

Clinical and nutritional evaluation of phenylketonuric patients on tetrahydrobiopterin monotherapy

Nilo Lambruschini^a, Belén Pérez-Dueñas^b, Maria Antonia Vilaseca^{c,*}, Anna Mas^d,
Rafael Artuch^c, Rosa Gassió^b, Lilian Gómez^a, Alejandra Gutiérrez^a,
Jaume Campistol^b

^a Nutrition Department, Hospital Sant Joan de Déu, Universitat de Barcelona, Barcelona, Spain

^b Neuropediatrics Department, Hospital Sant Joan de Déu, Universitat de Barcelona, Barcelona, Spain

^c Biochemistry Department, Hospital Sant Joan de Déu, Universitat de Barcelona, Barcelona, Spain

^d Pharmacy Department, Hospital Sant Joan de Déu, Universitat de Barcelona, Barcelona, Spain

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Abstract

The clinical, nutritional, and neuropsychological data of 11 mild/moderate PKU patients after one year of treatment with BH4 are evaluated. BH4 monotherapy was introduced at 5 mg/kg/day in 14 PKU patients. In 11/14 patients, Phe tolerance increased significantly from 356 ± 172 to 1546 ± 192 mg/day ($p = 0.004$), and special PKU formula was gradually reduced until complete removal. In them, mean plasma Phe concentrations remained below $360 \mu\text{mol/L}$ at 5 mg BH4/kg/day (7 mg/kg/day in one patient). BH4 therapy was stopped in three patients (V388M/P362T and R243Q/IVS10-11G > A genotypes) because it was not possible to improve Phe tolerance and to remove formula intake. Serum micronutrients were not significantly different at the start of treatment and at one year follow-up, except for selenium, which increased significantly after one year of therapy ($p = 0.017$). Anthropometric, and nutritional measurements were within the age- and sex-specific percentiles for a healthy population after one year therapy. Neuropsychological follow-up indicated that intelligence scores persisted within normal limits. In terms of patients' genotype, we confirmed that the P275S mutation combined with R408W was associated with long-term BH4 responsiveness, while the combination of P362T/V388M, and R243Q/IVS10-11G > A resulted in poor metabolic control in long-term BH4 therapy. In summary, our data confirm that BH4 is a safe, and effective therapy in a selected group of mild, and moderate PKU patients who respond to the BH4 loading test. Low doses of BH4 in monotherapy permit withdrawal of the special formula and guarantee a good clinical and nutritional outcome with no adverse side effects in PKU patients.

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Introduction

Phenylketonuria (PKU; OMIM 261600) is an autosomal recessive metabolic disease caused by a deficiency of phenylalanine hydroxylase (PAH, EC 1.14.16.1), a hepatic

enzyme which catalyses the conversion of phenylalanine (Phe) to tyrosine, using tetrahydrobiopterin (BH₄) as coenzyme [1]. The mainstay of dietary treatment is restriction of the Phe intake which in practice means restriction of nearly all protein-rich foods, and supplementation with Phe-free amino acid mixtures [2]. The description by Kure et al. [3] of four phenylketonuric patients with known mutations on the *PAH* gene who responded to BH4 supplementation by lowering plasma Phe concentrations

* Corresponding author. Fax: +34 93 2803626

E-mail address: vilaseca@hsjdbcn.org (M.A. Vilaseca).

opened a wide field of research into this novel therapeutic strategy in PKU [4]. Since then, many mutations of the *PAH* gene have been reported to be BH₄ responsive [5–9], and several investigation groups are currently studying the reasons for this responsiveness [10–12]. Furthermore, BH₄ treatment of some responsive patients resulted in successful control of blood Phe levels with a progressive relief or withdrawal from the Phe restricted diet [13–19]. However, in spite of a growing experience in BH₄ therapy, few data concerning the clinical and nutritional evolution have been reported.

Working at a reference centre for PKU in Catalonia (Spain), we systematically investigated BH₄ responsiveness in patients at diagnosis from the neonatal screening, as well as in PKU patients on a Phe restricted diet [20], so as to be able to offer this alternative therapy to the responsive patients. After the selection of the responsive patients, the initiation of BH₄ treatment would bring about an increase in Phe tolerance as well as removal of the special PKU formula.

Here, we present the clinical, nutritional, and neuropsychological evaluation after one year of treatment with BH₄ of 11 mild/moderate PKU patients.

