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CLINICAL RESEARCH ARTICLE

Metabolic control during the neonatal period in phenylketonuria: associations with childhood IQ

Geertje B. Liemburg[1], Stephan C. J. Huijbregts[2]✉, Frank Rutsch[3], Reinhold Feldmann[3], Rianne Jahja[1], Josef Weglage[3], Ulrike Och[3], Johannes G. M. Burgerhof[4] and Francjan J. van Spronsen[1]

BACKGROUND: In phenylketonuria, treatment and subsequent lowering of phenylalanine levels usually occur within the first month of life. This study investigated whether different indicators of metabolic control during the neonatal period were associated with IQ during late childhood/early adolescence.

METHODS: Overall phenylalanine concentration during the first month of life (total "area under the curve"), proportion of phenylalanine concentrations above upper target level (360 µmol/L) and proportion below lower target level (120 µmol/L) during this period, diagnostic phenylalanine levels, number of days until phenylalanine levels were <360 µmol/L, and lifetime and concurrent phenylalanine levels were correlated with IQ scores of 64 PKU patients (mean age 10.8 years, SD 2.9).

RESULTS: Overall phenylalanine concentration and proportion of phenylalanine concentrations >360 µmol/L during the first month of life negatively correlated with IQ in late childhood/early adolescence. Separately, phenylalanine concentrations during different periods within the first month of life (0–10 days, 11–20 days, 21–30 days) were negatively correlated with later IQ as well, but correlation strengths did not differ significantly. No further significant associations were found.

CONCLUSIONS: In phenylketonuria, achievement of target-range phenylalanine levels during the neonatal period is important for cognition later in life, also when compared to other indicators of metabolic control.

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IMPACT:

- In phenylketonuria, it remains unclear during which age periods or developmental stages metabolic control is most important for later cognitive outcomes.
- Phenylalanine levels during the neonatal period were clearly and negatively related to later IQ, whereas no significant associations were observed for other indices of metabolic control. This emphasizes the relative importance of this period for cognitive development in phenylketonuria.
- No further distinctions were observed in strength of associations with later IQ between different indicators of metabolic control during the neonatal period. Thus, achievement of good metabolic control within 1 month after birth appears "safe" with respect to later cognitive outcomes.

INTRODUCTION

Phenylketonuria (PKU; OMIM 261600) is caused by an autosomal recessively inherited deficiency of the hepatic enzyme phenylalanine hydroxylase (PAH),[1,2] normally converting phenylalanine (Phe) into tyrosine (Tyr). Biochemically, the defect is characterized by strongly increased blood and cerebral Phe concentrations and reduced to normal Tyr concentrations.[1]

If left untreated, PKU is associated with neurological and behavioral problems, such as severe intellectual disability, epilepsy, and anxiety disorders.[3,4] An early diagnosis of PKU following neonatal screening and immediate start of a Phe-restricted diet to reduce blood Phe concentrations prevent most of the neurological problems,[1] while some patients benefit from treatment with tetrahydrobiopterin (BH4), the natural co-substrate of PAH.[1] Long-term effects of Phe ammonia lyase,[5–7] a recently Food and Drug Administration- and European Medicines Agency-approved new treatment for (adult) PKU patients, on neurocognitive outcome still need to be investigated.

Early and continuous treatment prevents severe intellectual disability, but the outcome still remains suboptimal.[8,9] Higher Phe concentrations have been associated with lower intelligence quotient (IQ) and problems in executive and social functioning at different ages, with childhood Phe levels often being more predictive of outcomes later in life than recent or concurrent Phe

[1]Division of Metabolic Diseases, Beatrix Children's Hospital, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands. [2]Department of Clinical Child and Adolescent Studies/Leiden Institute for Brain and Cognition, Leiden University, Leiden, The Netherlands. [3]Department of Pediatrics, Münster University, University Children's Hospital, Münster, Germany. [4]Department of Epidemiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands. ✉email: shuijbregts@fsw.leidenuniv.nl

