

Original

# Quality of dietary control in phenylketonuric patients and its relationship with general intelligence

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Abstract

**Objectives:** Assessment of the quality of dietary treatment of phenylketonuria (PKU) patients and investigation of its relationship with the general intelligence of the patients.

**Methods:** Cross-sectional and longitudinal study of 105 PKU treated patients. The index of dietary control (IDC) was calculated as the phenylalanine (Phe) data reduction in half-year medians and the mean of all medians throughout the patient's life. We calculated four different IDCs related to age: IDC-A (< 6 years), IDC-B (6-12 years), IDC-C (13-18 years) and IDC-D (> 18 years). To evaluate the fluctuation of Phe values we calculated the standard error of the estimate of the regression of Phe concentration over age. Development quotient was calculated with the Brunet-Lezine test (< 4 years). Intelligence quotient was evaluated with the Kaufman Bit Intelligence Test (K-Bit), Wechsler Intelligence Scale for Children-Revised (WISC-R) and Wechsler Adult Intelligence Scale Third Edition (WAIS III).

**Results:** Cross-sectional study: The IDC in age groups were significantly different and so were the number of patients with good, acceptable and poor IDC related to age ( $p < 0.001$ ). Sampling frequency was good in 72, acceptable in 23 and poor in 10 patients. The general intelligence ( $101 \pm 10$ ) correlated negatively with the IDC ( $p < 0.0001$ ) and Phe fluctuations ( $p < 0.004$ ). Longitudinal study: Significant differences were observed between the IDC through the patients' lifetime except in the adolescent/adult period.

**Conclusions:** 85% of PKU patients showed good/acceptable quality of dietary control. General intelligence correlates with the IDC at all ages, which highlights the importance of good control to achieve good prognosis.

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Key words: Phenylketonuria. Phenylalanine. Dietary treatment. Intelligence quotient. Recommendations.

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## CALIDAD DEL CONTROL DE LA DIETA EN PACIENTES FENILCETONÚRICOS Y SU RELACIÓN CON LA INTELIGENCIA GENERAL

Resumen

**Objetivos:** Evaluación de la calidad del control dietético en pacientes con fenilcetonuria (PKU) e investigación de su relación con el nivel de inteligencia.

**Métodos:** Estudio transversal y longitudinal de 105 pacientes PKU en tratamiento dietético. El índice de control de la dieta (IDC) se ha calculado como la reducción de los valores de fenilalanina (Phe) a las medianas de cada 6 meses y la media de todas las medianas a lo largo de la vida del paciente. Se han calculado cuatro diferentes IDC según la edad: IDC-A (< 6 años), IDC-B (6-12 años), IDC-C (13-18 años) and IDC-D (> 18 años). Para evaluar las fluctuaciones de los valores de Phe hemos calculado el error estándar de la regresión estimada de la concentración de Phe según la edad. El índice de inteligencia se ha evaluado mediante el test de Brunet-Lezine (< 4 años) y el coeficiente de inteligencia mediante Kaufman Bit Intelligence Test (K-Bit), Wechsler Intelligence Scale for Children-Revised (WISC-R) y Wechsler Adult Intelligence Scale Third Edition (WAIS III).

**Resultados:** Estudio transversal: El IDC en los diferentes grupos de edad es significativamente diferente y también lo son el número de pacientes con un IDC bueno, aceptable y malo en relación con la edad ( $p < 0,001$ ). La frecuencia de controles de Phe fue buena en 72 pacientes, aceptable en 23 y mala en 10. La inteligencia general ( $101 \pm 10$ ) se correlaciona negativamente con el IDC ( $p < 0,0001$ ) y con las fluctuaciones de Phe ( $p < 0,004$ ). Estudio longitudinal: Se han observado diferencias significativas entre IDC a lo largo de la vida de los pacientes a excepción del período adolescencia/edad adulta.

**Conclusiones:** El 85% de pacientes PKU mostraron una calidad del control de la dieta buena/aceptable. Los niveles de inteligencia general se correlacionan con el IDC en todas las edades, lo que muestra la importancia del buen control de la dieta para lograr un buen pronóstico.

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Palabras clave: Fenilcetonuria. Fenilalanina. Control dietético. Coeficiente de inteligencia. Recomendaciones.

