



Submitted on: 01/06/2024

Approved on: 01/14/2024

ORIGINAL ARTICLE

Anthropometric, metabolic parameters and serum phenylalanine levels in patients with phenylketonuria: a cohort study

Hugo Raphael Resende Cruz¹, Luana Teles de Resende², Jeverton de Santana Santos³, Fábio Augusto de Moraes Prado Santos³, Osmar Mendes Peixoto Filho³, Adriana Barbosa de Lima Fonseca³

Keywords:

Phenylalanine,
Anthropometry,
Metabolism,
Nutritional status.

Abstract

Objective: To investigate anthropometric and metabolic parameters and serum phenylalanine levels in patients with phenylketonuria. **Method:** Cohort study, in a public secondary health service. All patients with phenylketonuria, between four and 21 years of age, followed at the service between January 2017 and January 2020 were included. Patients with a late diagnosis of phenylketonuria, chronic osteometabolic diseases, previous chronic kidney disease or use of medications with laboratory interference. Anthropometric measurements and laboratory tests were confirmed. Data were collected through medical records and analyzed using descriptive measures and comparative analyses. **Results:** Of the 20 patients in the sample, 15 (75%) were male and 5 (25%) were female. The average age obtained was 13.26 years (SD $\pm$ 3.98), with an average weight, height and BMI of 33.76 kg (SD $\pm$ 14.55, 125 cm (SD $\pm$ 20.48) and 16.71 kg/m² (SD $\pm$ 3.56), respectively. The average height between males and females varied, being 125.9 cm (SD $\pm$ 20.48) and 122.3 cm (SD $\pm$ 22.61), in that order. Laboratory analysis demonstrated changes in HDL levels, with an average of 27.75 mg/dL (SD $\pm$ 11.84). SD $\pm$ 3.68). **Conclusions:** The results point to the need for an approach more comprehensive, which encompasses aspects such as continuous monitoring of phenylalanine levels, strict adherence to a restrictive diet and multidisciplinary monitoring.

¹ Universidade Federal de Sergipe, Departamento de Medicina - Lagarto - Sergipe - Brazil.

² Universidade Tiradentes, Coordenação de Medicina - Aracaju - Sergipe - Brazil.

³ Universidade Federal de Sergipe, Departamento de Medicina - São Cristóvão - Sergipe - Brazil.

Correspondence to:

Hugo Raphael Resende Cruz

Universidade Federal de Sergipe. Av. Gov. Marcelo Déda, 13 - São José, Lagarto - SE, Brazil. 49400-000. E-mail: hugorcruz1@gmail.com

INTRODUCTION

Inborn errors of metabolism are hereditary disorders resulting from genetic mutations that affect enzymes or proteins involved in metabolic pathways. These mutations lead to failures in nutrient processing, energy production, or elimination of metabolic waste, resulting in the accumulation of toxic substances or a deficiency of essential metabolic products¹. A notable example of an inborn metabolic disorder is phenylketonuria (PKU), an autosomal recessive disease caused by mutations in the enzyme phenylalanine hydroxylase (PAH), leading to impaired metabolism of the amino acid phenylalanine (Phe). Consequently, phenylalanine accumulates in plasma, causing harmful effects on multiple body systems, including the nervous system, skin, and urine composition^{1,2}.

Epidemiological data related to phenylketonuria may vary across the literature due to different factors, such as genetic variation, newborn screening coverage, and methodological approaches adopted in studies. In Brazil, according to the Ministry of Health, prevalence has been reported to range from 1:15,000 to 1:25,000 live births³. One study on newborn screening for PKU in Sergipe, Brazil, reported 1 case per 8,690 individuals and showed that despite adequate screening coverage and early diagnosis, there were delays in treatment and challenges in metabolic control⁴.

Early diagnosis and dietary intervention are crucial components in preventing irreversible brain damage resulting from phenylketonuria². A systematic review addressed the impact of PKU in Latin America. The authors identified an estimated prevalence of 18% for intellectual disability, 15% for motor delay, and 35% for speech impairment in patients diagnosed early. In addition, this analysis identified gaps in neuropsychological needs and socioeconomic challenges that were not adequately addressed, highlighting the importance of directing interventions to tackle these complex issues and provide substantial improvements⁵.