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levels.[10–14] Therefore, advised upper target Phe concentrations have decreased over time, especially for younger PKU patients.[15,16] Currently, Phe concentrations within the target range of 120–360 µmol/L are the most commonly advised, especially for patients <12 years of age.[17] For the period between 0 and 12 years, there are several studies indicating that the first 6 or 7 years of life are the most important for achieving optimal outcomes later in life.[12,14,18,19] During this period of life, the fastest cognitive development takes place, although this continues into young adulthood, and more complex abilities, and those involving motivational or emotion regulation, have later developmental peaks as well.[20,21] Still, the building blocks or foundations of these (complex) abilities develop strongly from birth onwards, which suggests that the first months of life of PKU patients may represent an especially vulnerable period when Phe levels are not within the target range. Indeed, Burgard et al. reported higher IQ scores in patients who started treatment within 3 weeks if compared with start of treatment between 3 and 6 weeks,[22] while Van der Schot et al.[23] found significant negative correlations between the mental development at 1 and 2 years of age and Phe concentrations >500 µmol/L during the first 30 days of life. Smith et al.[24] showed that IQ fell progressively by roughly four points for each 4 weeks' delay in starting treatment in a large cohort of patients where treatment was started within 4 months after birth (1031 children born between 1964 and 1980 (808 followed prospectively). On the other hand, they showed that "too low phenylalanine concentrations" (<120 µmol/L, which is the maximum value that would be expected in healthy individuals) for a period of several months during the first 2 years of life also resulted in suboptimal outcome. Although it is difficult to translate the results of these studies, which have generally been performed several decades ago, to the present day situation, where metabolic control in PKU patients has strongly been improved and occasions where treatment is started and metabolic control is achieved after 1–2 months are rare, they clearly indicate that an earlier achievement of acceptable Phe levels (including a minimum) might benefit later cognitive development. As noted, newborn screening takes place, and treatment is started within 1 month after birth in most countries. Moreover, acceptable Phe levels are generally achieved within weeks after the start of treatment. A question that remains is whether variation within the first 30 days of life has an effect on later cognitive development. If this is the case, programs on neonatal screening for PKU and its early diagnostic and treatment strategies may have to be sharpened further. Therefore, the present study's research question was: Is neonatal metabolic control in PKU patients related to IQ during late childhood/early adolescence? Whereas the term neonatal metabolic control mainly represents average Phe levels and Phe levels outside the recommended range during the first month of life, there are several other factors that might influence these Phe levels, and that could therefore also be considered indicators of neonatal metabolic control. These include diagnostic Phe levels and number of days until Phe levels were <360 µmol/L. A higher diagnostic Phe level may be representative of the extent of PAH dysfunction (and thus of the specific genetic mutation) and could result in a later achievement of Phe levels within the target range.

Based on the evidence for beneficial effects of lowering Phe levels as quickly as possible after a PKU diagnosis, and the evidence for the importance of the neonatal period for cognitive development, it was expected that Phe levels during the first month of life, as well as diagnostic Phe and number of days until target range Phe levels were reached, would be related to IQ later in life. It was also investigated whether the strength of Phe–IQ correlations varied within the first postnatal month. In case only Phe levels during the first 10 days of life were related to later IQ, or when such correlations would be significantly stronger than those for the remainder of the neonatal period (i.e., between 10 and 30 days), it could be argued that neonatal screening should be further advanced, and treatment should be started as early as possible during the neonatal age (i.e., "the earlier the better"). Finally, it was expected that lifetime and concurrent Phe levels (i.e., on the day of testing) would also be related to IQ scores. This hypothesis was based on previous findings.[9–14,17–19] Considering the continued cognitive development throughout childhood and early adolescence, no big differences were expected in Phe–IQ correlations between the neonatal and later childhood periods.

METHODS

Participants

Forty-four Dutch patients and 20 German patients were included in this study (mean age 10.8 years, SD 2.9 years, range 6–18 years, 30 males and 34 females). All patients were detected through neonatal screening for PKU (mean: 6.7 days, SD 3.5, range 0–20 days) and continuously treated from that moment onwards until the moment of neurocognitive testing.

The study was approved by the medical ethical committees of the participating treatment centers and has been registered in the CCMO Register in the Netherlands (NL38932.042.11). The Dutch patients were part of the PKU-COBESO study.[14,25] German patients had participated in various studies at the University of Münster,[26,27] which were all approved by the local METC. Participants and/or parents gave written informed consent to participate in the study and for using their data in this report.

Measurements

The IQ scores were obtained with the Wechsler Intelligence Scale for Children (WISC). In the Netherlands, an abbreviated version of the WISC-III was used consisting of two subtests: Block Design (performance IQ) and Vocabulary (verbal IQ).[28] In Germany, children completed the Hamburg–Wechsler-Intelligenztest (HAWIK-III and IV), which is a German translation of the WISC.[29] Its subtests measured speech, logical thinking, working memory, and processing speed. All (standardized) subtest scores (and the total IQ score) of the WISC/HAWIK fall into a normal distribution with an average of 100 (SD 15).

