



# Metabolic Control in Patients With Phenylketonuria Pre- and Post-Sapropterin Loading Test

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## Abstract

In Portugal, tetrahydrobiopterin (BH4)-responsive patients with phenylketonuria (PKU) are identified using a loading test (LT). Phenylalanine/natural protein (Phe/NP) intake is increased to elevate blood Phe prior to the LT. In a longitudinal retrospective study, the impact of Phe/NP titration post-LT in 58 patients ( $19.6 \pm 8.2$  years) with PKU during 4 study periods (SPs) was examined. In SP1 (2010-2013), patients were diet treated only; in SP2 (2014), the Phe/NP titration was followed by the LT in SP3 (2015). In SP4 (2016), patients received diet treatment only ( $n = 49$ ) or BH4 + diet ( $n = 9$ ). The median percentage blood Phe within the target range was higher in SP1 versus SP4 (64 [28-85] vs 45 [0-66];  $P < .001$ ). Our results suggest that transient Phe/NP titration, associated with a deliberate increase in NP, may adversely affect metabolic control. Controlled studies are necessary to examine the longer term impact of temporary increased NP with BH4 LT in non-BH4-responsive patients.

## Keywords

phenylketonuria, BH4 loading test, metabolic control

## Introduction

Phenylketonuria (PKU) is an autosomal recessive inborn error of phenylalanine (Phe) metabolism, affecting 1:10 000 people in Europe, caused by deficiency of phenylalanine hydroxylase (PAH) enzyme.<sup>1,2</sup> The conversion of Phe (either from diet or endogenous catabolism) into tyrosine is compromised, resulting in increased blood Phe. In PKU, diet is the primary treatment, involving a severe restriction of Phe and natural protein (NP) and replacement of non-Phe protein with a protein substitute (PS) and low-protein foods (including special low-protein foods) to satisfy energy needs.<sup>3,4</sup> In Portugal, PS are available as Phe-free amino acid supplements, low Phe glycomacropeptide-based PS, or large neutral amino acids.<sup>5</sup>

Maintaining life-long adherence to this restricted diet is challenging, especially in older children.<sup>6</sup> It is well established that there is deterioration in metabolic control over time, particularly in late adolescence and adulthood.<sup>6-9</sup>

More recently, it has been established that the administration of pharmacological doses of tetrahydrobiopterin (BH4), the PAH

cofactor, available as a commercial formula of sapropterin dihydrochloride (Kuvan), may help improve metabolic control, improving dietary Phe tolerance in a subset of patients with mild

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or moderate PKU.<sup>10–12</sup> Tetrahydrobiopterin responsiveness is established either by genotype or with a loading test (LT).<sup>1</sup> Although different methodologies are used to evaluate BH4 responsiveness, a 48-hour BH4 LT protocol is commonly advocated in Europe, analyzing blood Phe before and after a single daily dose of sapropterin (20 mg/kg/d) on 2 consecutive days.<sup>13,14</sup>

In Portugal, the Portuguese Society for Metabolic Disorders (SPDM) specifies that all potential BH4 responders should be identified using a LT. Prior to the LT, Phe/NP intakes are increased to elevate blood Phe to >480  $\mu\text{mol/L}$ . Sometimes this procedure is challenging both for patients and professionals<sup>15</sup> associated with undefined individual Phe tolerance prior to LT and patient ability to eat the amount of higher protein foods that may be necessary to increase blood Phe. The length of time that it takes to increase Phe levels varies between individual patients. Opinion varies on the source of NP that should be given. No published studies have conducted controlled trials to investigate the effect of short-term increase in dietary Phe/NP intake on long-term blood Phe, particularly in non-BH4 responders. In a longitudinal, retrospective study over 6 years, we aimed to examine the impact of Phe/NP titration on metabolic control post-LT in patients with PKU.