Phenylketonuria has a significant impact on the lives and dietary habits of affected individuals due to the restrictive, lifelong treatment required. The therapeutic approach involves adopting a protein-restricted diet, including reduced intake of natural proteins, supplemented with special amino acid mixtures. The main goal of this intervention is to ensure metabolic balance while providing the energy and nutrients needed for appropriate growth⁶. Lack of strict adherence to the diet, combined with its restrictive nature, may result in several complications, especially in children and adolescents who are developing autonomy in dietary choices³.

Despite recent progress in PKU research, inconsistencies in the literature persist, particularly regarding anthropometric measures and biochemical parameters that influence cardiovascular disease risk in these patients. Current evidence indicates that, despite advances in dietary therapy, ideal growth outcomes are still not achieved in PKU⁷. Therefore, the aim of this study is to investigate anthropometric and metabolic

parameters and serum phenylalanine levels in patients with phenylketonuria.

MATERIALS AND METHODS

This is a cohort study conducted at a referral outpatient clinic for the care of children with phenylketonuria in the state of Sergipe. The service is linked to the Brazilian Unified Health System (SUS) and is responsible for active case finding of suspected cases, diagnostic confirmation, treatment, and specialized multidisciplinary follow-up.

Inclusion criteria were patients diagnosed with phenylketonuria, aged 4 to 21 years, followed at the service from January 2017 to January 2020. Patients with late diagnosis of PKU, chronic osteometabolic diseases, pre-existing chronic kidney disease, or use of medications that could interfere with biochemical test results were excluded.

Anthropometric measurements were performed by the outpatient clinic pediatrician at an approximate frequency of every 4 months. Weight was recorded in kilograms (kg) using an electronic scale with 100-gram (g) precision. Height was measured in centimeters (cm) using a vertical stadiometer with millimeter (mm) precision. Body mass index (BMI) was calculated using the formula $BMI = \text{weight (kg)} / \text{height squared (m}^2\text{)}$, and values were evaluated according to percentiles from the World Health Organization (WHO) growth charts.

Laboratory tests were performed in the hospital's own clinical laboratory. Blood samples were collected for phenylalanine (Phe) measurements every 4 months, in addition to annual collections for triglycerides, total cholesterol and fractions, fasting glucose and basal insulin, as well as bone metabolism markers. Samples were collected in appropriate tubes after a 10- to 14-hour fast, and tests were performed using routine laboratory and colorimetric enzymatic methods. The phenylalanine cutoff values used were 2 to 6 mg/dL at all ages, according to Vocley et al.⁸.

Data were collected from medical records. After collection, data were transferred to an Excel for Windows 10 spreadsheet, and descriptive measures and comparative analyses were performed. Data normality was assessed using the Kolmogorov–Smirnov test. Fisher's exact test was applied for associations between variables, and unpaired t-test and Kruskal–Wallis tests were used for comparisons between variables. The significance level adopted was $p < 0.05$.

The study was approved by the Research Ethics Committee (opinion number 3676244, CAAE 17507219.6.0000.5546), and participants were invited to sign the Informed Consent Form and/or an Assent Form. Measures were adopted to ensure participants' confidentiality and anonymity.

RESULTS

After applying the inclusion and exclusion criteria, a sample of 20 patients was obtained, with most (75%) being

male. The mean age of the sample was 13.26 years (± 3.98) and the median was 12.85, with 30% of patients in the 6 to 10 years age group and 70% in the 10 to 20 years age group. General participant data are presented in Table 1.

In the anthropometric analysis, eight measurements of weight, height, and BMI were obtained for each patient. Most patients had weight, height, and BMI within the expected parameters, with mean values of 33.76 kg (± 14.55), 125 cm (± 20.48), and 16.71 kg/m² (± 3.56), respectively.

Regarding weight, patients were evaluated and classified into three groups: adequate weight for age, high weight for age, and underweight for age. Of the measurements obtained, most showed adequate weight for age. Comparing sexes, the mean weight for males was 33.82 (SD ± 16.19), whereas for females the mean was 33.57 (SD ± 9.36).