Diagnostic blood Phe concentration, number of days until target range Phe levels were reached, and all Phe concentrations during first month after birth were collected. Also, lifetime Phe levels were retrieved (only for the Dutch patients) and concurrent Phe levels on the day of IQ testing were determined (all patients).

Statistical analyses

As Phe levels (and number of measurements) during the first month of life tend to vary strongly, the area under the curve (AUC) was used to analyze the Phe concentrations during this time. Since patients were diagnosed from 4 days onwards, an estimated course from birth until the fourth day of life was needed. For this, reference data were used from McCabe et al.[30], who collected blood Phe concentrations of PKU patients at birth, during the first 12 and 24 h, and after 2, 3, and 4 days.

All blood Phe concentrations were graphically plotted against time using the program RStudio.[31] The surface under the curve was estimated by the following formula:

$$\sum_{i=1}^{n-1} \frac{(C_i + C_{i+1}) \cdot (T_{i+1} - T_i)}{2}$$

In this calculation, C_1 and C_2 are successive blood Phe concentrations and T is the day the blood sample was taken, T_2 minus T_1 being the period of time between two blood samples.

First, the total surface under the curve was estimated after which the proportion of Phe levels above the most commonly recommended upper target limit of 360 µmol/L (AUC area above 360 µmol/L) was calculated. Also, the proportion of Phe values <120 µmol/L was calculated to study the effect of (too) low blood Phe concentrations. To study the influence of Phe levels during the first month in more detail, we also divided the month into various periods, i.e., day 0–10, day 10–20, and day 20–30. Correlations were calculated between IQ and the abovementioned indices of metabolic control during the first month of life. Correlations (Pearson's r) with diagnostic Phe level, number of days it took to achieve target range Phe levels, and lifetime and concurrent Phe levels were also calculated. Lifetime Phe levels, i.e., the mean of half-year median Phe levels from 1 month after

birth until the day of testing were available for 44 PKU patients, whereas the *N* for all other analyses was 64. For calculation of the correlations, IBM SPSS Statistics 25 was used. Comparisons between (strength of) correlations were performed according to Eid, Gollwitzer, and Schmitt.[32,33]

RESULTS

The average total IQ of the PKU patients in this study was 100.1 (SD 11.8, range 74–127.5). Details regarding metabolic control, especially during the first month of life, are provided in Table 1.

IQ correlated negatively with AUC >360 µmol/L during the first month of life ($r = -0.287$, $p = 0.012$, as well as with total AUC during the first month ($r = -0.275$, $p = 0.015$). No significant correlation was found between AUC <120 µmol/L during the first month and IQ ($r = 0.026$, $p = 0.422$). Also, no significant correlations were found between IQ and diagnostic Phe level ($r = -0.07$, $p = 0.303$), highest Phe level during the first month of life ($r = -0.135$, $p = 0.145$), days (from birth) until metabolic control was achieved (excluding the 1 patient for whom 82 days were reported, see Table 1) ($r = -0.128$, $p = 0.167$), lifetime Phe level ($r = -0.059$, $p = 0.352$), or blood Phe concentrations at the day of testing ($r = -0.096$, $p = 0.454$).

To study the effect of Phe values during the neonatal period on later IQ in more detail, the first month was divided into three periods (0–10, 10–20, and 20–30 days). AUC >360 µmol/L between 0 and 10 days was significantly related to later IQ ($r = -0.217$, $p = 0.045$), whereas a trend was observed for total AUC between 0 and 10 days ($r = -0.200$, $p = 0.059$). AUC >360 µmol/L between 10 and 20 days was significantly related to later IQ ($r = -0.271$, $p = 0.016$) as was total AUC between 10 and 20 days ($r = -0.238$, $p = 0.032$). The correlation between AUC >360 µmol/L between 20 and 30 days and later IQ was a trend ($r = -0.189$, $p = 0.070$), while the correlation with total AUC between 20 and 30 days did not reach significance ($r = -0.132$, $p = 0.153$). These results suggested that Phe levels during the middle portion of the neonatal period (around day 20) had the strongest influence on later IQ. However, in absolute sense the correlations did not differ substantially, and comparisons of (strength of) correlations with IQ did not reveal significant differences: AUC >360 between 0 and 10 days versus 10–20 days versus 20–30 days, $z = 0.288$, $p = 0.387$).[33] Similar to results for the overall neonatal period, no significant correlations between IQ and AUC <120 µmol/L during the separate periods within the first month of life were observed.

Table 1. PKU patients' metabolic control during the first month of life ($n = 64$).

