Master's Thesis

Association of Phenylalanine Levels and Dietary Control with Cognitive Performance in Early-Treated Phenylketonuria: A Retrospective Longitudinal Study from Childhood to Adolescence

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Abstract

Background: Phenylketonuria (PKU) is a metabolic disorder characterised by impaired phenylalanine (Phe) metabolism. Early diagnosis and dietary management can prevent neurological impairment, but the impact of long-term dietary control on cognitive performance from early childhood to adolescence is not yet fully understood. ***Objective:*** This study investigated the association between Phe levels and cognitive performance in early-treated PKU patients from early childhood to early adolescence. ***Methods:*** Data from 64 PKU patients (35 female, 29 male) treated at Heidelberg University Hospital were analysed. Cognitive performance was assessed using reaction time (RT) tasks. Historical and concurrent Phe levels were collected from the clinic's databases and indices of dietary control (IDC) were formed from mean half-year median Phe levels. The relationship between IDCs, concurrent Phe levels and cognitive performance was examined by multiple linear regression analyses. ***Results:*** The longitudinal analysis included 35 patients (from the initial sample of 64; 21 female, 14 male) tested at two time points (T1: mean age 8.7 years; T2: mean age 13.5 years). Lower concurrent Phe levels at T1 were significantly associated with better cognitive performance at T1, especially in simple RT and visual search tasks. No significant association was found between Phe levels after age ten and cognitive performance at T2, but cognitive performance at T1 correlated significantly with performance at T2. ***Conclusions****:* Maintaining low Phe levels in early childhood is important for optimised cognitive outcomes in young PKU patients, emphasising the importance of strict dietary control during critical developmental periods. The results of this study did not support the need for continuation of a strict dietary control during adolescence. Further research is required to explore the potential relaxation of dietary restrictions in adolescence and the long-term cognitive development.

Keywords: Phenylketonuria, Dietary Control, Cognitive Performance, Children, Longitudinal.

Association of Phenylalanine Levels and Dietary Control with Cognitive Performance in Early-Treated Phenylketonuria: A Retrospective Longitudinal Study from Childhood to Adolescence

PKU is an autosomal-recessive transmitted metabolic disease characterised by a defect in the metabolism of the essential amino acid Phe (Burlina et al., 2015). The prevalence of PKU varies significantly across ethnicities and geographical locations, affecting approximately one in 24,000 individuals globally (Elhawary et al., 2022), and one in 100,000 in Europe (Zuñiga Vinueza, 2023). About 0.45 million individuals worldwide are currently diagnosed with PKU (Hillert et al., 2020). PKU can be categorised in three groups based on the severity of the enzyme deficiency: classical PKU (Type 1), mild PKU (Type 2) and mild hyperphenylalaninemia (HPA; Type 3) (Williams et al., 2008). Despite the widespread introduction of phenylketonuria (PKU) as a target disease in newborn screening programs in Germany since 1969 (Gramer et al., 2018) and the subsequent early and effective detection and treatment of patients, the cognitive performance in association with dietary adjustment of phenylalanine (Phe) levels for patients of different ages has not been thoroughly researched throughout PKU patients' early lifespan.

Normally phenylalanine is metabolised by the enzyme phenylalanine hydroxylase (PAH) into tyrosine (Mitchell et al., 2011). Due to a monogenetic defect in PKU patients this transformation is impaired or does not occur at all, leading to the accumulation of phenylalanine in the organism (Stern, 2000; Zuñiga Vinueza, 2023). The resulting high Phe levels can potentially harm the white matter of the brain (Dyer, 1999). If left untreated, PKU results in severe neurological dysfunction and intellectual disability, motor deficits, and behavioural difficulties (Blau et al., 2010; De Lucca et al., 2017). However, early diagnosis and start of metabolic treatment can effectively prevent severe cerebral impairment and improve neurological outcomes (Van Wegberg et al., 2017).

The condition is managed by a carefully calculated phenylalanine-reduced, semisynthetic diet with an additional intake of a phenylalanine-free amino acid mixture supplemented with vitamins, trace elements, and minerals (Evans et al., 2014; MacDonald et al., 2020). To effectively prevent intellectual disability, it is important to initiate dietary Phe restriction already shortly after birth (Blau et al., 2010). Nevertheless, maintaining such a strict diet can be challenging, particularly for adolescents and young adults, leading to poor adherence (Blau et al., 2010). Consequently, PKU patients often still demonstrate lower Intelligence Quotient (IQ) scores and perform less proficiently across several cognitive domains compared to healthy controls (Koch et al., 2002; Romani et al., 2017).

Cognitive performance, encompasses several executive functions (EF) and stages of information processing, including the encoding of stimuli, working memory, decision making,

attention and organisation of motor function (Canton et al., 2019). These processes require structural and functional integrity of specific gray and white matter, and circuits including the cortex, basal ganglia, and thalamus, such as the cortico-striato-thalamocortical loop (Leisman & Melillo, 2013). Given the characteristic cerebral abnormalities of myelination in PKU and in analogy to white matter changes in other neurological diseases, PKU patients might be at risk for neurobehavioral impairments in RTs, information processing, or abnormal development of cognitive functions (Anderson et al., 2007).

A connection between average Phe levels during treatment and cognitive outcomes has been suggested for adulthood (Jahja et al., 2017; Bartus et al., 2018), but there is limited research on the long-term relationship between Phe levels and cognitive performance in children and adolescents. During the first ten years of life (infancy and childhood) the brain's vulnerability appears to be greatest due to essential developmental processes, such as synaptogenesis, dendritic arborisation, and myelination (Semple et al., 2013). Especially for this developmental period negative relationships between blood Phe levels and IQ test scores have been demonstrated (Fonnesbeck et al., 2013). On average, early treated PKU patients show reductions in general IQ test scores of half a standard deviation per 300 µmol/l increase in Phe levels during childhood compared to metabolically healthy control subjects (Burgard, 2000; Waisbren et al., 2007).