Materials and methods

Patient selection

We investigated BH₄ responsiveness in a group of 73 PKU patients. The differential diagnosis of hyperphenylalaninemia was performed in all patients and a defect in BH₄ synthesis or recycling was excluded. In seven patients, the BH₄ loading test was applied at diagnosis from the neonatal screening before starting the Phe restricted diet, following the protocol described by Blau et al. [4], while in 66 patients the combined Phe/BH₄ loading test was applied as previously described [20]. Fourteen patients were initially selected owing to good response to the BH₄ loading test (a decrease of 45–94% in plasma Phe). Nine patients were mild PKU (tolerance: 400–600 mg Phe/day), four patients moderate PKU (tolerance: 350–400 mg Phe/day), and one classic PKU (tolerance: <350 mg Phe/day). All of them were on a Phe-restricted diet supplemented with special PKU formula at the start of BH₄ treatment.

All children or their guardians in this study 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. Compassionate use authorization for the BH₄ loading test and treatment was obtained from the health authorities.

BH₄ therapy protocol

BH₄ was obtained in 50 mg tablets from Schircks Laboratories (Jona, Switzerland). An initial dose of

5 mg/kg/day was applied to the selected PKU patients. Since the elimination half-life time of orally administered BH₄ is about 4 h [21], the BH₄ was administered in three daily doses. Phe restricted diet was progressively liberalised by adding 200 mg Phe/day every week for two months, while formula was gradually reduced (from a mean \pm SD of 51 \pm 40 g/day) until complete removal was achieved. BH₄ therapy was discontinued when tolerance could not be increased more than 400 mg Phe/day and formula could not be completely removed. Patients were clinically and nutritionally evaluated monthly throughout the BH₄ treatment.

Anthropometric and nutritional examination

Anthropometric evaluation was performed by the measurement of weight (kg) and height (cm). Assessment of nutritional status was performed on the basis of brachial areas of fat and muscle. Brachial area was calculated by measuring arm circumference. Brachial muscular area (mm²) was calculated as the ratio of triceps skinfold thickness and arm circumference, while brachial adipose area (mm²) was expressed as the difference between the brachial area and the brachial muscular area. Values obtained for all these measurements were compared to previously established age- and sex-specific percentiles for healthy population [22].

Tolerance evaluation

Tolerance is defined as the highest Phe intake tolerated while keeping blood Phe levels within the safe range (120–360 μ mol/L). Tolerance was assessed before the start of BH₄ therapy and whenever an increase in daily Phe-intake was introduced. A three-day questionnaire was used to calculate the Phe intake with the DietSource 2.0 Sanutrin Program (Novartis Consumer Health).

Biochemical procedures

Metabolic control: Plasma phenylalanine and tyrosine were analysed by ion exchange chromatography with ninhydrin detection (Biochrom 20, Pharmacia Biotech, Cambridge, England) [23]. Controls were performed weekly until complete introduction of natural proteins and removal from formula, every fortnight for the first three months and monthly for the last nine months. The index of dietary control for the last year before the start of BH₄ therapy and for the one year BH₄ treatment was calculated as the mean of the median of all Phe values for one year.

Nutritional control: Serum albumin was analysed by standard procedures with a Cobas Integra 700 Analyser (Roche Diagnostics). Serum vitamins E and A were determined by HPLC with UV detection [24], and B vitamins (folate, B₁₂), and ferritin by a competitive

protein-binding chemiluminescent assay (Centaur, Bayer). Serum oligoelements (Se, Zn) were measured by atomic absorption spectrometry. Urinary biopterin and neopterin were determined by HPLC with fluorescence detection (Perkin Elmer, Serie 200, Norwalk, CT, USA) [25]. Vitamin and oligoelement daily intake was calculated by the DietSource 2.0 Sanutrin Program (Novartis Consumer Health).

Neuropsychological evaluation

Development quotients were calculated with the Brunet–Lezine test in patients younger than 3 years of age after BH4 therapy ($N=4$). Intelligence measurement was evaluated with the Kaufman Assessment Battery (K-ABC) (patients from 3 to 6, 5 years), and Wechsler Intelligence Scale for Children-Revised (WISC-R) (patients older than 6.5 years) ($N=4$) before and after BH4 therapy. Results were expressed as T score: mean 100; SD 85–115. We asked the families about the incidence of sleep disorders, hyperkinesia or other behaviourally associated problems. Neuropsychological evaluation was performed immediately before and one year after the introduction of BH4 therapy.