## Methods

### Participants

In 2015, 66 patients with PKU completed a LT from the Reference Center of Inherited Metabolic Diseases of Centro Hospitalar do Porto. Eight patients were excluded from the study: 5 were late diagnosed (late treated patients with inconsistent dietary adherence), 2 had insufficient clinical data and dietary records, and 1 had Down syndrome with severe neurological impairment affecting dietary management.<sup>16</sup> The final sample included 58 ( $n = 58$ ) early treated patients (4–34 years;  $19.6 \pm 8.2$  years; 50% females). Disease severity was classified according to the neonatal blood Phe, at newborn screening, as stated in the Portuguese Consensus<sup>17</sup>: hyperphenylalaninemia (blood Phe <6 mg/dL), mild PKU (blood Phe  $\geq 6$  mg/dL and  $\leq 20$  mg/dL), and classical PKU (blood Phe >20 mg/dL). There were 30 (51.7%) patients with mild PKU and 28 (48.3%) patients with classical PKU. The blood Phe target range was  $\leq 6$  or  $\leq 8$  mg/dL with patients aged <12 or  $\geq 12$  years, respectively.<sup>17</sup>

The final sample of patients was studied in 4 different study periods (SPs). During SP1 (2010–2013), all patients were on a low Phe diet. In SP2 (2014), the NP prescription was increased to define maximum Phe tolerance to establish a blood Phe >480  $\mu\text{mol/L}$ , according to SPDM protocol. During this period, in patients whose blood Phe was <480  $\mu\text{mol/L}$ , NP intake was increased initially by adding regular cow milk to the PS. This was then followed by adding controlled amounts of regular foods, such as bread, pasta, or other dairy products. The PS was maintained and the intake of special low-protein foods was decreased as equivalent regular foods were introduced. A time period of 8 weeks was allocated to stabilize blood Phe >480  $\mu\text{mol/L}$  in order to initiate the test.

In the SP3 (2015), the preparation for LT was continued and patients were gradually enrolled, so all of them completed the test during the year 2015. Additionally, some responders started BH4 treatment immediately after the LT. In the SP4 (2016), patients were either exclusively diet treated ( $n = 49$ ) or on BH4 treatment in combination with diet ( $n = 9$ ).

### Study Design

This is a longitudinal retrospective study, with data collected from 2010 to 2016. Gender, birthdate, newborn screening blood Phe, disease severity, genotype, date of LT, and BH4 responsiveness were collected from electronic patient clinical records. Participants were identified by a code, preventing patient identification. There was no control group as all patients with PKU had a LT.

### Data Collection

**Anthropometry.** Data on anthropometry were collected from clinical records from the final nutritional appointment of each SP. Weight and heights were measured when patients were in light clothing only, without shoes and accessories. Seca measuring scales (accuracy = 0.5 kg) and a stadiometer (measuring scale = 1 mm) were used. Body mass index (BMI) was calculated as weight (kg)/height<sup>2</sup> (m) and classified by World Health Organization criteria.<sup>18</sup> Anthro (Version 3.2.2) and Anthro Plus (Version 1.0.4) software were used to calculate BMI  $z$  scores for patients aged between 0 to 5 and 5 to 19 years, respectively. For patients aged 0 to 5 years, overweight was defined when BMI  $z$  score was >2 standard deviations (SDs).<sup>19,20</sup> In patients aged between 5 and 19 years, overweight was characterized when BMI  $z$  score was >1 SD.<sup>20,21</sup>

**Nutritional intake.** Data on dietary intake were collected from the final nutritional appointment of each SP. Natural protein (g/kg/d), protein equivalent (PE) from PS (g/kg/d), and total protein (TP; g/kg/d) intakes were calculated. Additionally, data on quantitative NP intake (g/kg/d) were collected from the last register before the LT.