According to Jahja et al. (2017) the most commonly noted cognitive deficits in treated PKU patients are found in EF. Typically, EF include domains like cognitive flexibility, working memory, and inhibitory control (Sabhlok et al., 2022). Some authors assume that processing speed, which refers to the rate at which an individual can perceive, process, and respond to information, is a component of EF (Brown et al., 2011). Others, like Sabhlok et al. (2022), view information processing speed as a fundamental ability that can influence EF. Individuals who have a slower processing speed, cannot process information fast enough to activate executive or decision-making functions before new task demands arise (Sabhlok et al., 2022).

Significant variability in cognitive outcomes was found among adult individuals with PKU, who generally performed well on learning tasks but struggled with processing speed and complex executive functions (Palermo et al., 2017). In neuropsychological studies, particularly in the measurement of RTs, a significant but reversible relationship between increased RTs and blood phenylalanine levels at the time of testing was found (Schmidt et al., 1994; Albrecht et al., 2009). When it comes to measuring and comparing cognitive performance over time, RT measures seem more appropriate than IQ measures, especially for PKU patients. IQ is a measure of global intellectual abilities, that depicts the quality of one's solution of different more or less complex cognitive tasks (verbal and non-verbal knowledge and understanding, logical reasoning), as well as the amount of resolved items (power test) (Mackintosh, 2011). On the other hand, elementary cognitive processes measured by RT paradigms (i.e.,

sustained attention, scanning of stimuli, binary decisions) qualify as sensitive measures especially for the speed of information processing (Albrecht et al., 2009). An additional advantage of RT measures is the mostly language- and age- independent implementation of the tests (Albrecht et al., 2009). In contrast, IQ test batteries are based on different theoretical concepts, designed and validated for different age groups, making longitudinal comparisons challenging, as patients of different age groups receive different IQ test questions and tasks, and IQ values can be calculated from different scores, influenced by changes over time (e.g., the Flynn effect) (Must et al., 2009).

Despite the established relationship between Phe levels and processing speed (Albrecht et al., 2009), so far only few longitudinal studies have investigated the development of cognitive abilities in PKU patients in relation to their Phe concentration, measuring EF (Sharman et al. 2015; Mastrangelo et al. 2015; Jahja et al., 2017). Sharman et al. (2015) were the first to longitudinally examine EF in adolescents with hyperphenylalaninemia. Two sibling pairs with mild hyperphenylalaninemia underwent neuropsychological testing in early childhood and early adolescence. By early adolescence, none of the four children showed EF impairment, displaying a typical developmental trajectory despite phenylalanine exposure. The longitudinal study by Mastrangelo et al. (2015) found that white matter abnormalities on MRI increased with age and poor dietary control, but these abnormalities did not significantly affect cognitive outcomes (IQ) in 24 early-treated PKU patients during adolescence and early adulthood, with about 30% of their variability unexplained by known factors. Jahja et al. (2017) found that in 21 PKU patients, lower childhood phenylalanine levels were significantly associated with better executive functioning and mental health in adulthood, highlighting the importance of maintaining phenylalanine levels below 360 µmol/L during childhood for better long-term outcomes, despite limited evidence for continued influence after childhood.

Studies on the relationship between Phe levels and cognitive performance mostly had very small sample sizes and/or lacked control groups (Sharman et al. 2015; Mastrangelo et al. 2015; Jahja et al., 2017). Moreover, the few longitudinal studies on cognitive functioning in PKU mainly focused on IQ rather than on the speed of information processing (Schuler et al., 1996; Nardecchia et al., 2015). Furthermore, no longitudinal studies of cognitive performance in PKU patients across different age groups, particularly during the critical developmental period of infancy and childhood, have been identified. It remains unclear from the literature to what extent a strict diet is critical for PKU patients during adolescence and adulthood (van Wegberg et al, 2017).

Even though a strict diet can reduce the impact of the metabolic disease on cognitive development, it is important to note that having to maintain a very strict diet and monitor all their intake, can have a profound impact on children's lives (Bosch et al., 2015). In a large international study, Bosch et al. (2015) reported a relatively good health-related quality of life

(QOL) in PKU patients. Nevertheless, the greatest PKU-QOL impact scores were associated with the emotional consequences of PKU, concerns about blood Phe levels, feelings of guilt from not adhering to dietary restrictions or Phe-free amino acid supplement intake, and anxiety about blood Phe levels during pregnancy. Adhering to a strict diet will become especially difficult during adolescence, as children become more independent from their parents and strive to fit in with their peers. Additionally, as adolescents spend more time away from home, maintaining parental control over their diet is likely to be harder to maintain.

In the long term, it would be desirable to explore if dietary restrictions could be somewhat relaxed for young adolescents, which would make everyday life easier, especially for the young patients and their caregivers. This study was conducted to contribute a step in this direction by examining the longitudinal association between dietary control, concurrent Phe levels and cognitive performance in young PKU patients.

Aim of the Study

This study aimed to explore the association between historical and concurrent Phe levels, and cognitive performance, specifically processing speed, in a longitudinal sample of young PKU patients who were treated since the neonatal period and tested at two different time points (T1 and T2) before and after the age of 10. This exploration gives rise to the following research questions:

1. How do historical and concurrent Phe levels at T1 influence specific cognitive performance measures, including simple reaction time, continuous reaction time and stability, and reaction time with different cognitive loads, in early-treated PKU patients under the age of ten?

2. How are the historical Phe levels between T1 and T2, along with concurrent Phe levels at T2 and cognitive performance at T1, associated with cognitive performance in the same group of PKU patients after the age of ten years at T2?

Methods

Design and Participants

The current observational study concerns a retrospective analysis of longitudinal data that was collected between 1970 and 2016 as part of routine monitoring of patients with PKU in the Center for Paediatric and Adolescent Medicine at Heidelberg University Hospital.