## Introduction

Phenylketonuria (PKU) (McKusick 261600) is an inborn error of phenylalanine (Phe) metabolism resulting from deficient activity of phenylalanine-4-monooxygenase (EC 1.14.16.1), the hepatic enzyme that catalyses the synthesis of tyrosine from phenylalanine.<sup>1</sup> Phenylalanine accumulation in plasma and tissues with decreased tyrosine biosynthesis seems to be involved in the pathogenesis of PKU. Treatment of PKU patients consists of restriction of phenylalanine intake, which means a natural protein-restricted diet supplemented with a phenylalanine-free amino acid mixture enriched with some essential micronutrients, such as vitamins, minerals and trace elements.<sup>2</sup> Alternative treatment with tetrahydrobiopterin (BH4) also results in lowering plasma Phe in BH4-responsive patients.<sup>3,4</sup> Early treatment of PKU prevents severe neurological damage, although a slight reduction in intelligence quotient compared with control populations and/or specific executive function deficits may arise, especially when careful dietary compliance is not achieved.<sup>1,5-8</sup> Quality of dietary treatment appears to be, therefore, a most important condition for good prognosis.<sup>9</sup> Different indices of dietary control (IDC)<sup>10</sup> and parameters of Phe fluctuation<sup>11</sup> have been computed from Phe levels during certain periods of time or throughout life for evaluation of long-term compliance. Several guidelines for the management of PKU have been published with recommendations about frequency of monitoring and desirable plasma Phe concentrations,<sup>12-15</sup> although there is a lack of internationally accepted guidelines, especially for the management of adolescent/adult patients<sup>16</sup>. Therefore, the experience of a reference centre in the management of PKU patients may yield further information.

## Objectives

Our aim was to assess the quality of dietary treatment of PKU patients and to determine its relationship with the general intelligence of the patients.

## Material and methods

### Patients

We did a cross-sectional and longitudinal study of 105 PKU patients who were selected from a group of 192 hyperphenylalaninemic patients periodically controlled in our hospital (Reference Centre for PKU in Catalonia) [sex: 59 females and 46 males; mean age: 15.7 (SD: 11.6; range: 3 months-40 years)]. Inclusion criteria were: a) phenylalanine-4-monooxygenase deficiency confirmed by differential diagnosis and mutation analysis, and b) pre-treatment plasma Phe concentrations higher than 360  $\mu\text{mol/L}$ . Exclusion criteria were: a) treatment refusal (7

adult diagnosed patients), b) death owing to causes other than PKU (2 patients), and c) change of residence to other parts of the country and control in other PKU reference centres (5 patients).

PKU diagnosis was performed in 76 patients through newborn screening and 29 patients were late diagnosed. All patients were treated with a Phe-restricted diet just after diagnosis. In 10 BH4-responsive patients<sup>17</sup> diet was replaced by BH4 therapy (5-15 mg/kg/day) for the three years prior to the study. However, since the IDC was not significantly different in these patients treated with Phe-restricted diet ( $280 \pm 66 \mu\text{mol/L}$ ) or with BH4 ( $298 \pm 53 \mu\text{mol/L}$ ),<sup>4</sup> we included them in the study.

All children or their guardians signed an informed consent agreement in accord with the Helsinki Declaration of 1964, revised in Edinburgh in 2000. Our hospital ethics committee approved the study.

## Methods

**Biochemical methods:** Plasma and dried blood spot Phe concentrations were analysed by ion exchange chromatography (Biochrom 30, Pharmacia Biotech). The frequency of Phe measurements varied from every week (< 2 month of age), or every two weeks (2 months-4 years old) to every month (> 4 years of age). We considered it correct when the proportion of samples expected was > 90%, acceptable >75% and poor < 75%.

