolerance can be reliably assessed. In contrast, the pre-treatment Phe concentration as well as Phe tolerance before the age of two years are not or only weakly associated with Phe tolerance at 10 years.
Before discussing the results some methodological issues need to be addressed. The period of time between 1974 and 1996 was taken because during that time period no change was made in the target Phe range in plasma, and the target concentration was 200-500 μmol/L in all age groups in the Dutch PKU population. The original classification of Güttler was based on the upper limit of Phe of 600 μmol/L at 5 years of age. As recommended Phe concentrations in young patients were lowered, the upper concentration of Phe used by Güttler was first decreased to 420 and later to 300 μmol/L. This however, was done without changing the ranges of the Phe tolerances within the classification, probably because the effect of lowering the target ranges on the tolerance is not well known.

For a large number of patients, data on dietary Phe intake and weight were not available within the chosen small margin around the seven time points (1 month before and after) and patients therefore were excluded at that time point for further analysis. In addition, when the mean Phe concentration was out of range around a certain time moment, data on tolerance were not included at that specific time moment. This especially applied to 5 and 10 years of age were data of 130 and 95 patients were available, respectively. Part of this loss of patient numbers can be explained by the smaller number of patients that had reached that age at the time of the study, part of this loss of patient numbers is explained by the fact that Phe concentrations were out of the target range at that time point. While the mean Phe concentration was within the target range of 178 patients at 1 month of age, the mean Phe concentration of only 111 and 74 patients were within the target range at 5 and 10 years of age, respectively.

The figures of Phe intake in our retrospective study were based on the prescribed intake, rather than the actually consumed intake, because the actually consumed intake figures (e.g. calculation of recorded 3 days food intake, dietary history) could not be obtained in all centres. It should be taken into account that, also reported consumed intake is not 100% reliable. The influence of this is unclear. Probably the actual Phe intake and consequently the tolerance at an older age may be higher than the prescribed amount. However, this does not necessarily need to change the correlations. When children grow older the influence of parents on dietary intake of their child will diminish. Therefore, we expect that at older ages (after the age of two years) this bias towards the null value will be even higher than at younger ages. It is therefore unlikely that this bias has any influence on our conclusions. It should further be taken into account that both in the study of Güttler in 1980, and the present study in the Netherlands, Phe tolerance is based on total Phe intake, whereas in some other countries Phe of low protein food is not included in the calculation of the Phe tolerance.

We found that the Phe tolerance at 10 years can be predicted adequately from 2 years of age rather than at 5 years of age. This shows that notwithstanding the impact of intercurrent illness and growth, there is a clear relationship between the Phe tolerance at early age and 10 years of age. This study aimed to predict the Phe tolerance at 10 years. We do not suggest that Phe tolerance does not change after 10 years of age. Apart from the activity of PAH various factors may influence...
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Phe tolerance during childhood. Where growth and intercurrent illnesses are the most important factors in childhood, after childhood, these will probably be mainly illnesses, adolescent growth spurt, and changes in body composition, as well as differences in the target Phe concentrations. None of these factors, however, has been studied in PKU so that they remain hypothetical.

Correlations between pre-treatment Phe concentrations and data on tolerance values throughout the studied period were weak (Table 1). This is in line with the finding that the tolerance before 2 years of age showed weak correlations with tolerance at 10 years of age. This implies that to construct a predictive parameter before 3 years of age, non-clinical in-vivo parameters are necessary. For this reason the early use of a Phe loading-test with $^{13}$C-Phe measuring $^{13}$CO$_2$ production, as performed by Treacy et al, may be useful$^{24}$.

In conclusion, the Phe tolerance at 10 years can be adequately predicted with the Phe tolerance as early as 2 years of age. This might help parents and treatment teams to estimate the severity of natural protein restriction for a larger period of time during childhood. Further studies are necessary, especially with patients above 10 years of age.

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