	Mean	SD	Min	Max
Concurrent Phe (µmol/L)[a]	413	233	130	1250
Historical Phe (µmol/L)[b,c]	312	90	199	707
Diagnostic Phe (µmol/L)	1105	658	250	2938
Time of diagnostic Phe (days of life)	6.7	3.5	0	20
Highest Phe concentration during first month (µmol/L)	1293	693	302	3136
Days from birth until Phe <360 µmol/L	14	10.8	3	82[d]
Phe area under the curve (AUC) day 0–30	14014	5991	6018	36039
AUC >360 µmol/L day 0–30	6454	5565	110	27258
Proportion AUC >360 µmol/L day 0–30[e]	0.39	0.19	0.02	0.76
AUC <120 µmol/L day 0–30	359	415	0	1634
Proportion AUC <120 µmol/L day 0–30[e]	0.03	0.05	0	0.27

[a]Blood Phe concentration on the day of (IQ) testing.
[b]$N = 44$, as data were only available for Dutch patients.
[c]Mean of half year median Phe levels from birth until the day of testing.
[d]One PKU patient in sample where achievement of target level Phe was reported at day 82. Next longest period was day 31. Descriptive statistics without this participant: mean: 12.8, SD: 6.2, min 3, max 31. Median on both occasions: 12.
[e]Proportions of area under the curve (AUC) outside Phe target range (120–360 µmol/L).

DISCUSSION

This study explored relationships between several different indicators of metabolic control during the first month of life and IQ in children and adolescents with PKU. Associations between lifetime Phe levels and IQ and between Phe levels on the day of testing and IQ were also examined. The main finding was that a lower IQ during late childhood and early adolescence was related to higher blood Phe concentrations during the first month of life. Specifically, the proportion of Phe levels >360 µmol/L (the upper target limit for children with PKU) was negatively related to later IQ. Low Phe levels (<120 µmol/L) in the first weeks of life were unrelated to later IQ, although it should be noted that these occurred much less frequently than high Phe levels, while none of the other indicators of metabolic control, including lifetime and concurrent Phe levels, were related to later IQ either. These (contrasting) results appear to signify the relative importance of the neonatal period for later cognitive development in PKU patients. However, before discussing the results in more detail, some (methodological) limitations should be addressed. First, the number of measurements and the time between measurements varied somewhat between patients, the effects of which were counteracted as much as possible by using the AUC approach. Second, as measurements of the first days until the diagnostic Phe concentration were lacking, we had to use reference values of McCabe et al.[30] to roughly estimate those levels. Another limitation is that IQ was used as the sole measure for cognitive functioning in this population. Acknowledging the fact that deficits in executive functioning can still be present when IQ is within the normal range[32] and that childhood correlations of Phe measures with IQ measures are usually less strong than those with executive function,[34–36] studying the relation between executive functioning and early Phe measures could be of further value. Also, only metabolic control was investigated with respect to its effect on IQ.

Other interesting factors in this respect such as socioeconomic status, parenting, or IQ of family members, which are likely to modulate any influence of metabolic control on cognitive outcomes, could not be taken into account in this study. Also, metabolic control beyond the neonatal period could be investigated in more detail. For example, there is accumulating evidence showing that variability or fluctuations in Phe levels predict cognitive outcomes beyond historical or concurrent Phe levels[37] and also that (historical) Phe levels during sensitive periods for cognitive development may have a particularly strong influence on cognitive outcomes. Whereas our data did not allow for a specific investigation of this, it remains possible that such alternative measures of (historical) metabolic control are more strongly related to cognitive outcome measures than the ones we had available for the post-neonatal period. As noted, we did opt for the AUC approach when analyzing our data, as Phe fluctuations and differences in the number of Phe assessments are particularly prominent during the neonatal period. Finally, the Dutch and German PKU patients who participated in the present study generally had very good metabolic control, with the majority showing lifetime and concurrent Phe levels within the treatment target range. Whereas this is obviously a good thing, methodologically it may have resulted in relatively small variability in (post-neonatal) Phe levels, which, in turn, reduces the chances

of significant associations between Phe levels and outcome measures.