**Metabolic control.** All patient blood Phe measurements conducted in SP1, SP2, SP3, and SP4 were collected from the patient database. Blood Phe was measured from blood spots by tandem mass spectrometry. The samples were collected by patients in the morning when fasted. Patients were advised to take weekly blood spots. Blood Phe measurements in each of the 4 SP were calculated for median, mean, and SD of blood Phe. Furthermore, for each period and each patient, the percentage of blood Phe measurements within target range was calculated. Whenever patients reached 12 years ( $n = 14$ ), the upper target range was adjusted, so results were correctly interpreted for age.<sup>17</sup> Additionally, for patients who were not BH4 responsive and so not taking BH4 treatment in 2016, the percentage of blood Phe measurements within target range before and after the LT was compared. In contrast, for patients who

**Table 1.** Gender, Age, and Disease Severity of Patients Studied.

Sample size	N = 58
Gender	Female: n = 29 (50%) Male: n = 29 (50%)
Age (at LT <sup>a</sup> —2015)	19.6 ± 8.2 years (minimum = 4 years; maximum = 34 years) <19 years: n = 24 (41.4%) ≥19 years: n = 34 (58.6%)
Disease severity	Mild PKU: n = 30 (51.7%) Classical PKU: n = 28 (48.3%)

Abbreviations: LT, loading test; PKU, phenylketonuria.

<sup>a</sup>LT—tetrahydrobiopterin (BH4) loading test.

were BH4 responsive and on BH4 treatment in 2016, the percentage of blood Phe measurements within target range before the LT and after the BH4 introduction was compared.

### Ethical Statement

This study and its data collection were approved by the ethics committee of Centro Hospitalar do Porto, on May 18, 2015, to the investigation project “Trends in Nutritional Status of patients with phenylketonuria”, with the reference 2015.101 (092-DEFI/087-CES). Written informed consent was obtained from each patient or caregiver.

### Statistical Analysis

IBM SPSS Statistics 24 for Windows was used for statistical analyses. Kolmogorov-Smirnov test was done to evaluate normal distribution of variables. Categorical variables were presented as absolute values or percentage, and continuous variables were presented as mean ± SDs or as medians [P<sub>25</sub>-P<sub>75</sub>], according to its distribution. Wilcoxon test and Mann-Whitney test were used to identify differences when non-normal distribution was found. The level of significance considered was  $P < .05$ .

### Results

The patient’s characteristics are described in Table 1.

#### Change in NP Intake

The NP intake (g/kg/d) remained similar between the 4 SPs (0.58 ± 0.3 vs 0.53 ± 0.3 vs 0.50 ± 0.3 vs 0.55 ± 0.3), with a trend to a lower PE intake (g/kg/d; 1.12 ± 0.4 vs 0.95 ± 0.3 vs 0.92 ± 0.3 vs 0.84 ± 0.3), which is reflected in a lower TP intake (g/kg/d) in SP4 (1.72 ± 0.4 vs 1.49 ± 0.3 vs 1.46 ± 0.4 vs 1.41 ± 0.3). However, from SP1 to LT, NP (g/kg/d) intake was increased by 0.14 ± 0.5 (Table 2).

#### Change in Blood Phe

Median blood Phe was lower in SP1 than in SP4 (6.80 [4.70-10.30] vs 7.91 [6.53-11.11];  $P < .001$ ; Table 3). The percentage

**Table 2.** Natural Protein Intake Differences Between LT and SP1.

Protein intake	SP1	LT
Natural protein intake, g/kg/d	0.58 ± 0.3	0.72 ± 0.4
Difference	NP <sub>LT</sub> - NP <sub>2013</sub> = 0.14 ± 0.5	

Abbreviations: LT, loading test; NP, natural protein; SP1, study period 1.

**Table 3.** Metabolic Control of Patients During the 4 Periods of the Study.

Metabolic Control	SP1	SP2	SP3	SP4
Median [Phe], mg/dL	6.80	7.98	8.20	7.91
	P <sub>25</sub> = 4.70	P <sub>25</sub> = 5.85	P <sub>25</sub> = 6.80	P <sub>25</sub> = 6.53
	P <sub>75</sub> = 10.30	P <sub>75</sub> = 10.58	P <sub>75</sub> = 10.25	P <sub>75</sub> = 11.11
Phe measurements within target range, %	64	47	38	45
	P <sub>25</sub> = 28	P <sub>25</sub> = 7	P <sub>25</sub> = 18	P <sub>25</sub> = 0
	P <sub>75</sub> = 85	P <sub>75</sub> = 76	P <sub>75</sub> = 62	P <sub>75</sub> = 66

Abbreviations: P<sub>25</sub>, 25th percentile; P<sub>75</sub>, 75th percentile; Phe, phenylalanine; SP, study period.