In total, 658 PKU patients were registered in the database of the clinic at the time of analysis (see Figure 1). Cognitive performance tests were administered in 199 patients (114 female, 85 male; aged 5.2-35.7 years at test day) between 1986 and 2016 during individual control visits in the outpatient clinic. The frequency of the visits is adjusted to the individual needs of the patient. The visits are usually more frequent in early childhood and less frequent with increasing age depending on the course of the treatment. Of these 199 tested individuals, 151 (76%) were diagnosed as PKU type I, 38 (19%) as type II, 6 (3%) as type III, and the types of 4 patients were not specified in the database. All patients were diagnosed by newborn screening and were treated since the neonatal period (≤28 days after birth).

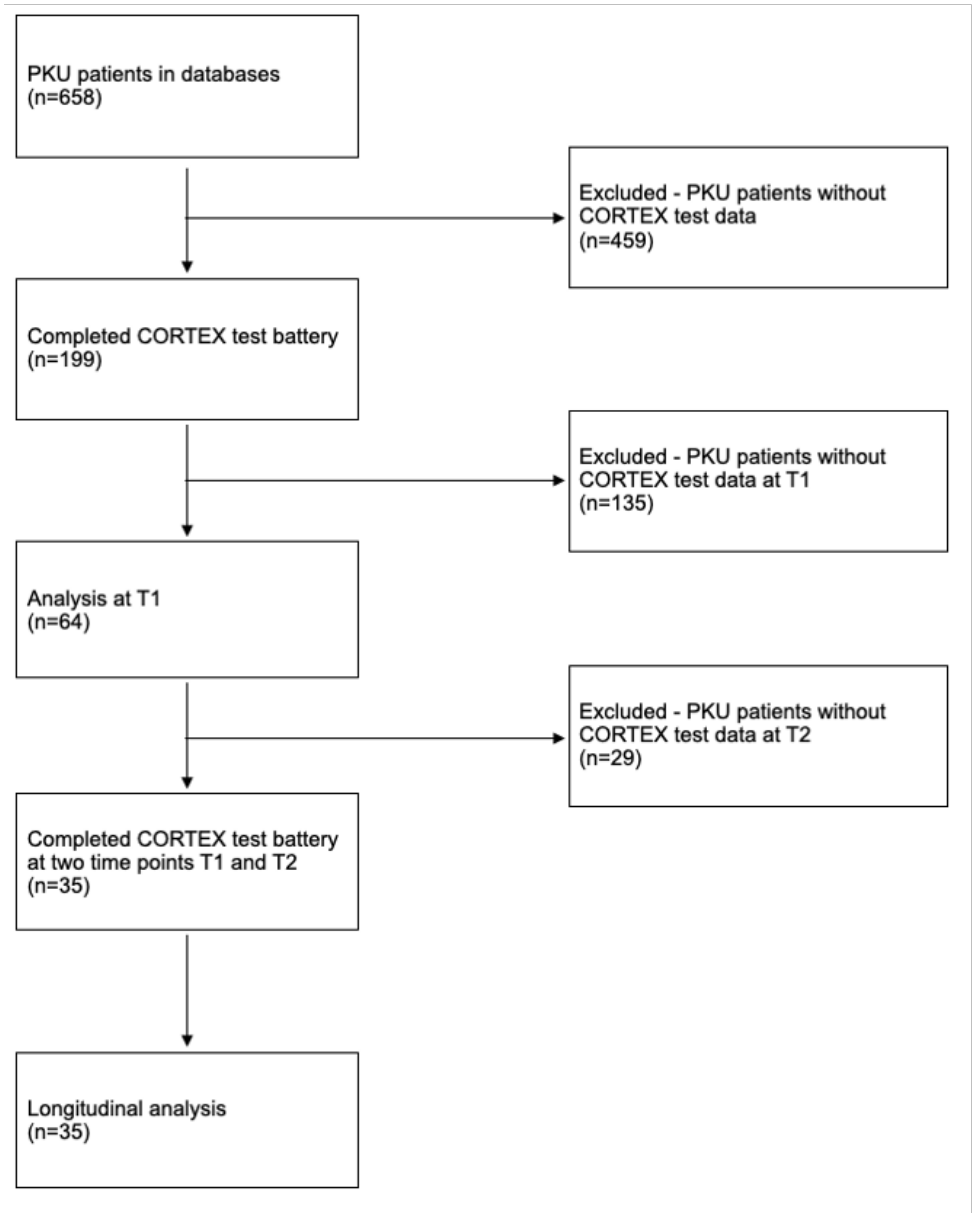
Sixty-four patients were tested with cognitive performance tests before the age of ten, at around 8 years (T1), and 35 (55%) of them were retested at around 13 years (T2). To examine whether a strict Phe diet should be maintained after the age of 10 years, data from these 35 patients, who were tested both before the age of 10 and again at around 13 years, were included in the longitudinal statistical analysis. For all 35 PKU patients who were included in the longitudinal analysis sufficient historical and concurrent Phe values were available in the databases for analyses.

The cognitive performance data of patients were standardised to those of healthy subjects, who were tested with the same test battery as the patients in childhood (n=181; age range 7.8-9.8 years at T1 (M=8.85; SD=0.41) and in early adolescence at T2 (M=13.34; SD=0.65; 11.8-14.9 years)) (Jahja et al., 2017). Data of healthy children and adolescents were collected in local schools of various educational levels from 1994 to 2016, initially for the evaluation of other metabolic diseases (Boy et al., 2015).

The study was approved by the Institutional Ethics Committee of the Medical Faculty of Heidelberg University (No. S-070/2016). All data were collected after obtaining written informed consent from the PKU subjects or their parents, as well as from the healthy subjects. The data were pseudonymised before conducting the statistical analysis.

Figure 1

Flowchart of PKU Patients and Sample Selections for the Analyses



Measures

The cognitive performance of PKU patients was assessed in relation to their historical dietary control and concurrent Phe levels at two time points.

Cognitive Performance

A computer-based psychological test battery called “Cortex”, developed by the clinic's research team for testing subjects with rare inherited metabolic diseases, was administered at both time points, T1 and T2. The aim of the battery is to evaluate basic cognitive processes, largely devoid of educational influences, using the same information processing tasks across the age range from childhood to adulthood (Boy et al., 2015). The test battery originally consists of six computer-based tests that measure various fundamental aspects of information processing, allowing for quantitative analyses of these processes (Boy et al., 2015).