Statistical analysis

Statistical analyses were performed using the statistical package SPSS (version 11.0). Wilcoxon test for paired data was used to compare the tolerance, micronutrient values, nutrient daily intake and neuropsychological data before and after BH4 treatment. Students *t* test was applied to compare individual blood Phe values for each patient before and after BH4 therapy. Differences were considered significant when $p < 0.05$.

Results

The metabolic and genetic data as well as the results of the Phe/BH4 loading test of the 14 patients selected for BH4 therapy are summarised in Table 1.

BH4 treatment was introduced at 5 mg/kg/day in the 14 selected patients. In a group of 11 patients, Phe tolerance increased significantly from 356 ± 172 mg/day (mean \pm SD; range: 201–600) to 1546 ± 192 mg/day (range: 1240–1801) (Wilcoxon test; $p = 0.004$). PKU formula could be completely removed in these patients. However, in three of the 14 selected patients BH4 therapy was unsuccessful. In patient 5, treatment with BH4 (10 mg/kg/day), special formula (70 mg/day of Maxamaid XP) and an increase of 400 mg/day Phe intake resulted in poor metabolic control (mean Phe \pm SD: 652 ± 38 μ mol/L). This patient carries a combination of a null mutation (IVS10-11G > A) and a BH4 responsive mutation in the loading test (R243Q). In siblings 10 and 14 treatment with BH4 (10 mg/kg/day), special formula (60 mg/day of Maxamaid XP) and an increase of 600 mg/day Phe intake also raised mean plasma Phe values (416 ± 64 μ mol/L). These siblings carry a combination of two BH4 responsive missense mutations (V388M/P362T). Since it was not possible to improve Phe tolerance and to remove formula intake despite high doses of BH4, therapy was stopped in these three patients (in agreement with their parents).

The indexes of dietary control before and during BH4 treatment of PKU patients are summarised in Table 2. Overall, the IDC in 10 patients were within the safe range with BH4 therapy at 5 mg/kg/day (Fig. 1). In patient 12, Phe values decreased to the safe range when BH4 was increased to 7 mg/kg/day (Phe values 350 ± 84 μ mol/L), when compared with the previous

Table 1
Metabolic and genetic data and results of the Phe/BH4 loading test of the 14 PKU patients selected for BH4 treatment

Patient number	Metabolic PKU phenotype	Genotype		Phe at 0 h of BH4-loading-test (μ mol/L)	Phe at 21 h of BH4-loading-test (μ mol/L)	% BH ₄ response
		Allele 1	Allele 2			
1	Mild	IVS10-11G > A	D415N	612	37	94.0
2	Mild	R408W	E390G	950	153	83.9
3	Mild	Y168H	V388M	925	150	83.8
4	Mild	Y414C	n.i.	672	148	78.0
5 ^a	Classic	IVS10-11G > A	R243Q	2134	545	74.5
6	Mild	R241Q	n.i.	668	185	72.3
7	Moderate	V388M	R241H	1330	359	72.0
8	Mild	IVS10-11G > A	E178G	972	288	70.4
9	Mild	S349P	E390G	1186	402	66.1
10 ^a	Moderate	V388M	P362T	1068	511	52.2
11	Mild	F55L	delF39	737	365	50.5
12	Moderate	R408W	P275S	903	455	49.6
13	Mild	R261X	R241H	892	477	46.5
14 ^a	Moderate	V388M	P362T	930	505	45.7

^a In patients 5, 10, and 14 BH4 treatment was stopped. Patients 10 and 14 are siblings. Details of the BH4-loading test were previously reported [20].

Table 2
Index of dietary control (IDC) of patients on Phe-restricted diet and on BH4 therapy and free Phe-diet

Patient number ^a	IDC on Phe-restricted diet	IDC on BH4 therapy	Significance (Students <i>t</i> test)
1	194 ± 93	226 ± 83	ns
2	434 ± 133	344 ± 99	<i>p</i> < 0.001
3	252 ± 76	177 ± 53	<i>p</i> < 0.001
4	290 ± 94	332 ± 116	<i>p</i> < 0.028
6	291 ± 68	358 ± 103	<i>p</i> < 0.001
7	295 ± 47	307 ± 79	ns
8	255 ± 93	218 ± 60	ns
9	412 ± 84	345 ± 86	<i>p</i> < 0.006
11	301 ± 240	332 ± 159	ns
12	254 ± 108	350 ± 84	<i>p</i> < 0.001
13	302 ± 67	297 ± 62	ns

Students *t* test was applied to compare individual blood Phe values for each patient before (*n* = 40) and after BH4 therapy (*n* = 40). All patients received BH4 at 5 mg/kg/day except for patient 12 who received the cofactor at 7 mg/kg/day.