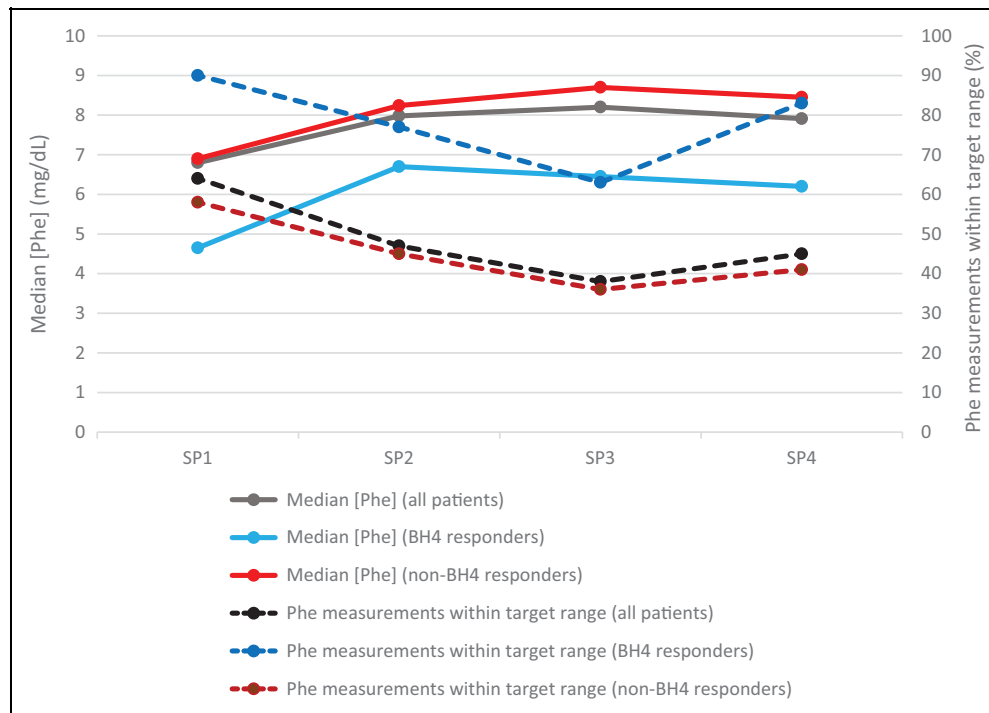
of blood Phe measurements within target range was higher in SP1 compared with SP4 (64% [28-85] vs 45% [0-66];  $P < .001$ ; Table 3). In SP4, 16 (27.6%) patients did not have any blood Phe measurement within the target range compared with 6 (10.3%) patients in SP1. Changes in blood Phe control throughout the study are illustrated in Figure 1.

In SP1, there were almost twice as many patients in good metabolic control (39.7%) compared to SP4 (22.4%; Table 4). Table 5 presents data on percentages of blood Phe measurements within target range at SP1 and SP4, for patients on BH4 treatment + diet and diet treatment only. In these subgroups, the median differences between SP1 and SP4 were -1 [-46 to 22] and -17 [-33 to 0], not reaching statistical significance ( $P = .408$ ). The percentage of blood Phe measurements within target range in the BH4 treated + diet group in 2016, after starting BH4 (responders, n = 9), remained stable when compared to the SP1 pre-LT phase (85 [51-95] vs 81 [58-88];  $P = .953$ ). In the non-BH4 responder group (n = 49) who were prescribed a low Phe diet, there was a post-LT deterioration of metabolic control compared with the pre-LT phase (44 [5-63] vs 51 [22-71];  $P = .001$ ).

Thirty-one (53.4%) of the 58 patients were already adults (aged ≥18 years) at SP1. Their metabolic control deteriorated from SP1 to SP4, median blood Phe increased (9.80 [6.90-12.50] vs 10.35 [7.10-14.30];  $P = .037$ ), and the percentage of blood Phe measurements within target range was reduced (32% [3-77] vs 3% [0-60];  $P = .026$ ).