Three RT tasks from the test battery were used to investigate the cognitive performance of patients at time points T1 and T2 (see Table 1). The RT tasks include simple visual reaction time (measuring motor speed) and speed tests with constant low (continuous performance in a binary choice task) or increasing cognitive demand (visual search with 1, 2, and 3 targets). The continuous performance test measures both the mean level of performance and the stability of performance over time. All tests were administered individually to each patient.

In the simple reaction time (SRT) task, a sequence of 80 black squares appears on the screen, requiring the participant to press a button as quickly as possible. Inter-stimulus intervals are randomised to prevent strategy effects. Test scores are calculated as the mean of 80 reactions (40 with the dominant hand and 40 with the non-dominant hand) (SRT Speed). Lower scores indicate faster RTs.

Continuous performance was measured using a choice reaction time paradigm (CRT). A sequence of 600 patterns consisting of three, four, or five squares is presented. The participant must press a button with the dominant hand after four squares (hits) and a button with the non-dominant hand after three or five squares. Test scores are the mean RT for hits (CRT Speed), measuring the level of performance, and the standard deviation of single hit times (CRT SD), measuring the stability of performance.

In the visual search (VS/LDT; Letter Detection Task), the time to decide whether a squared pattern of four different letters contained a set of target letters was measured. Three loads were tested: one (LDTk), two (LDTkr), or three target letters (LDTkrs). Test scores were the mean RTs for correct choices in each of the three loads.

Table 1

CORTEX test battery: Computer-based Information Processing Tests

Test No.	Short Description	Abbreviation
1	Visuomotor simple reaction time	SRT
2	Sustained concentration in a choice reaction time paradigm (Continuous Reaction Time)	CRT
3	Visual search: Choice reaction time with increasing cognitive load (Letter Detection Task)	LDT

Blood Phenylalanine Measurements

Blood samples were taken from PKU patients during their individual regular control visits at the outpatient clinic and Phe values were determined in the laboratory to be registered in the databases and monitored throughout patient treatment.

Historical Phe levels were extracted from the clinic's databases for the purpose of this study. Indices of dietary control (IDC) were calculated as the mean of all half-year median Phe levels up to the testing day (Jahja et al., 2017). IDC T1 was calculated from birth to the day of testing at T1, and IDC T2 from the day of testing at T1 up to T2 (Jahja et al., 2017).

Blood samples had also been collected on the days of neuropsychological testing at both T1 and T2 and were as well retrieved from the clinic's databases to determine concurrent Phe levels (PheConT1 and PheConT2).

Statistical Analysis

The data analysis was performed using IBM SPSS Statistics, version 29, following an exploratory approach as outlined by Smith et al. (1990a, b). Initially, t-tests were conducted to ensure baseline comparability of cognitive performance scores at T1 between PKU patients with and without subsequent T2 data, suggesting that cognitive performance at the initial time point was comparable regardless of whether patients had follow-up data at T2.

For the main analysis, multiple linear regressions were performed to examine the associations between cognitive performance at T1 and T2 and four indicators of dietary control (PheConT1, IDC T1, PheConT2, IDC T2). Regression models were separately analysed for each of the six dependent variables (SRT Speed, CRT Speed, CRT Stability, LDT Load 1, LDT Load 2, LDT Load 3), using z-scores calculated from age-matched test scores of healthy controls as norm references, with higher z-scores indicating better performance (lower RTs) (Jahja et al., 2017). A 'percent correct hits' ratio was calculated and included in the regression analyses as a 'weighted least squares' variable to correct for the speed-accuracy tradeoff in the continuous performance (CRT) and visual search (LDT) tasks.

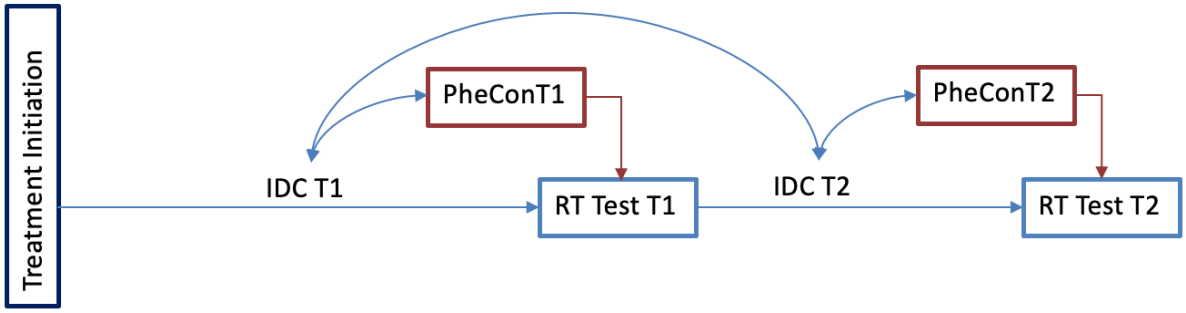
Assumption checks for the regression analyses included tests for heteroscedasticity through visual inspection of Q-Q plots (Figure A1 in the Appendix) and multicollinearity, assessed via a correlation matrix of predictor variables (Table 3) and VIF values (Table A1 in the Appendix). Statistical significance was set at an alpha level of 0.05.

Regarding multiple testing, the inter-correlation of the IDCs, as shown in the correlation matrix (Table 3), suggests that these measures are not entirely independent. Given this and the exploratory nature of the study, a decision was made against applying a correction for multiple testing. While this increases the risk of Type I errors, it allows for the identification of potentially meaningful associations between dietary control and cognitive performance that could justify further exploration. Jahja (2017) pointed out that applying a strict correction for multiple testing could hide meaningful associations by setting an overly conservative threshold for the p-value, which could result in only non-significant correlations being reported.

Pre-processing of the data included correcting missing values and ensuring that all relevant cases were analysed to obtain reliable results.

Figure 2

Theoretical Model for Statistical Analysis



The model in Figure 2 illustrates the assumed correlations of historical and concurrent Phe levels at T1 and T2 with each other and the dependent variables (RT test results) to help understand how Phe levels influence cognitive performance over time and across different stages of development in PKU patients. Curved arrows symbolise potential correlations, while straight arrows demonstrate presumed directions of effect.