To summarise plasma Phe values along patients' life we plot them as a longitudinal history of dietary control (fig.1 A and B). Moreover, we calculate the IDC as the Phe data reduction in half-year medians and the mean of all medians<sup>18</sup> throughout the patient's life. Since in previous studies we found that IDC increases significantly with age ( $r = 0.636$ ,  $p < 0.001$ ),<sup>19</sup> we calculated four different IDCs related to age: IDC-A (< 6 years), IDC-B (6-12 years), IDC-C (13-18 years) and IDC-D (> 18 years). We consider it to be good control with IDC-A < 360  $\mu\text{mol/L}$  and IDC-B, C and D < 480  $\mu\text{mol/L}$ , acceptable control with IDC-A < 480  $\mu\text{mol/L}$  and IDC-B, C and D < 600  $\mu\text{mol/L}$  and poor control when IDC was higher than those values. To evaluate the fluctuation of Phe values we calculated the standard error of the estimate (SEE) of the regression of Phe concentration over age.<sup>11</sup>

**General intelligence measurement:** Development quotients were calculated with the Brunet-Lezine test in patients younger than 4 years of age (N = 21). Intelligence quotient (IQ) was evaluated with the Kaufman Bit Intelligence Test (K-Bit) (patients from 4 to 6 years) (N = 6), Wechsler Intelligence Scale for Children-Revised (WISC-R) (patients from 6 to 18 years) (N = 35), and Wechsler Adult Intelligence Scale Third Edition WAIS III (patients older than 18 years) (N = 6). Late diagnosed patients were excluded from this part of the study. Intelligence measurement was not available for 8 patients.