^a Patients 5, 10, and 14 were excluded from the table.

5 mg /kg /day (Phe values 442 ± 58 μmol/L) (*p* < 0.001). This patient harbours one null mutation (R408W) and one mutation with unknown residual activity (P275S).

The biochemical data for evaluation of the nutritional status (oligoelements, and vitamins) were not significantly different at the start of treatment and at one year follow-up, except for selenium, which was significantly increased after one year of therapy (*p* = 0.017) (Table 3). No differences were observed in vitamin, and oligoelement daily intakes before and after BH4 therapy (data

Table 3
Biochemical data of the 11 PKU patients before BH4 therapy, at six months of therapy and at one-year

Biochemical data	Before BH4 treatment	After one-year therapy	Significance (Wilcoxon)
Phe (μmol/L)	382 ± 229	442 ± 141	ns
Tyr (μmol/L)	48 ± 10	44 ± 14	ns
Albumin (g/L)	46 ± 5	45 ± 3	ns
Ferritin (μg/L)	30.3 ± 12.5	24.6 ± 5.0	ns
Zn (μg/dl)	84.5 ± 15.7	84.1 ± 13.7	ns
Se (μg/L)	61.6 ± 21.1	85.0 ± 21.4	<i>p</i> = 0.017
Vit A (μmol/L)	1.3 ± 0.3	1.4 ± 0.5	ns
Vit E (μmol/L)	25.9 ± 6.8	21.5 ± 5.6	ns
Folate (nmol/L)	35.5 ± 0.7	32.9 ± 13.4	ns
Vit B ₁₂ (pmol/L)	577 ± 314	495 ± 134	ns
Urine % B/Pter ^a	39.4 ± 12.3	69.6 ± 17.7	<i>p</i> = 0.028

^a % B/Pter, % biopterin/(biopterin + neopterin) in urine.

not shown). Selenium daily intake was not significantly different (Wilcoxon test) before (mean: 47.1 μg/day) and one year after treatment (mean: 56.2 μg/day). The percentage of biopterin in urine was significantly higher on BH4 therapy compared with basal values (*p* = 0.028).

Anthropometric measurements of the 11 treated patients on Phe restricted diet and after BH4 treatment are summarised in Table 4. All values were within the age- and sex-specific percentiles for a healthy population.

As regard the neuropsychological evaluation, developmental quotient in patients younger than 3 years were within normal values after BH4 therapy (104 ± 3;

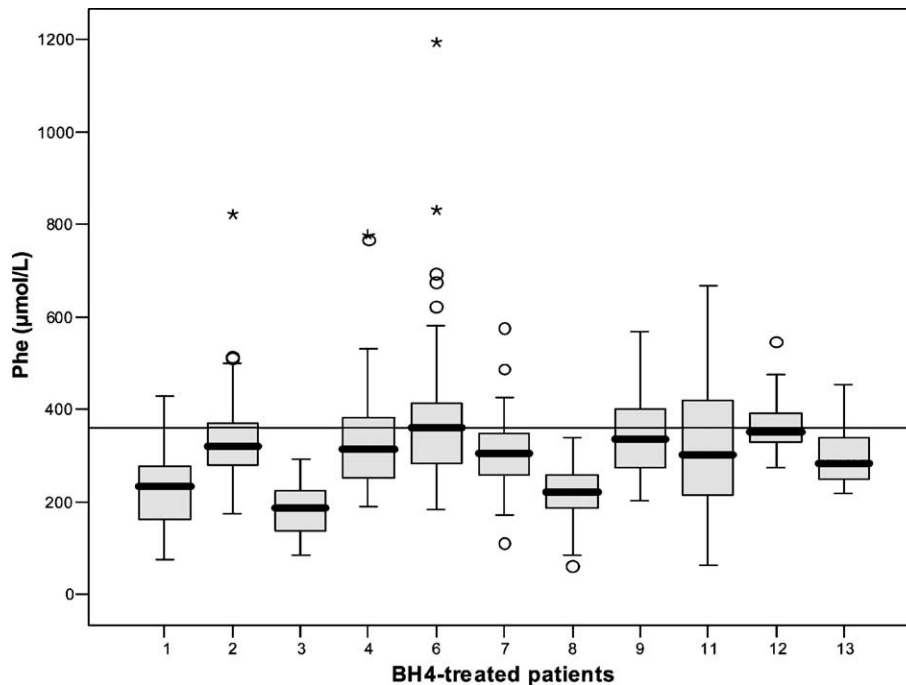


Fig. 1. Plasma Phe concentrations during one year treatment with BH4 in 11 PKU patients. The length of the boxes indicates the interquartile space (p25–p75); the horizontal line inside the box represents the median (p50) and the error bars indicate the adjacent values—that is, the maximum and minimum values of the distribution that may not be considered abnormal. The circles indicate the outliers and the stars the extreme values.

