Neurocognitive Evidence for Revision of Treatment Targets and Guidelines for Phenylketonuria

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Objectives To compare the neurocognitive outcomes of patients with phenylketonuria (PKU) to determine whether decreasing phenylalanine (Phe) levels to <240 is preferable to the use of 360 μmol/L as an upper-target Phe level. An additional aim was to establish the influence of biochemical indices other than Phe on neurocognitive outcomes.

Study design Patients with PKU (n = 63; mean age 10.8 ± 2.3 years) and healthy controls (n = 73; mean age 10.9 ± 2.2 years) performed computerized tasks measuring neurocognitive functions (inhibitory control, cognitive flexibility, and motor control). Lifetime and concurrent blood Phe levels, Phe-to-tyrosine ratio (Phe:Tyr), and Phe variations were examined in relation to neurocognitive outcomes using nonparametric tests and regression analyses.

Results Patients with PKU with Phe levels ≤240 μmol/L and healthy controls performed equally well. Patients with Phe levels between 240 and 360 μmol/L and ≥360 μmol/L performed more poorly than did controls across tasks. Patients with Phe levels ≤240 μmol/L performed significantly better than patients with levels between 240 and 360 μmol/L on tasks measuring inhibitory control and cognitive flexibility. Absolute Phe levels and Phe variation were the best predictors of motor control, whereas Phe:Tyr were the best predictors of inhibitory control.

Conclusions The results of this study suggest that upper Phe targets should be lowered to optimize neurocognitive outcomes. Moreover, Phe variation and Phe:Tyr appear to be of additional value in treatment monitoring. *(J Pediatr 2014;164:895-99)*.

The intellectual disability, neurologic problems (eg, epilepsy), motor deficits, and behavioral problems of phenylketonuria (PKU) can be largely prevented by the early introduction of dietary phenylalanine (Phe) restriction after neonatal screening.[1-3] However, average IQ in patients with treated PKU is still 8-10 points lower than normal, and patients perform less accurately and more slowly than do controls on several neuropsychologic tasks.[4-7] They particularly show impaired executive functioning and motor control, over and above any intellectual disability.[5-12] High brain Phe levels most likely affect cerebral myelination and decrease levels of several neurotransmitters such as dopamine and serotonin; both of these have been associated with executive and motor function deficits.[13-16]

Cognitive impairments in treated PKU patients have been associated with concurrent blood Phe levels and, even more strongly and consistently, with lifetime blood Phe levels.[1,5,8] Notwithstanding the positive effects of decreasing blood Phe levels, the upper target Phe level varies not only between countries but also from center to center.[1] For the first decade of life, recommendations for upper target Phe levels usually vary between 240 and 360 μmol/L.[5,17] These guidelines, however, are not based on studies comparing outcomes at Phe levels <240 μmol/L, between 240 and 360 μmol/L, and >360 μmol/L.

At present, treatment guidelines for PKU primarily advise on absolute upper target Phe levels, thereby leaving other suggested biochemical measures, such as fluctuations of Phe values[18,19] and the Phe-to-tyrosine (Tyr) ratio (Phe:Tyr)[20,21] without advised treatment target. Phe variation has been related to outcome measures (predominantly IQ) in patients with PKU and their children.[18,22] The interest in Phe variation is growing as it appears that new therapies may increase stability in blood Phe levels.[23-25] Phe:Tyr might be of interest considering the fact that Tyr levels are more closely related to dopamine levels than are Phe levels.[26] Studies investigating Phe variation in relation to cognitive outcomes show mixed results or did not take into account the generally strong associations between absolute Phe levels and Phe variation. Recent studies on Phe:Tyr showed that high lifetime ratios rather than absolute Phe levels were significantly related to impairment in executive functioning.[20,21] It

FL	Flanker
Phe	Phenylalanine
Phe:Tyr	Phenylalanine:tyrosine
PKU	Phenylketonuria
PU	Pursuit
SAD	Sustained Attention-Dots
SSV	Shifting Attentional Set-Visual
Tyr	Tyrosine

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should be noted, however, that Phe:Tyr also strongly relates to absolute Phe levels.

This study aimed to address the following questions: (1) Should blood Phe of 240 μmol/L be the upper target of treatment compared with 360 μmol/L? and (2) What is the relative importance of lifetime Phe, concurrent Phe, variation in lifetime Phe levels, and lifetime and concurrent Phe:Tyr in predicting cognitive outcome in early and continuously treated children and adolescents with PKU?