As for height, patients were classified as "height adequate for age" or "short stature for age," and most had height measurements within the expected range. The mean height for males was 125.9 cm (SD ± 20.48), while for females it was 122.3 cm (SD ± 22.61).

BMI analysis was based on the last anthropometric parameter assessed, which was classified as eutrophic (normal weight), overweight, obesity, thinness, and severe thinness. Most patients were classified as eutrophic. Patients with elevated BMI (overweight or obesity) accounted for 25%, whereas those with low BMI accounted for 10% of participants. The mean BMI was 21.61 kg/m² (± 1.97) for eutrophic individuals, 18.1 kg/m² (± 0.31) for individuals with thinness, and 14.47 kg/m² (± 1.64) for individuals with severe thinness.

The mean BMI for males was 16.89 (SD ± 3.69), whereas for females it was 16.17 (SD ± 3.49). Table 2 presents the mean BMI of each patient across the eight assessment points, according to sex and age.

When BMI was analyzed at two time points, a total of 15 (75%) individuals had adequate BMI at the first study

measurement, decreasing to 11 (55%) eutrophic individuals at the last measurement. Patients with BMI above the expected range at the beginning of data collection totaled 3 (15%), of whom 1 (5%) was overweight and 2 (10%) had obesity; this increased to 5 (25%) by the end of data collection, with 3 (15%) overweight and 2 (10%) with obesity. The findings for thinness and severe thinness were 1 (5%) for each at baseline, increasing to 2 (10%) patients with thinness and none with severe thinness at the end of the period. When height was analyzed, adequate height for age was observed in 19 (95%) and 14 (70%) patients at the beginning and end of the study, respectively, with 1 (5%) and 3 (15%) patients below the desired height in the same periods.

Laboratory tests were performed annually to assess patients' metabolic profile. The final mean values of the tests were calculated. The mean absolute values were: glucose 65.25 mg/dL (± 23.36), aspartate aminotransferase (AST) 21.32 U/L (± 10.27), alanine aminotransferase (ALT) 17.17 U/L (± 8.57), LDL 56.10 mg/dL (± 24.22), HDL 27.75 mg/dL (± 11.84), total cholesterol 50.60 mg/dL (± 45.53), triglycerides 56.85 mg/dL (± 36.36), total calcium 7.95 mg/dL (± 2.83), ionized calcium 3.42 mg/dL (± 1.71), albumin 3.29 g/dL (± 1.35), and phosphorus 3.44 mg/dL (± 1.66).

Among the tests monitored, HDL levels were predominantly low, accounting for 90% of all patients based on the mean values across the collections analyzed. Likewise, mean total calcium and ionized calcium values were below the expected range in 40% and 50% of patients, respectively, in the samples in question.

Serum phenylalanine was measured every four months, with eight serum phenylalanine measurements performed for each patient. The overall mean phenylalanine level was 11.5 mg/dL (± 3.68). Most patients had inadequate serum phenylalanine levels during the follow-up period, as shown in Table 3 below:

Table 1. General data of the participants evaluated. Values are presented as absolute (n) and relative (%) frequencies.

General data	n (%)
Age group	
0–2 years	0 (0%)
2–5 years	0 (0%)
5–10 years	6 (30%)
10–20 years	12 (60%)
>20 years	2 (10%)
Sex	
Male	15 (75%)
Female	5 (25%)

Source: Prepared by the authors (2023).

Table 2. Data on the mean BMI of each participant across the eight measurements. Values are presented as mean $\pm$ standard deviation. BMI = body mass index; SD = standard deviation; p = participant.

Age group	Mean BMI (SD)	
	Boys	Girls
0-2 years	-	-
2-5 years	-	-
5-10 years	p1 - 15.2 (0.57) p2 - 21.8 (0.85) p3 - 15.7 (0.46) p4 - 17.1 (0.68) p5 - 16.4 (0.66) p6 - 18.0 (1.13)	-
10-20 years	p7 - 15.2 (0.82) p8 - 15.9 (0.55) p9 - 15.6 (0.49) p10 - 25.9 (0.72) p11 - 17.7 (0.65) p12 - 20.3 (1.05) p13 - 19.5 (1.19)	p16 - 20.2 (1.29) p17 - 21.3 (2.06) p18 - 15.4 (1.42) p19 - 16.7 (0.79) p20 - 22.2 (2.24)
> 20 years	p14 - 24.3 (0.59) p15 - 18.5 (0.70)	-

Source: Prepared by the authors (2023).