There are only few reports that pay attention to metabolic control during the first month of life in PKU patients.[22–24,38,39] Van der Schot et al.[22] used growth curve modeling in 33 patients to estimate the surface >500 µmol/L as a measure for elevated Phe concentrations. They found significant negative correlations between the mental development at both 1 and 2 years of age and elevated blood Phe concentrations during the first 30 days of life. Zeman et al.[38] found that dietary treatment introduced in the first 3 weeks of life was associated with better school performance and IQ in a sample of 81 adolescents with PKU compared to when dietary treatment was started after 3 weeks. It is not entirely clear what the variability in treatment starting time (after 3 weeks) was: obviously, it could make a big difference whether treatment was started in the fourth week of life or after several months. Such detail was provided by Burgard et al.[22], who compared IQ scores of patients who started treatment within 3 weeks to those of patients starting between 3 and 6 weeks. Comparable results were obtained by Gonzalez et al.[39], who, like Zeman et al.[38], not only had a sample with large variation regarding start of treatment (with only 25% of the late-treated group (>2 months) having an IQ in the normal range) but also showed that PKU patients treated before 1 month had higher IQ scores than those treated between 1 and 2 months of life. We aimed to extend this knowledge by zooming in on the first month of life and by including not only absolute Phe levels but also the recommended target range for Phe levels during childhood (including infancy). Phe levels (also proportion of Phe levels >360 µmol/L) during the first 10 days of life, and particularly between 10 and 20 days, appeared to be most strongly related to later IQ, although comparisons with Phe levels between 20 and 30 days did not show statistically significant differences. Therefore, these results show some support for "the earlier, the better" hypothesis for achievement of metabolic control within the first month of life but insufficient support to substantiate that detrimental effects will occur when metabolic control is achieved later in the first month of life. Contrasts with other (including later) indicators of metabolic control are more convincing and emphasize the importance, once more, to achieve metabolic control in the first month of life in PKU.

As noted before, treatment guidelines generally not only include an upper target limit, i.e., 360 µmol/L for children up to the age of 12 in European guidelines[15] and throughout life in US guidelines,[16] but also advise target ranges for Phe level, including a minimum level as well, as Phe is also an important amino acid required for adequate (physical) growth.[40,41] The minimum blood Phe concentration is generally set at 120 µmol/L, which is the maximum Phe level observed in healthy individuals. In PKU patients, Smith et al.[24] found that IQ fell progressively with four IQ points for each 5 months with Phe concentrations <120 µmol/L in the first 2 years of life. In Tyrosinemia Type 1, a genetic disorder where the same metabolic pathway is affected as in PKU, low Phe levels in the beginning of life were shown to be related to later cognitive, behavioral, and social outcomes as well.[40,41] In the present study, however, we found no evidence for associations between low Phe levels (<120 µmol/L) during the first month of life and later IQ. This is in agreement with earlier findings by Van der Schot and colleagues.[23] Possibly, the negative effect of low Phe concentrations only becomes evident after longer lasting periods of time with low Phe.

In conclusion, our data suggest that, as long as treatment is started within the first month of life, there are no indications that, within this month, an earlier lowering of Phe levels makes a further difference, at least to later IQ scores. The effects of (the start of) metabolic control during the first month of life in relation to other outcomes, including executive and social–cognitive functioning, should be investigated further. Still, the present results confirm that current clinical practice is working well. Even though our findings do not suggest antedating the moment of neonatal screening for PKU would provide further improvement of long-term outcomes in PKU, clinicians should remain aware that the introduction of new medication and screening procedures (e.g., the BH4 loading test) might put pressure on the whole chain of actions to get patients treated as early as possible. Currently, there are hardly any patients left whose treatment is started after the first month of life, but it is important to keep it this way. Our results indicate that good metabolic control in this period of time is essential for later cognitive–behavioral outcomes.

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AUTHOR CONTRIBUTIONS

G.B.L., S.C.J.H., F.R., J.G., M.B. and F.J.v.S. contributed to conception and design of the study and the analysis and interpretation of data. G.B.L., F.R., R.F., R.J. and U.O. contributed to acquisition of data. All authors contributed to drafting the article or revising it critically for intellectual content.

COMPETING INTERESTS

S.C.J.H. has participated in strategic advisory boards and received grants and honoraria as a consultant and/or speaker from Biomarin, Merck- Serono, Homology Medicines, and Nutricia. R.J. has received honoraria as a speaker and consultant from Merck- Serono and Biomarin. F.J.v.S. has received research grants, advisory board fees, and speaker's honoraria from Nutricia Research, Merck-Serono, Biomarin, Codexis, Alexion, Vitaflo, MendeliKABS, Promethera, SOBI, APR, and ARLA Foods Int. G.B.L., F.R., R.F., J.W., U.O. and J.G.M.B. declared that they do not have potential conflicts of interest.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Participants and/or parents gave written informed consent to participate in the study and for using their data in this report.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Stephan C. J. Huijbregts.

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