Results

In the following section the results from the conducted analyses are presented. First, demographics and metabolic outcomes of the PKU patients are described, including age, gender, PKU type, and Phe levels. Second, the relationships between different indicators of dietary control and concurrent Phe levels are presented in a correlation matrix and descriptive statistics of the cognitive outcomes are summarised in a table. Third, results of the regression analyses are presented, examining the relationship of dietary control, concurrent Phe levels and cognitive performance across two critical time points.

Metabolic and Cognitive Outcomes

For the 35 patients with data available at both test points, the mean age was 8.8 years (SD=0.5) at T1, and 13.6 years (SD=1.0) at T2, with relatively more females than males in the longitudinal sample; descriptive statistics are provided in Table 2. Among these patients, 27 were diagnosed with classical PKU (Type I; pre-treatment Phe levels ≥ 1200 µmol/L), while 8 had mild PKU (Type II; pre-treatment Phe levels between 600 and 1200 µmol/L). No Type III patient data was available for analysis, as these patients usually don't need to stick to a strict diet and are not monitored continuously.

The mean concurrent Phe levels were 8.3 mg/dL (SD=5.6) at T1 and increased significantly to 12.8 mg/dL (SD=5.5) at T2, which was expected, as patients relax their diet to a certain extent with increasing age. The IDC showed a mean of 5.5 mg/dL (SD=2.3) at T1, with an overall increase to an IDC score of 11.1 mg/dL (SD=3.8) between T1 and T2.

Table 2

Descriptive Statistics of PKU Patients (N=35)

	PKU longitudinal sample
Age at T1 M±SD (range)	8.8±0.5 (8.1-10.3)
Age at T2 M±SD (range)	13.6±1.0 (12.3-16.2)
Gender (male:female)	14:21
Biochemical PKU phenotype (n)	PKU1 (27); PKU2 (8)
Concurrent Phe T1 M±SD (range)	8.3±5.6 (1.0-21.3)
Concurrent Phe T2 M±SD (range)	12.8±5.5 (4.1-29.6)
IDC T1 M±SD (range)	5.5±2.3 (2.3-11.4)
IDC T2 M±SD (range)	11.1±3.8 (5.8-18.0)

IDC: Index of dietary control.
PKU phenotype based on diagnostic Phe measurement.
All Phe values are reported in milligrams per deciliter (mg/dL).

The correlation matrix presented in Table 3 illustrates the relationships between different indicators of dietary control and concurrent Phe levels at two time points (T1 and T2) for the 35 patients whose data were available at T2. Significant correlations are indicated with asterisks. The analysis revealed several significant associations, notably a strong positive correlation between concurrent Phe levels at T1 (PheConT1) and the index of dietary control at T1 (IDC T1). Furthermore, IDC T1 was found to be very strongly correlated with IDC T2, suggesting that patients with higher dietary control scores at T1 tended to also have higher dietary control scores at T2.

Table 3

Pearson Correlations between Phe Indices of longitudinal Sample (N=35)

	PheConT1	IDC T1	PheConT2	IDC T2
PheConT1	1.000			
IDC T1	0.749**	1.000		
PheConT2	0.416*	0.481**	1.000	
IDC T2	0.638**	0.706**	0.716**	1.000

**Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Descriptive statistics for cognitive outcomes in PKU patients are provided in Table 4. Results for these patients at T1 and T2 were normed to the outcomes of age-matched healthy controls by calculating z-scores. These standardised values provide a way to compare cognitive outcome scores across different ages. The mean values indicate that, despite early treatment, patients consistently underperformed relative to healthy peers at both time points. As the sample size of available patient data sets (N=35) is relatively small for statistical analyses and all patient's performances should be taken into account, "outliers" were included in the calculations, which resulted in some high values for the means and standard deviations. In general, the results show that cognitive performance at T2 slightly approaches the outcomes of healthy subjects, which may indicate that performance of PKU patients converges with age to that of healthy individuals despite their condition. The statistical analyses of the relationship between Phe levels and cognitive performance will be presented in the next paragraphs.

Table 4

Descriptive Statistics of Cognitive Outcomes at T1 and T2

	T1			T2		
	N	M	SD	N	M	SD
Simple Reaction Time (Speed)	35	-1.10	1.64	34	-0.72	1.50
Continous Reaction Time (Speed)	27	-1.57	18.76	27	-0.96	14.42
Continous Reaction Time (Stability)	27	-0.82	1.10	27	-5.74	5.84
Letter Detection Task (Speed, Load 1)	32	-0.88	21.0	31	-0.10	1.13
Letter Detection Task (Speed, Load 2)	32	-0.85	18.90	31	-0.41	1.63
Letter Detection Task (Speed, Load 3)	32	-0.73	11.33	31	-0.51	1.60

M = Mean; SD = Standard Deviation.

Associations between Dietary Control and Cognitive Performance

To answer the research questions, multiple linear regression analyses were conducted to assess the impact of dietary control and concurrent Phe levels (IDC T1, PheCon T1) on each cognitive performance score (SRT Speed, CRT Speed, CRT Stability, LDT Speed with three loads) at T1. Next, further regression analyses were performed to explore the influence of dietary control from T1 to T2, along with performance at T1 and the concurrent Phe level at T2, on cognitive performance at T2. Our primary aim for the longitudinal analysis was to elaborate whether Phe levels have a different impact on cognitive test performance at T1 relative to T2.

Assumption checks did not indicate any significant violations, with residuals approximately normally distributed, indicating no heteroscedasticity. Multicollinearity was not a main concern, as all VIF values remained below 2.4. Both Q-Q plots and VIF values are shown in Figure A1 and Table A1 in the Appendix.

Impact of Phe levels on cognitive performance at T1

"How do historical and concurrent Phe levels at T1 influence specific cognitive performance measures, such as simple reaction time, continuous reaction time and stability over time, and reaction time with different cognitive loads in early-treated PKU patients under the age of ten?"