Table 3. Percentual (%) de pacientes com níveis séricos inadequados de fenilalanina durante os oito momentos avaliados (M).

Measurements (M)	% of patients with inadequate Phe levels
M1	89,48%
M2	88,30%
M3	94,45%
M4	86,70%
M5	92,86%
M6	84,62%
M7	88,90%
M8	94,12%

Source: Prepared by the authors (2023).

DISCUSSION

The association between overweight and obesity in individuals with phenylketonuria (PKU) has been debated in the literature. In the present study, approximately one quarter of the evaluated patients had an elevated BMI at their last visit. In a recent systematic review, the authors observed that excess weight was a frequent finding in children and adolescents with PKU, especially after eight years of age. Among the associated factors, a higher intake of calorically dense foods due to protein restriction and a lack of encouragement to engage in physical activity due to social withdrawal were highlighted^{4,9}. In a cross-

sectional study including 94 adolescents with PKU, several variables were investigated, including body fat percentage, BMI, and metabolic control. The results showed that factors such as female sex, excess weight, and higher protein intake were associated with higher body fat levels⁹.

It should be noted that, although BMI is widely used as an outcome measure of growth, it has limitations, since the relative contribution of lean and fat mass to body weight cannot be assessed. Based on BMI alone, some patients may appear relatively stable in terms of weight despite changes in the underlying fat-to-lean ratio¹⁰. In this regard, waist circumference correlates with parameters related to

cardiovascular risk in patients with PKU, suggesting that it may be a more accurate indicator of cardiovascular risk when compared with BMI¹¹.

Most patients in this study had height appropriate for age. Similarly, one study reviewed the growth of children with PKU and concluded that this parameter was normal at birth and during childhood, although height was significantly lower in the first four years of life compared with reference populations. In contrast, patients with mild hyperphenylalaninemia, who do not require dietary restriction, did not show impaired growth⁶.

Regarding the lipid profile, analysis of laboratory tests identified mean LDL, triglycerides, and total cholesterol values within the expected range for age. However, the mean HDL level was considered low for the age of the evaluated sample. Similarly, the study by Almeida et al. (2020) showed that most patients met anthropometric and biochemical parameters, except for lower HDL levels in 59.3% of patients¹². Considering that, in addition to clinical parameters, low HDL levels and isolated high LDL levels have been identified as favorable indicators of cardiovascular risk¹², patients with PKU require attention to clinical and laboratory monitoring to prevent metabolic changes, as well as to avoid excessive weight gain and its implications.

Diet plays a fundamental role in the management of phenylketonuria, and carbohydrate intake may influence glycemic control. However, in this study, elevated blood glucose levels were not observed in the monitored individuals, differing from suggestions in the literature that dietary restrictions may result in higher glycemic levels due to increased caloric intake⁹. Restriction of natural proteins favors—and may even physiologically stimulate—the consumption of carbohydrate-rich foods, especially simple carbohydrates, leading to a higher glycemic-index diet and increasing the occurrence of metabolic abnormalities and excess weight^{3,13}.

Despite the risks associated with high carbohydrate intake in the literature, few studies have investigated carbohydrate metabolism in patients with PKU. In a multicenter study, Couce et al.⁹ evaluated 83 patients aged 4 to 52 years and found that metabolic control was adequate in 71.9%. The authors noted that patients at higher risk of carbohydrate intolerance and insulin resistance were adults and overweight.

Patients with phenylketonuria may also have an increased risk of micronutrient deficiencies¹⁴. In this study, mean total and ionized calcium levels showed a slight reduction. In addition, the mean phosphorus level was also considered low, raising the question of whether these patients were adequately adhering to the prescribed diet. It should be emphasized that in children with PKU, as in the general population, bone health assessment should be performed by bone densitometry¹⁵. The relationship between PKU and the risk of bone disease is recognized; however, the effectiveness of calcium supplementation as prevention still lacks solid evidence and is not widely adopted in clinical practice¹⁶.