**Statistical analysis:** Statistical analyses were performed using the package SPSS (version 15.0). *Cross-*

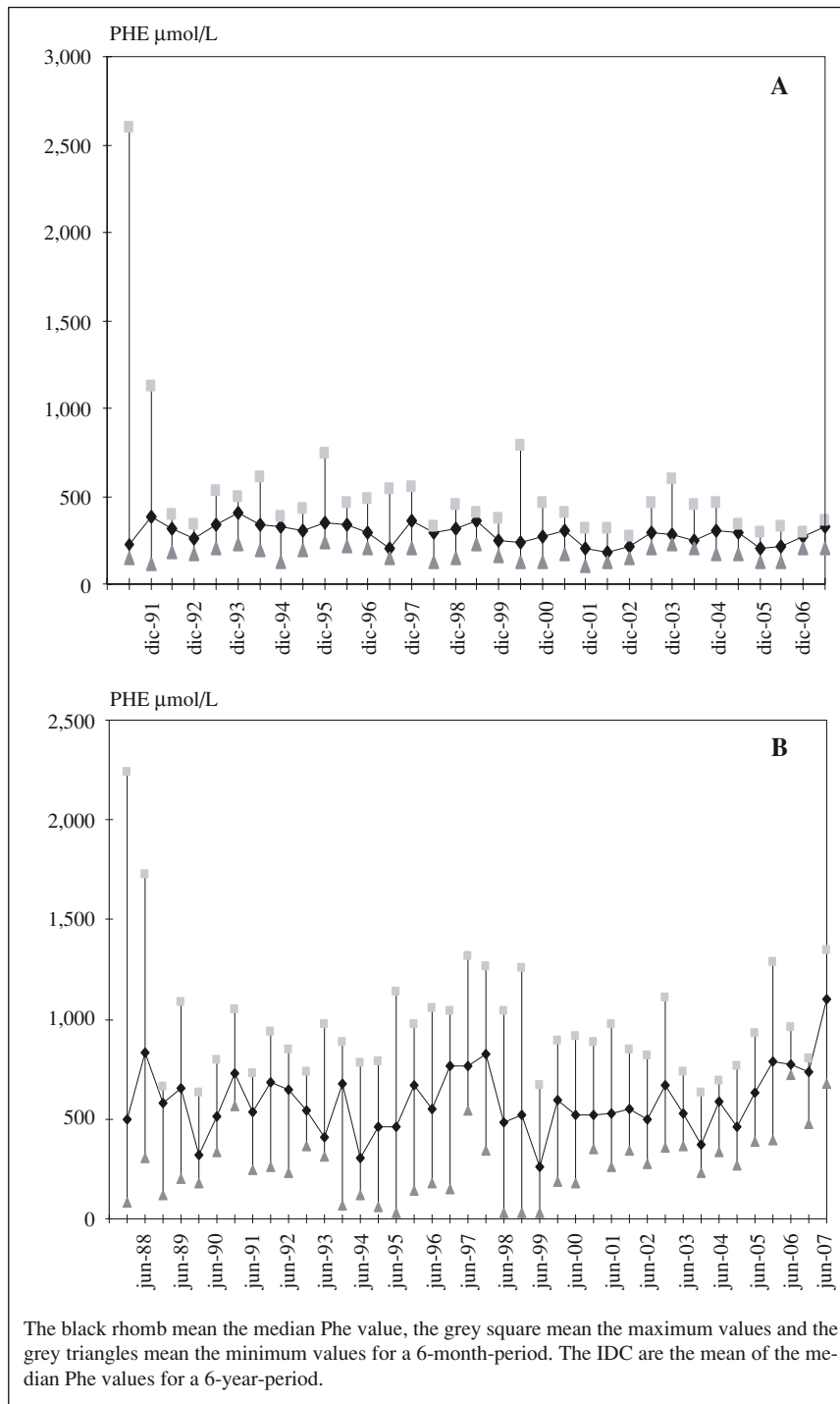


Fig. 1.—A) Patient with good dietary control. B) Patient with poor dietary control.

*sectional study:* The ANOVA test with Bonferroni correction was used to compare the IDC among the A, B, C and D groups. Pearson Chi square test was applied to search for association between categorical variables: (good, acceptable and poor IDC at different age groups and frequency of sampling). Pearson test was used to determine the correlations between the quantitative variables studied (IDC, SEE, and general intelligence). *Longitudinal study:* Student-T test for paired data was

used to analyse the differences between IDC (A, B, C, and D) from the same patient throughout life. Statistical significance was accepted at  $P < 0.05$ .

## Results

*Cross-sectional study:* The IDCs in the different age groups were significantly different (ANOVA with Bon-

**Table I**  
Cross-sectional study of the index of dietary control (IDC:  $\mu\text{mol/L}$ ) in the different age groups among the 105 PKU patients

IDC (years)	Number	IDC (mean)	IDC (SD)	Good*	Acceptable*	Poor*
A (<6)	30	300	91	26	4	0
B (6-12)	16	317	113	15	1	0
C (12-18)	20	558	282	9	6	5
D (>18)	39	598	239	17	10	11
Total patients	105			68	21	16

SD: standar deviation.

\*IDC good: IDC-A < 360  $\mu\text{mol/L}$  and IDC-B, C and D < 480  $\mu\text{mol/L}$ .

\*IDC acceptable: IDC-A < 480  $\mu\text{mol/L}$  and IDC-B, C and D < 600  $\mu\text{mol/L}$ .

\*IDC poor: IDC-A > 480  $\mu\text{mol/L}$  and IDC-B, C and D > 600  $\mu\text{mol/L}$ .

Significance: ANOVA (Bonferroni correction): IDC A & B and C & D: non-significant differences; A & C, D and B & C, D ( $p > 0.001$ ).

ferroni correction;  $p < 0.001$ ) (table I; fig. 2). The number of patients with good, acceptable and poor IDC was significantly different related to age (Pearson Chi square test:  $p < 0.001$ ) (table I). However, no significant differences were observed regarding sex in any age group. Frequency of sampling was correct in 72 patients, intermediate in 23 and poor in 10 patients, although there were no significant differences related to age (table II). The IDC was significantly different in patients with good ( $383 \pm 186 \mu\text{mol/L}$ ), intermediate ( $550 \pm 258 \mu\text{mol/L}$ ) and poor frequency of sampling ( $829 \pm 162 \mu\text{mol/L}$ ) (ANOVA with Bonferroni correction;  $p < 0.001$ ).

Excluding the late-diagnosed PKU patients ( $N = 29$ ), the general intelligence of PKU patients was  $101 \pm$

10 (range: 80-129) and showed a negative correlation with the present IDC of the patients ( $r = -0.468$ ;  $p < 0.0001$ ), an even stronger negative correlation with the IDC-A of these patients ( $r = -0.501$ ;  $p < 0.0001$ ), but no correlation with pre-treatment Phe values. General intelligence showed a significantly negative correlation with the SEE ( $r = -0.347$ ;  $p < 0.004$ ). A significant correlation was observed between the IDC and the SEE ( $r = 0.618$ ;  $p < 0.0001$ ). Pre-treatment Phe values only showed a correlation with the SEE ( $r = 0.391$ ;  $p < 0.001$ ).