This research question was investigated using multiple linear regression analyses. The analysis at T1 showed that higher concurrent Phe levels were significantly associated with slower processing speed, as shown by the significant regression model for SRT (F (2, 32) = 5.756, p = .007, R^2 = .27). This result indicates that increased Phe levels contribute to longer reaction times and influence cognitive performance.

Processing speed in the LDT was significantly influenced by concurrent Phe levels across different cognitive loads. Specifically, the overall regression models indicated significant effects for LDT Load 2 (F (2, 29) = 5.277, p = .011, R^2 = .27) and LDT Load 3 (F (2, 29) = 4.415, p = .021, R^2 = .23). The overall regression model for LDT Load 1 did not reach statistical significance (F (2, 29) = 2.627, p = .089, R^2 = .15). However, higher concurrent Phe levels were still significantly associated with slower reaction times, indicating a potential effect. These results demonstrate that higher Phe levels are associated with slower processing speed under varying cognitive demands. Table 5 shows the regression coefficients.

Table 5

Regression analyses at T1 with Impact of Historical and Concurrent Phe Levels on Cognitive Performance Measures

Dependent Variable	N	Predictor	Unstandardised B	SE	Standardised β	t	*p*
Simple Reaction Time (Speed)	35	Concurrent Phe	-.222	.067	-.756	-3.308	.002*
		IDC T1	.323	.164	.452	1.976	.057
Continous Reaction Time (Speed)	27	Concurrent Phe	-.207	.114	-.541	-1.821	.081
		IDC T1	.322	.274	.349	1.175	.252
Continous Reaction Time (Stability)	27	Concurrent Phe	-.116	.064	-.536	-1.812	.083
		IDC T1	.161	.153	.311	1.051	.304
Letter Detection Task (Speed, Load 1)	32	Concurrent Phe	-.226	.099	-.567	-2.286	.030*
		IDC T1	.421	.238	.440	1.771	.087
Letter Detection Task (Speed, Load 2)	32	Concurrent Phe	-.265	.082	-.734	-3.210	.003*
		IDC T1	.392	.200	.448	1.959	.060
Letter Detection Task (Speed, Load 3)	32	Concurrent Phe	-.134	.053	-.605	-2.536	.017*
		IDC T1	.101	.126	.191	.800	.430

IDC T1: Index of dietary control at T1; SE: Standard error.

Impact of Phe levels on cognitive performance at T2

"How are the historical Phe levels between T1 and T2, along with concurrent Phe levels at T2 and cognitive performance at T1, correlated with cognitive performance in the same group of PKU patients after the age of ten years at T2?"

This second research question was explored using multiple linear regression analyses at T2. The results indicated significant effects in the overall regression models for CRT Speed (F (3, 23) = 10.028, p < .001, R^2 = .57), CRT Stability (F (3, 23) = 4.138, p = .017, R^2 = .35), and LDT Load 2 (F (3, 27) = 3.491, p = .029, R^2 = .28). Even if the overall models for the remaining variables did not become significant, there is an indication that cognitive performance at T1 was a significant predictor of performance at T2 for several tasks. Specifically, SRT Speed, CRT Speed, CRT Stability, LDT Load 1, and LDT Load 3 at T1 all significantly predicted their respective measures at T2, as detailed in Table 6.

These findings suggest that cognitive performance at T1 is a significant predictor of cognitive outcomes at T2. The analysis at T2 showed that neither historical Phe levels (between T1 and T2) nor concurrent Phe levels were significantly associated with cognitive outcomes (Table 6). These results indicate that increased Phe levels in early adolescence do not contribute to longer reaction times and do not have a significant influence on cognitive performance after the age of ten years in our study participants.

Table 6

Regression analyses at T2: The Impact of Historical and Concurrent Phe Levels and T1 Performance on Cognitive Outcomes

Dependent Variable	N	Predictor	Unstandardised B	SE	Standardised β	t	*p*
Simple Reaction Time (Speed)	34	Concurrent Phe	.035	.064	.129	.540	.593
		IDC T2	.019	.095	.048	.200	.843
		SRT T1	.374	.154	.412	2.436	.021*
Continous Reaction Time (Speed)	27	Concurrent Phe	-.071	.062	-.215	-1.144	.264
		IDC T2	.081	.089	.171	.912	.371
		CRT T1 (Speed)	.570	.108	.728	5.291	<.001**
Continous Reaction Time (Stability)	27	Concurrent Phe	.225	.294	.183	.763	.453
		IDC T2	-.623	.417	-.352	-1.496	.148
		CRT T1 (Stability)	2.992	.920	.563	3.254	.003*
Letter Detection Task (Speed, Load 1)	31	Concurrent Phe	-.014	.053	-.067	-.257	.799
		IDC T2	.053	.080	.173	.657	.517
		LDT T1 (Speed, Load 1)	.192	.092	.381	2.084	.047*
Letter Detection Task (Speed, Load 2)	31	Concurrent Phe	-.076	.070	-.254	-1.074	.292
		IDC T2	.072	.108	.162	.665	.512
		LDT T1 (Speed, Load 2)	.427	.143	.510	2.984	.006*
Letter Detection Task (Speed, Load 3)	31	Concurrent Phe	-.099	.071	-.346	-1.397	.174
		IDC T2	.086	.107	.201	.802	.430
		LDT T1 (Speed, Load 3)	.487	.228	.375	2.137	.042*

IDC T2: Index of dietary control at T2; SRT T1: Simple Reaction Time at T1; CRT T1: Continuous Reaction Time at T1; LDT T1: Letter Detection Task at T1; SE: Standard error.

Summary

The regression analyses indicated that Phe levels significantly affected cognitive test performance before the age of ten but not thereafter. Specifically, concurrent Phe levels during childhood were significant predictors for several cognitive performance measures, while Phe levels in early adolescence showed no significant association with cognitive outcome. Furthermore, cognitive performance in childhood significantly predicted cognitive outcomes in early adolescence.