Accurate control of blood Phe levels is crucial to prevent cognitive, neuropsychological, and behavioral problems associated with phenylketonuria. Classification of the disease into phenotypes based on previous Phe levels—such as classic PKU, mild PKU, and hyperphenylalaninemia—allows anticipation of complications such as microcephaly in children and neurological conditions such as attention-deficit/hyperactivity disorder (ADHD) at different stages of life. In addition, monitoring serum phenylalanine levels is essential to guide clinical decisions, such as dietary adjustments or enzyme replacement therapy, to improve disease control¹⁷.

In this study, the analysis of 20 patients with phenylketonuria highlights the complexity of achieving normal phenylalanine levels during follow-up, indicating a greater need to increase awareness on the topic. This was observed both in the final mean and in individual measurements, resulting in elevated levels for all patients. Studies show that dietary control in phenylketonuria tends to decrease in older children and adolescents, with difficulties reported by parents due to children starting school, resulting in changes in phenylalanine levels after 10 years of age^{18,19}. Another factor that may explain the lack of control of Phe levels is difficulty accessing and following up with specialized professionals, such that the absence of a referral system for PKU services may contribute to a lack of guidance for patients regarding the relevance of the diet²⁰.

An important limitation of this study is the lack of dietary assessment, which is essential to monitor patients adequately, along with the lack of continuous follow-up and detailed documentation in medical records. In addition, the complexity of comparing results with other PKU studies due to differences in patient ages and age groups adopted in methodologies underscores the need for future research, including long-term evaluations of the effects of phenylalanine restriction and adherence to the diet.

In summary, this study evaluated anthropometric and metabolic parameters and phenylalanine levels in patients with phenylketonuria. Most patients had adequate nutritional status, despite low HDL levels and inadequate control of serum phenylalanine levels. These findings highlight the need for a more comprehensive approach to optimize metabolic control, emphasizing the importance of constant monitoring and dietary intervention, with multidisciplinary follow-up, to prevent adverse effects and improve the quality of life of patients with phenylketonuria.

REFERENCES

1. Schiengens KA, Weiß KJ, Dokoupil K, et al. Ernährung bei angeborenen Stoffwechselerkrankungen – ein Spagat zwischen Genuss und Therapie. Bundesgesundheitsbl. 2020;63:864-871. DOI: <http://www.doi.org/10.1007/s00103-020-03168-x>
2. Kanufre VC, Soares RDL, Alves MRA, Aguiar MJB, Starling ALP, Norton RC. Metabolic syndrome in children and adolescents with phenylketonuria. J Pediatr (Rio J). 2015;91(1):98-103. DOI: <http://www.doi.org/10.1016/j.jped.2014.06.006>