*Longitudinal study:* Taking into account the IDC throughout patient life, significant differences (Student T-test for paired samples) were observed between the

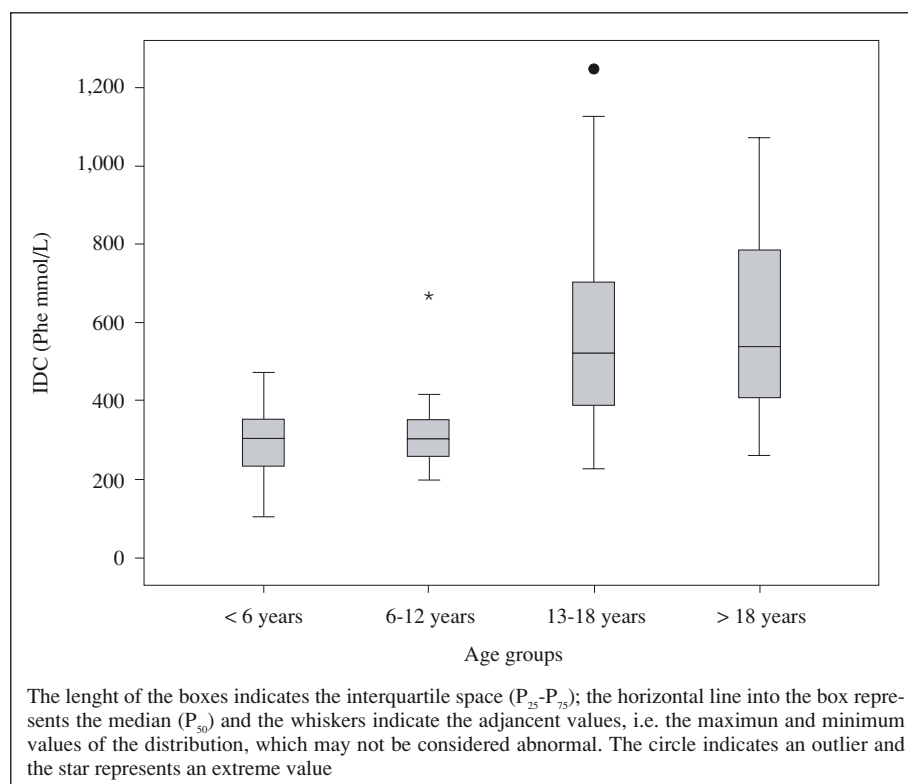


Fig. 2.—Index of dietary control in the different age groups.

**Table II**  
Sampling frequency (% of recommended) in the different age groups of PKU patients

Frequency of sampling*	Good (> 90%)	Acceptable (> 75%)	Poor (< 75%)	Total
< 6 years	22	7	0	29
6-12 years	14	1	1	16
12-18 years	14	4	3	21
> 18 years	22	11	6	39
Total patients	72	23	10	105

\*No significant differences related to age (Pearson Chi square test).

IDC A & B ( $p < 0.01$ ), and B & C ( $p < 0.012$ ), but not between C & D (table III).

## Discussion

Although the metabolic control of PKU patients includes many biochemical parameters involved in their nutritional status that may be deficient owing to the natural protein restricted diet,<sup>2</sup> here we focused only on the blood Phe levels throughout life. The biochemical follow-up of PKU children involves so much data on Phe control that it can become confusing for the clinicians in charge of the management of the patients. To compute all data and plot it as a longitudinal history of dietary control (based on the half-year medians of Phe values) is very useful for the clinical staff and for the family when it comes to evaluating the evolution of that control.<sup>10</sup> Medians are better than means since they correct for the sporadic decompensation caused by illness (cough, diarrhoea, or other infections). The mean of half-year medians is a useful index for correlation with clinical and neuropsychological data, which is indispensable in patients' follow-up.<sup>8,9,13,20</sup> However, since in previous studies we observed that IDC increases significantly with age,<sup>19</sup> we calculated here four different IDCs related to patient age (A, B, C, and D). Moreover, a fluctuation index (SEE) also seems necessary for the complete evaluation of the quality of diet, since the same IDC might be associated with a calm but increasing profile or an erratic but even profile.<sup>11</sup>