#### Overweight and Obesity

There was no change in the prevalence of overweight or obesity in patients <5 years, whereas it increased in adults (Table 6). None of the patients who were overweight or obese in 2013 became normal weight in 2016 (data not shown). This was irrespective of treatment.



**Figure 1.** Change in blood Phe. Phe denotes phenylalanine.

**Table 4.** Metabolic Control Quality.<sup>a,b</sup>

Metabolic Control (% of Patients per Class)	SP1		SP4	
	<75% "Bad Control"	≥75% "Good Control"	<75% "Bad Control"	≥75% "Good Control"
	60.3	39.7	77.6	22.4

Abbreviations: Phe, phenylalanine; SP, study period.

<sup>a</sup>Phe measurements within target range ≥75%—"good control."

<sup>b</sup>Phe measurements within target range <75%—"bad control."

**Table 5.** Metabolic Control of Patients on and Without BH4 Treatment in 2016.<sup>a</sup>

Metabolic Control (% of Phe Measurements Within Target Range)			
On BH4 Treatment in 2016 (n = 9) <sup>b</sup>		Without BH4 Treatment in 2016 (n = 49)	
SP1	SP4	SP1	SP4
90	83	58	41
P <sub>25</sub> = 65	P <sub>25</sub> = 48	P <sub>25</sub> = 21	P <sub>25</sub> = 0
P <sub>75</sub> = 97	P <sub>75</sub> = 91	P <sub>75</sub> = 80	P <sub>75</sub> = 57
Median differences = -1 [-46 to 22]		Median differences = -17 [-33 to 0]	

Abbreviations: BH4, tetrahydrobiopterin; P<sub>25</sub>, 25th percentile; P<sub>75</sub>, 75th percentile; Phe, phenylalanine; SP, study period.

<sup>a</sup>This table only represents patients on medication in 2016.

<sup>b</sup>Some patients have responded to loading test but only initiated medication during/after 2016 or stopped medication (n = 6).

## Discussion

This was a longitudinal retrospective study that suggested that patients receiving a BH4 LT, with a temporary increase in NP pretest, had worse Phe control 1 year posttest, although there was a trend toward a slight improvement with time. Both the percentage of blood Phe measurements within target range at SP1 compared with SP4 and the contrast between percentage of patients under good metabolic control in SP1 and SP4 support our conclusions.

We recognize that many factors may have led to deterioration in blood Phe control in addition to BH4 LT. Diet treatment for patients with PKU is very restrictive and PS's have a strong taste and odor, so adherence is challenging.<sup>7,8</sup> After the age of 10 years, dietary adherence is particularly difficult, associated with transition to self-care, rebellion, and hunger.<sup>6,7</sup> Accordingly, a progressive deterioration in metabolic control with age, particularly in adolescents, is seen in most patients.<sup>1,6,8</sup> During adolescence, there is usually some diet relaxation and food pleasure and socialization becomes more important, and in adulthood, the time constraints and stress associated with food preparation balanced with work, study, or parenthood are difficult to align.<sup>9</sup> Considering that during the 4 SPs our cohort of patients increased in age, it seemed more appropriate to analyze the percentage of blood Phe measurements within target range rather than median blood levels, as target ranges are already age adjusted.<sup>17</sup>

We expected that metabolic control of non-BH4 responders could be maintained post-LT, once the usual low Phe diet was introduced, but most of the nonresponders had deterioration in their metabolic control. Generally, adults with PKU who

**Table 6.** Anthropometric Data of Patients Studied in SPI Versus SP4.

Anthropometry	SPI			SP4		
	0-5 Years (n = 3)	5-19 Years (n = 28)	>19 Years (n = 27)	0-5 Years (n = 0)	5-19 Years (n = 24)	>19 Years (n = 34)
BMI	–	–	22.8 ± 4.6	–	–	23.9 ± 4.7
BMI z score	0.30 ± 0.6	0.23 ± 1.1	–	–	0.17 ± 1.2	–
Overweight/obesity	n = 0	n = 9	n = 6	–	n = 9	n = 11
Overweight/obesity prevalence (%)		25.9%			34.5%	

Abbreviations: BMI, body mass index; SP, study period.

usually follow the same restrictive dietary patterns from day-to-day are anticipated to maintain long-term consistent blood Phe concentrations so the increase in their blood Phe following an unsuccessful BH4 LT was unexpected.