3. Tummolo A, Carella R, Paterno G, Bartolomeo N, Giotta M, et al. Body composition in adolescent PKU patients: beyond fat mass. *Children (Basel)*. 2022;9(9):1353. DOI: <http://www.doi.org/10.3390/children9091353>
4. Camatta GC, Kanufre VDC, Alves MRA, Soares RDL, Norton RC, Aguiar MJB, et al. Body fat percentage in adolescents with phenylketonuria and associated factors. *Mol Genet Metab Rep*. 2020;23:100595. DOI: <http://www.doi.org/10.1016/j.ymgmr.2020.100595>
5. Sena BS, Andrade MIS, Silva APF, Dourado KF, Silva ALF. Excesso de peso e fatores associados em crianças e adolescentes com fenilcetonúria: uma revisão sistemática. *Rev Paul Pediatr*. 2020;38:e2018201.
6. Shakiba M, Alaei M, Saneifard H, Mosallanejad A. Avaliação de índices antropométricos em pacientes com fenilcetonúria. *Iran J Child Neurol*. 2020;14(2):27.
7. Fernandez-Crespo S, Vazquez-Agra N, Marques-Afonso AT, Cruces-Sande A, Martinez-Olmos MA, Araujo-Vilar D, et al. The value of waist circumference as a predictor of cardiovascular risk in adult patients with classic phenylketonuria. *Med Clin (Barc)*. 2023. DOI: <http://www.doi.org/10.1016/j.medcli.2023.06.023>
8. Vockley J, Andersson HC, Antshel KM, Braverman NE, Burton BK, Frazier DM, et al. Phenylalanine hydroxylase deficiency: diagnosis and management guideline. *Genet Med*. 2014;16(2):188-200. DOI: <http://www.doi.org/10.1038/gim.2013.157>
9. Couce ML, Sánchez-Pintos P, Vitoria I, De Castro MJ, Aldámiz-Echevarría L, Correcher P, et al. Carbohydrate status in patients with phenylketonuria. *Orphanet J Rare Dis*. 2018;13:103. DOI: <http://www.doi.org/10.1186/s13023-018-0847-x>
10. Green B, et al. Nutritional and metabolic characteristics of UK adult phenylketonuria patients with varying dietary adherence. *Nutrients*. 2019;11(10):1-13. DOI: <https://doi.org/10.3390/nu11102459>
11. Alghamdi N, Alfheaid H, Cochrane B, Adam S, Galloway P, Cozens A, et al. Mechanisms of obesity in children and adults with phenylketonuria on contemporary treatment. *Clin Nutr ESPEN*. 2021;46:539-543. DOI: <http://www.doi.org/10.1016/j.clnesp.2021.10.012>
12. de Almeida BNDF, Laufer JA, Mezzomo TR, Shimada NC, Furtado IHF, Dias MRMG, et al. Parâmetros nutricionais e metabólicos de crianças e adolescentes com fenilcetonúria. *Clin Nutr ESPEN*. 2020.
13. Moretti F, Pellegrini N, Salvatici E, Rovelli V, Banderali G, Radaelli G, et al. Dietary glycemic index, glycemic load and metabolic profile in children with phenylketonuria. *Nutr Metab Cardiovasc Dis*. 2017;27:176-182. DOI: <http://www.doi.org/10.1016/j.numecd.2016.11.002>
14. Kose E, Arslan N. Vitamin/mineral and micronutrient status in patients with classical phenylketonuria. *Clin Nutr*. 2019;38(1):197-203. DOI: <http://www.doi.org/10.1016/j.clnu.2018.01.034>
15. van Wegberg AMJ, MacDonald A, Ahring K, Belanger-Quintana A, Blau N, Bosch AM, et al. The complete European guidelines on phenylketonuria: diagnosis and treatment. *Orphanet J Rare Dis*. 2017;12(1):162. DOI: <https://doi.org/10.1186/s13023-017-0685-2>
16. Tanaka NYY, Turcato MF, Nicoletti CF, Nonino CB, Martins LD, Iannetta O, Guerreiro CT, Santos GG, Marchini JS. Effects of short-term calcium supplementation in children and adolescents with phenylketonuria. *J Clin Densitom*. 2018;21(1):48-53. DOI: <http://www.doi.org/10.1016/j.jocd.2017.02.001>
17. Silveira AM. Sobrepeso/obesidade e doença hepática gordurosa não alcoólica em adolescentes com fenilcetonúria [tese]. Belo Horizonte: Universidade; 2020. Disponível em: <https://pesquisa.bvsalud.org/portal/resource/pt/biblio-1397300>
18. Rovelli V, Longo N. Phenylketonuria and the brain. *Mol Genet Metab*. 2023;139(1):107583. DOI: <http://www.doi.org/10.1016/j.ymgme.2023.107583>
19. Karam SM, Jardim LB, Giugliani R, Horta BL. Triagem neonatal para hiperfenilalaninemia: um estudo de coorte. *Rev AMRIGS*. 2012;56(1):17-21.
20. Lamônica DA, Gejão MG, Anastácio-Pessan FL. Fenilcetonúria e habilidades de leitura e escrita. *Rev CEFAC*. 2015;17(1):143-150. DOI: <https://doi.org/10.1590/1982-0216201515313>