In the cross-sectional study, although there was a positive correlation between IDC of the total group and

age ( $r = 0.627$ ;  $p < 0.0001$ ), the quality of control was good in infancy and childhood (IDC-A and B) and clearly impaired in adolescence (IDC-C), although it remained stable in adult age (IDC-D). The longitudinal study confirmed this increasing trend when comparing the different IDCs in the same patient throughout life. In a previous study performed by our group in late-diagnosed patients or those who resumed Phe-restricted diet after a period of discontinuation, only half of the patients achieved an acceptable/good control despite their belief that their quality of life had improved with the diet.<sup>21</sup>

Recommendations for sampling frequency were followed by 95% (good/acceptable frequency) of the patients. There is a clear relationship between correct sampling frequency and good IDC. All these results agree with evaluation studies performed in other reference centres,<sup>22</sup> although they only evaluate patients younger than 19 years of age. Interestingly, we found no relationship between patient age and sampling frequency, suggesting that adult patients accept management guidelines better than adolescents. Sex seems not to play a significant role in the quality of control in PKU patients, even in adults, among whom some women spent long periods of time with very good control owing to future pregnancies [women ( $N = 24$ ):  $583 \pm 217 \mu\text{mol/L}$  versus men ( $N = 15$ ):  $679 \pm 276 \mu\text{mol/L}$ , not significantly different].

The lack of correlation between the IDC and plasma Phe levels at diagnosis, observed by other authors as well,<sup>23</sup> suggests that the quality of treatment is not related to the severity of the PKU phenotype. Conversely, a highly significant relationship was observed between Phe fluctuations (SEE) and pre-treatment Phe

**Table III**  
Longitudinal study of the index of dietary control (IDC:  $\mu\text{mol/L}$ ) throughout patient life

IDC	Mean	SD	IDC	Mean	SD	Number	*Significance
A	359	106	B	398	143	43	$p < 0.010$
B	477	201	C	528	250	32	$p < 0.012$
C	569	270	D	618	224	22	NS

SD: standar deviation.

\*Comparison by Student T-test for paired samples.

levels. Moreover, a significant relationship was observed between the SEE and the IDC, which suggests that fluctuations might also be partially corrected by accurate dietary control. This confirms the observations of other authors<sup>23</sup> in different PKU populations with diverse phenotypes, genotypes and even follow-up conditions, and indicates that the efforts made by the patients and their families for diet compliance can achieve good control of Phe levels, independently of the severity of the mutations.

According to our results, general intelligence shows a negative correlation with the IDC and the SEE but not with the pre-treatment Phe values. In previous extensive neuropsychological studies performed in 37 PKU patients we found that these patients showed lower values in intelligence and in visuospatial, fine motor, executive and attention functions when compared with a control population, and we also found that these cognitive functions were negatively associated with plasma Phe values in the first 6 years of life.<sup>8</sup> In another study we evaluated school performance in 26 PKU patients and found that they presented with more learning difficulties than controls. In that study, the index of dietary control for the last 6 months was significantly higher than the index for the first 6 years of life only in the patients with learning difficulties, which points to the importance of long-lasting good dietary control in PKU.<sup>24</sup> Moreover, grey and white matter volume changes related to the duration and strict observation of dietary treatment was demonstrated by our group in 27 treated PKU patients (mean age:  $20 \pm 7$  years).<sup>25</sup> In this study, significant correlations were observed between white matter volumes and concurrent Phe values, and between white matter and mean Phe values for the 12 months previous to the study in early treated PKU patients.<sup>25</sup> In the present study, of the whole PKU group (excluding late diagnosed patients), general intelligence correlates with the IDC from the first 6 years of age, but also with the IDC at any age.

These results point to the importance of improving the quality of dietary control of PKU at all ages, which can be achieved with a motivating policy designed by the follow-up unit including training plans, dietary and psychological support and frequent Phe controls.<sup>21</sup> The application of new treatment strategies will probably make it easier to achieve metabolic control of PKU patients.

In summary, 85% of PKU patients in our reference centre showed good/acceptable quality of dietary control. The lack of a relationship between the IDC and pre-treatment Phe values and the relation observed between the IDC and correct frequency of sampling point up the great importance of lifestyle in the quality of treatment in the face of the PKU biochemical phenotype. General intelligence correlates with the IDC not only in the first 6 years of age, but also with the IDC at any age, which highlights the importance of good control throughout life to achieve an improved prognosis.

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