Discussion

This study is among the first to investigate how historical dietary control and concurrent Phe levels affect cognitive performance from early childhood to early adolescence in a longitudinal sample of PKU patients. The practical implications of the findings for the patients' daily lives and for future research will be discussed and the study's strengths and limitations will be outlined in the subsequent paragraphs.

This study emphasises the importance of maintaining low Phe levels during early childhood for individuals with PKU. It is evident that an effective dietary control of Phe intake is associated with improved cognitive performance in young PKU patients, particularly in tasks measuring reaction time. However, the findings also indicate no significant benefit after the age of ten, indicating that a strict dietary control might be more important during childhood than during adolescence.

The first research question was "How do historical and concurrent Phe levels at T1 influence specific cognitive performance measures, including simple reaction time, continuous reaction time and stability, and reaction time with different cognitive loads, in early-treated PKU patients under the age of ten?". It was confirmed in the analyses that lower concurrent Phe levels are significantly associated with better performance on cognitive tasks such as simple reaction time and visual search. This is consistent with existing literature and guidelines, as an early and strict dietary treatment is advised to avoid neurological harm in PKU patients (Burlina et al. 2015; Van Wegberg et al., 2017). Jahja et al. (2017) and Bartus et al. (2018) postulated that maintaining low Phe levels during childhood is important for optimal cognitive outcomes in adulthood. Historical (cumulative) Phe levels did not have a significant effect on cognitive outcome, which might be due to the early and careful monitoring of the patients and their optimal dietary control since the neonatal period, and therefore relatively low cumulative Phe levels. There was no significant association between Phe levels and speed or stability in the continuous performance task. This might be due to the length of the test, as participants must respond to a sequence of 600 patterns, which can be too difficult for young children. One possibility to account for this difficulty would be to only include the first 200 stimuli of this test in statistical analyses.

The exploration of the second research question "How are the historical Phe levels between T1 and T2, along with concurrent Phe levels at T2 and cognitive performance at T1, associated with cognitive performance in the same group of PKU patients after the age of ten years at T2?" revealed no significant associations between dietary control during early adolescence, concurrent Phe levels, and cognitive outcomes in young adolescents. This finding suggests a possible relaxation of the dietary control during adolescence.

Additionally, cognitive performance in childhood was a significant predictor of cognitive performance in adolescence. This finding is important, as it encourages further investigation

of the long-term effects of early dietary treatment on cognitive abilities in adolescence and adulthood.

Strengths and Limitations

This study has several strengths, with the major one being its longitudinal design. This approach allowed to track changes in cognitive performance over time and across critical developmental periods from early childhood to early adolescence. By assessing PKU patients both before and after the age of 10, the study offered valuable insights into how historical and concurrent Phe levels are associated with cognitive outcomes at different moments in development. The longitudinal design lays a foundation for further testing of the PKU patient group in adulthood, which would allow an exploration of the effects of dietary control during childhood and adolescence on cognitive performance in adulthood. This exploration could be a next research step.

The sample size of this study is quite large compared to other longitudinal studies and with regard to the rarity of the metabolic disease PKU. The first researchers to longitudinally examine EF in adolescents with hyperphenylalaninemia were Sharman et al. (2015). They performed neuropsychological testing on two sibling pairs with mild hyperphenylalaninemia in early childhood and early adolescence. In another longitudinal study Mastrangelo et al. (2015) examined 24 early-treated PKU patients during adolescence and early adulthood and found that white matter abnormalities on MRI increased with age and poor dietary control, but these abnormalities did not significantly affect cognitive outcomes (IQ), which is also consistent with the findings of this study. The longitudinal study of Jahja et al. (2017) included 21 PKU patients and found lower childhood phenylalanine levels to be significantly associated with better executive functioning and mental health in adulthood. The present study seems to involve one of the largest PKU patient groups evaluated longitudinally to date. Even though the study sample is still quite small for statistical analyses, it is sufficiently informative to identify significant correlations.

The cognitive test battery “CORTEX” appears to be well-suited for exploring cognitive impairments in PKU patients, as increased Phe levels are mainly associated with lower performance speed (Palermo et al., 2017). Modifications of the test battery such as reducing test duration and incorporating a more modern, colourful, and engaging interface could help maintain children's attention and improve the accuracy of the results.

The study also had several limitations. Firstly, the longitudinal sample contained a disproportionate number of female and male participants. While no studies specifically addressing gender differences in the cognitive performance of PKU patients were identified, an impact of the gender imbalance on the results of this study cannot be ruled out.

Furthermore, all subjects were regularly monitored and had a good dietary control, which greatly limits the variance in Phe values and thereby may limit the generalisability of our findings. However, it is unrealistic to find less well-adjusted patients for whom sufficient values would be available to perform a (longitudinal) research study. Regarding the test battery, the continuous performance task was too long for young children and needs to be adjusted. Due to the duration children lost interest and sometimes discontinued the testing. However, the effectiveness of the test battery could even be further enhanced by improving its design to better suit younger children. The validity and reliability of the test battery, although specifically developed for testing cognitive functions in patients with different metabolic diseases, may not be as established as more widely used cognitive assessment tools, such as IQ tests. Future research could focus on improving these aspects of the test battery and thereby increasing its utility and credibility.

Conclusion

This study may have several implications for both the daily life of young patients with PKU and future research studies. Maintaining a strict dietary control during childhood is critical for cognitive development and thereby important for overall psychological well-being, yet at the same time it can be challenging, especially for adolescents. As Bosch et al. (2015) highlighted, the emotional burden of strict dietary adherence can significantly impact health-related QOL in PKU patients, as the anxiety over blood Phe levels, the feeling of guilt over dietary lapses, and the stress of continuous monitoring can lead to psychological distress.

Dietary recommendations for PKU patients have not been changed in several decades, as there was not enough research data available (van Wegberg et al, 2017). New treatment options are still being tested and researched, but a cure for the condition has not yet been found. Dietary control of Phe intake remains the main treatment option for PKU patients, and a lifelong treatment is still recommended by the latest European guidelines on phenylketonuria (van Wegberg et al, 2017).