The choice of foods for increasing NP intake for the Phe titration may have influenced the results. Increasing Phe intake using milk sources (as previously recommended) together with PS was not practical in older patients.<sup>15</sup> In many adult patients with PKU, a liquid PS was prescribed but it was not feasible to mix this with other NP sources. In milder patients with PKU, higher pre-LT Phe tolerance was observed, so foods with a high protein content was given to temporarily increase Phe/NP intake, for example, regular bread and pasta, milk, cheese, and yogurt. Although some patients were afraid to introduce high-protein foods into their daily routine, others enjoyed their taste.<sup>22</sup> We cannot rule out the negative psychological consequences on feeding behavior of giving additional NP and then withdrawing it again from nonresponders. The difficulty and disappointment in returning to their previous dietary restrictions should not be underestimated in nonresponders.<sup>15</sup> Although this aspect requires further study, it is naive to consider that it would have no impact. We also observed that some adult patients also decreased the frequency of blood spot monitoring (data not shown), which may be associated with lower motivation because of not responding to BH4 medication. The PKU European guidelines suggest that a LT should not be performed in patients with 2 known disease null mutations or 2 known BH4 responsive mutations.<sup>1</sup> Our results demonstrate that by enrolling every patient with PKU to a LT, independent of the genotype, as suggested by the SPDM, this may have potential adverse consequences on longer term metabolic control.

In the process of Phe titration, PS prescription was maintained during preparation for LT. However, considering the weight variations throughout the study (Table 6), stabilizing PS prescription resulted in a lower TP (g/kg) intake. Also, the observed weight changes may have justified an increase in PE prescription, to theoretically prevent any negative effect on metabolic control.

Our study has several limitations. First, there was no opportunity to identify the exact starting date of the LT preparation phase, because some patients already had a higher blood Phe prior to the LT. Also this was a retrospective study without a

control group, so we were unable to compare the results with a group of patients not undertaking a LT. Although we have used the percentage of blood Phe measurements within target range, which is already age adjusted, our group had a wide age range which may have influenced adherence and metabolic control. Finally, in SP4, the comparison between BH4 treated + diet and exclusively diet-treated patients should be interpreted carefully due to the small group of patients on drug treatment. However, this is the first study evaluating the effect of the LT procedures on metabolic control, involving patients from one reference treatment center and collecting data from the year following the LT, suggesting that prospective, controlled, and multicentric studies are needed to study this aspect further. It is also important to address the psychological impact of the LT. It is well established that in older patients with PKU following relaxed diets, they have difficulty recommencing strict dietary treatment, thereby this knowledge should be considered when conducting BH4 LT in PKU.

## Conclusion

In conclusion, our results suggest that a transient Phe and NP titration may further adversely affect metabolic control, particularly in nonresponders. Even though the use of BH4 has been described as a good coadjuvant for BH4 responders, there are many different protocols for assessing BH4 responsiveness. A multicenter and controlled study would be helpful to examine the long-term effect of different methodologies on metabolic control, including a review of length and strategy of preparation for the LT and the assessment of its relevance.

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## Declaration of Conflicting Interests


The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Anita MacDonald is a member of the European Nutritionist Expert Panel (Biomarin), member of Sapropterin Advisory Board (Biomarin), member of the Advisory Board entitled ELEMENT

(Danone-Nutricia), and member of an Advisory Board for Arla and Applied Pharma Research. Júlio César Rocha is member of the European Nutrition Expert Panel (Biomarin), of an Advisory Board for Applied Pharma Research, and he has received honoraria from Nutricia.