Methods

Sixty-seven patients with PKU participated in neuropsychologic examinations at the clinical centers where they received regular care. All had been treated, within 1 month after birth, and in accordance with Dutch guidelines.[27] Reliable or sufficient data on Phe and Tyr levels were unavailable for 4 patients; therefore, a total of 63 patients with PKU were included in the statistical analyses. Twenty-seven patients had pretreatment Phe levels >1200 μmol/L (classic PKU), 18 patients had pretreatment Phe levels of 600-1200 μmol/L (mild PKU), and 18 patients had Phe levels <600 μmol/L, of whom 6 patients started with 360-600 μmol/L (hyperphenylalaninemia) and 12 patients with pretreatment Phe levels <360 μmol/L (mild Hyperphenylalaninemia).[28] The mean age of the patients with PKU (30 boys, 33 girls) was 10.8 ± 2.3 years (range 6.0-15.2 years), similar to the mean age of the controls (n = 73, mean age 10.9 ± 2.2 years, range 6.6-15.5 years). Controls (44 boys, 29 girls) were recruited from the patients' families and friends or through newspaper advertisements.

A morning blood sample after overnight fasting was taken before the start of the assessment to determine concurrent Phe (mean 439.1 ± 248.7 μmol/L) and Tyr levels (mean 82.6 ± 54.2 μmol/L). From all Phe levels during each half-year of life, the median was taken, to take into account possible changes in frequencies of Phe measurements. The mean of all available half-year median Phe levels from birth until the day of testing constituted the lifetime Phe level (mean 336.8 ± 116.6 μmol/L). Phe variation was calculated by averaging the SDs of the half-year median Phe levels (mean 174.5 ± 66.9 μmol/L). Lifetime Phe:Tyr were also determined from the half-year median Phe and Tyr levels (mean 8.4 ± 6.9). The mean concurrent Phe:Tyr was 7.6 ± 6.1. To examine optimal upper target levels, a distinction was made between patients with lifetime Phe <240 μmol/L, between 240 and 360 μmol/L, and >360 μmol/L. Table I provides descriptive information on biochemical measures for the 3 PKU groups and descriptive statistics for the neuropsychological tasks for all groups. Written informed consent was obtained from patients' parents or caretakers before the start of the study. The study was approved by the ethical committees of the clinical centers and the Dutch National PKU Steering Committee.

The participants performed computerized neuropsychologic tasks (Amsterdam Neuropsychological Tasks[29]) measuring the executive functions inhibitory control ("Sustained Attention-Dots" [SAD], "Flanker" [FL], and "Shifting Attentional Set-Visual" [SSV]), cognitive flexibility (measured with the SSV task), and motor control ("Pursuit" [PU]). Because not all participants completed all tasks, there were some small variations in sample size for the different analyses.

In the SAD task, 3 (n = 200), 4 (n = 200), or 5 (n = 200) dots appear on a computer screen. Participants respond by pressing the left- or right-hand mouse button. A response bias is induced by requiring 1 of 2 possible responses twice as frequently (eg, press left when 3 or 5 dots appear, and right when 4 dots appear). Thus, this response becomes the "automatic" response, and inhibitory control is required when the "nonautomatic" response is required (Table I).

In the FL task, the response that has to be given depends on the color of the central stimulus. This central stimulus is surrounded by FL stimuli, which can be facilitating (part 1 of the task), neutral (parts 1 and 2 of the task), or interfering (part 2 of the task). Contrasts between performance in parts 1 and 2 of the task represent quality of inhibitory control.

The SSV task consists of 3 parts. In part 1, participants have to respond in accordance with the direction of movement of a green-colored square across a bar presented on the computer screen. In part 2, responses should be given opposite to the direction of movement of a red-colored square, and in part 3, the response depends on the color of the square after each movement. The contrast between performances in part 1 and part 2 represents inhibitory control, whereas the contrast between performances in part 1 and part 3 represents cognitive flexibility.

Motor control was measured by the PU task, in which participants try to keep the mouse cursor on an asterisk that is randomly moving across the screen. Accuracy of movement (mean deviation in millimeters from a moving stimulus) and stability of movement (SD of one's movement trajectory) were registered.

In general, higher scores reflect poorer outcomes, indicating greater differences in error rate or reaction time between experimental and control conditions, or greater movement deviations. Further detailed information on the tasks can be found elsewhere.[5,9,30]

Statistical Analyses

For the first set of analyses on which upper target level is most beneficial for neurocognitive functioning, nonparametric Kruskal–Wallis and Mann–Whitney tests were used, comparing performances of controls and patients with PKU with lifetime Phe ≤240 μmol/L, 240-360 μmol/L, and ≥360 μmol/L. Pearson correlations and stepwise regression analyses, starting in step 1 with all indices of dietary control that were significantly correlated with the outcome measures of interest, were conducted to determine which indices of dietary control were significant predictors of cognitive outcome. Analyses were conducted with the statistical program IBM SPSS Statistics 19 (Chicago, Illinois).