This study suggests that strict dietary control may have less impact on the cognitive outcomes in adolescents than previously assumed. Easing the dietary burden after the age of 10 could have a profoundly positive impact on young PKU patients, promoting better physiological, cognitive, and emotional outcomes, ultimately leading to improved overall well-being. Further research is required to explore the potential relaxation of dietary restrictions in adolescence and the long-term cognitive development into adulthood. In particular the finding that Phe levels do not appear to be predictive for cognitive performance in adolescence needs to be confirmed by further research, as the patients in this study had good dietary control anyway and it is not clear to what extent the diet can be relaxed without compromising the patient’s health.

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Appendix

Figure A1

Q-Q Plots of dependent Variables at T1

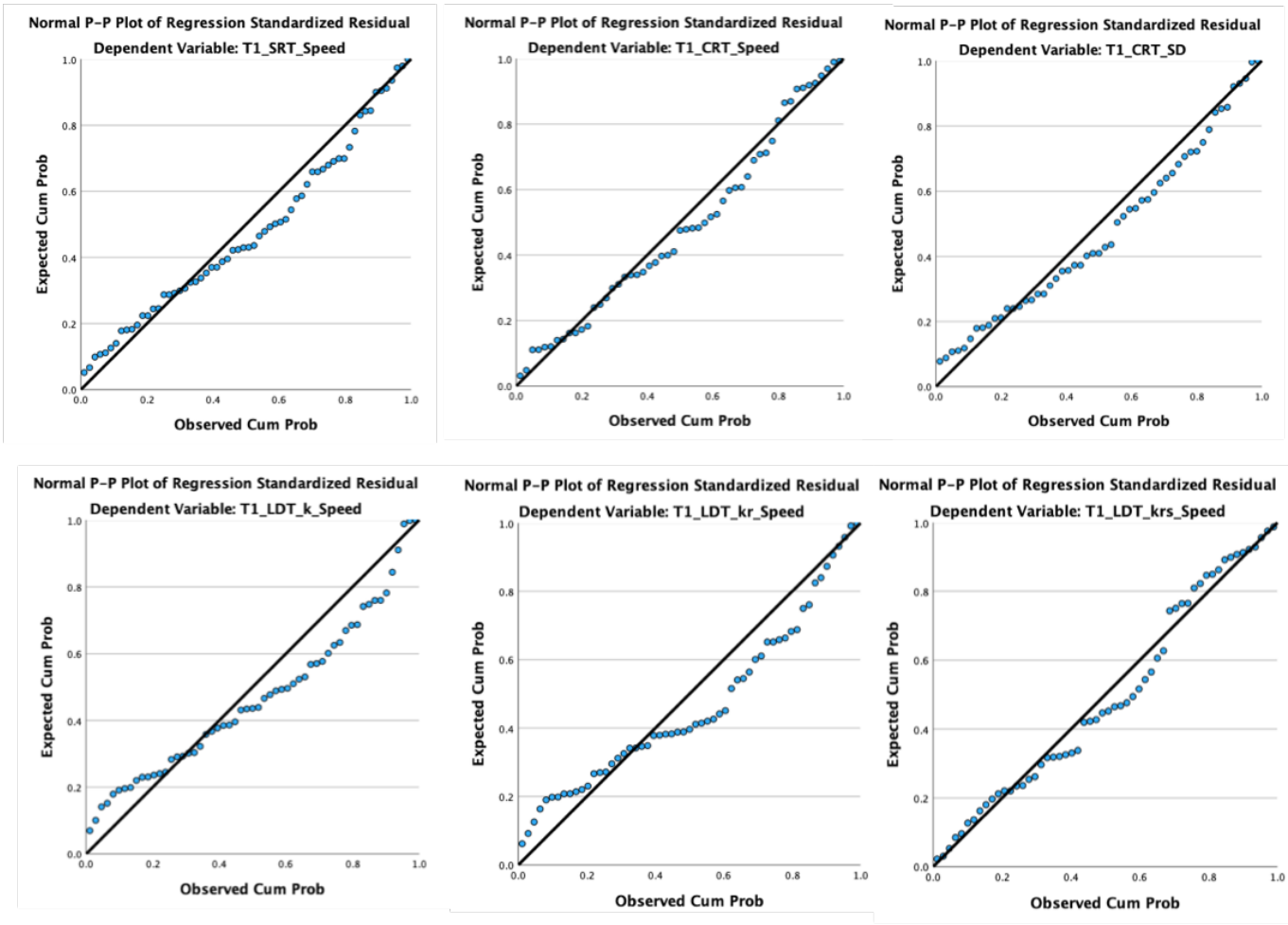

Table A1

Variance Inflation Factors of dependent Variables

	T1		T2		
	Tolerance	VIF	Predictor	Tolerance	VIF
Simple Reaction Time (Speed)	.439	2.275	Concurrent Phe	.482	2.073
			IDC T2	.468	2.139
			SRT T1	.956	1.046
Continous Reaction Time (Speed)	.413	2.420	Concurrent Phe	.535	1.870
			IDC T2	.533	1.876
			CRT T1 (Speed)	.995	1.005
Continous Reaction Time (Stability)	.415	2.408	Concurrent Phe	.494	2.026
			IDC T2	.509	1.964
			CRT T1 (Stability)	.942	1.061
Letter Detection Task (Speed, Load 1)	.474	2.111	Concurrent Phe	.474	2.110
			IDC T2	.460	2.175
			LDT T1 (Speed, Load 1)	.951	1.052
Letter Detection Task (Speed, Load 2)	.483	2.070	Concurrent Phe	.478	2.092
			IDC T2	.449	2.227
			LDT T1 (Speed, Load 2)	.914	1.094
Letter Detection Task (Speed, Load 3)	.465	2.152	Concurrent Phe	.483	2.072
			IDC T2	.470	2.127
			LDT T1 (Speed, Load 3)	.958	1.044

IDC T2: Index of dietary control at T2; SRT T1: Simple Reaction Time at T1; CRT T1: Continuous Reaction Time at T1; LDT T1: Letter Detection Task at T1; VIF: Variance Inflation Factor.