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### References

- van Spronsen FJ, van Wegberg AM, Ahring K, et al. Key European guidelines for the diagnosis and management of patients with phenylketonuria. *Lancet Diabetes Endocrinol.* 2017;5(9):743-756.
- Blau N, van Spronsen FJ, Levy HL. Phenylketonuria. *Lancet.* 2010;376(9750):1417-1427.
- Rocha JC, MacDonald A. Dietary intervention in the management of phenylketonuria: current perspectives. *Pediatric Health Med Ther.* 2016;7:155-163.
- MacDonald A, Rocha JC, van Rijn M, Feillet F. Nutrition in phenylketonuria. *Mol Genet Metab.* 2011;104:S10-S18.
- Pena MJ, Almeida MF, van Dam E, et al. Protein substitutes for phenylketonuria in Europe: access and nutritional composition. *Eur J Clin Nutr.* 2016;70(7):785-789.
- Walter JH, White FJ, Hall SK, et al. How practical are recommendations for dietary control in phenylketonuria? *Lancet.* 2002;360(9326):55-57.
- MacDonald A, van Rijn M, Feillet F, et al. Adherence issues in inherited metabolic disorders treated by low natural protein diets. *Ann Nutr Metab.* 2012;61(4):289-295.
- García MI, Araya G, Coo S, Waisbren SE, de la Parra A. Treatment adherence during childhood in individuals with phenylketonuria: early signs of treatment discontinuation. *Mol Genet Metab Rep.* 2017;11:54-58.
- Feillet F, MacDonald A, Hartung Perron D, Burton B. Outcomes beyond phenylalanine: an international perspective. *Mol Genet Metab.* 2010;99(suppl 1):S79-S85.
- Scala I, Concolino D, Della Casa R, et al. Long-term follow-up of patients with phenylketonuria treated with tetrahydrobiopterin: a seven years experience. *Orphanet J Rare Dis.* 2015;10:14.
- Thiele AG, Rohde C, Mutze U, et al. The challenge of long-term tetrahydrobiopterin (BH4) therapy in phenylketonuria: effects on metabolic control, nutritional habits and nutrient supply. *Mol Genet Metab Rep.* 2015;4:62-67.
- Thiele AG, Weigel JF, Ziesch B, et al. Nutritional changes and micronutrient supply in patients with phenylketonuria under therapy with tetrahydrobiopterin (BH4). *JIMD Rep.* 2013;9:31-40.
- Anjema K, van Rijn M, Hofstede FC, et al. Tetrahydrobiopterin responsiveness in phenylketonuria: prediction with the 48-hour loading test and genotype. *Orphanet J Rare Dis.* 2013;8:103.
- Blau N, Belanger-Quintana A, Demirkol M, et al. Optimizing the use of sapropterin (BH4) in the management of phenylketonuria. *Mol Genet Metab.* 2009;96(4):158-163.
- MacDonald A, Ahring K, Dokoupil K, et al. Adjusting diet with sapropterin in phenylketonuria: what factors should be considered? *Br J Nutr.* 2011;106(2):175-182.
- MacDonald A, Ahring K, Almeida MF, et al. The challenges of managing coexistent disorders with phenylketonuria: 30 cases. *Mol Genet Metab.* 2015;116(4):242-251.
- Rocha JC, Vilarinho L, Cabral A, Osório RV, Almeida MF. Consensus for the nutritional treatment of phenylketonuria. *Acta Paediatr Port.* 2007;38(1):44-54.
- Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. *World Health Organ Tech Rep Ser.* 1995;854:1-452.
- World Health Organization Multicentre Growth Reference Study Group. World Health Organization Child Growth Standards based on length/height, weight and age. *Acta Paediatr Suppl.* 2006;450:76-85.
- Rolland-Cachera MF. Childhood obesity: current definitions and recommendations for their use. *Int J Pediatr Obes.* 2011;6(5-6):325-331.
- de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. *Bull World Health Organ.* 2007;85(9):660-667.
- Gokmen Ozel H, Lammardo AM, Motzfeldt K, et al. Use of sapropterin in the management of phenylketonuria: seven case reports. *Mol Genet Metab.* 2013;108(2):109-111.