Table 4
Anthropometric parameters of the 11 patients on Phe-restricted diet and after BH4 treatment

Patient number ^a	Age (years)	Sex	Weight (kg)		Height (cm)		Brachial muscular area (mm ²)		Brachial adipose area (mm ²)	
			Diet	BH4	Diet	BH4	Diet	BH4	Diet	BH4
1	0.9	F	8.8	10.4	72.0	86.0	1120	1369	428	408
			P ₅₀	P ₅₀	P ₇₅	>P ₇₅		>P ₅₀		P ₂₅
2	2.2	F	9.9	12.0	76.5	87.0	1230	1628	319	523
			<P ₂₅	P ₂₅	<P ₂₅	P ₂₅	P ₅₀	P ₇₅	P ₅	P ₂₅
3	6.2	M	22.5	24.1	121.0	123.7	1788	2148	773	705
			P ₅₀	P ₇₅	>P ₇₅	P ₅₀ –P ₇₅	P ₅₀	P ₇₅	P ₅₀	P ₇₅
4	1.6	F	11.3	13.4	82.0	91.3	984	1167	352	857
			<P ₅₀	P ₅₀	<P ₅₀	P ₅₀	P ₁₀	P ₅₀	P ₁₀	P ₅₀
6	0.8	F	10.0	12.4	75.0	84.5	1382	1095	517	928
			P ₅₀	P ₇₅	P ₅₀	P ₅₀	P ₇₅	P ₂₅	P ₂₅	P ₇₅
7	10.7	M	35.9	37.8	146.5	150.5	2520	2448	1305	1733
			P ₇₅	P ₇₅	>P ₇₅	P ₇₅	P ₅₀	P ₅₀	P ₇₅	>P ₇₅
8	5.6	F	25.2	30.5	115	122	1755	2432	1567	1393
			>P ₇₅	>P ₇₅	>P ₇₅	>P ₇₅	>P ₇₅	>P ₉₅	>P ₇₅	P ₉₀
9	12.2	F	61.3	60.2	157	158	4505	4819	1914	1828
			>P ₇₅	>P ₇₅	P ₇₅	P ₅₀	>P ₉₅	>P ₉₅	>P ₅₀	>P ₅₀
11	0.2	M	6.9	10.6	61.3	78.5	1062	1216	600	683
			P ₇₅	P ₅₀	P ₅₀	P ₅₀		P ₅₀		P ₅₀
12	8.2	F	25.5	26.1	125.5	130	1832	1861	1021	1300
			P ₅₀	P ₅₀	P ₅₀	P ₅₀	>P ₂₅	P ₂₅	P ₅₀	P ₅₀
13	6.5	M	25.3	27.2	129.5	134.2	1700	1788	584	632
			>P ₇₅	P ₇₅	>P ₇₅	>P ₇₅	P ₅₀	P ₂₅	P ₅₀	P ₅₀

^a Patients 5, 10, and 14 were excluded from the table.

100–106) (mean \pm SD; range). Intelligence scores before BH4 treatment in older patients (102 \pm 9; 91–112) were not significantly different to values after 12 months of BH4 treatment (108 \pm 9; 96–118) (Wilcoxon test). Hyperkinesia, behavioral problems or sleep disorders were not described by the patients' families during BH4 treatment.

Discussion

There are still few reports on BH4 therapy in cofactor responsive PKU patients [13–19] and different treatment strategies have been applied in them [18]. Some patients have been treated after the neonatal screening, when BH4 loading test applied for the differential diagnosis of hyperphenylalaninemia revealed good response to the cofactor [13,15,16]. Special formula had not been introduced in these patients prior to BH4 treatment. Other authors described BH4 therapy combined with low Phe diet to moderately increase tolerance [17,18]. All of our BH4 responsive patients had already been in treatment with Phe restricted diet supplemented with the special PKU formula. Therefore, our strategy was to introduce BH4 therapy at low doses (5 mg/kg/day) and to increase Phe intake progressively, depending on the Phe tolerance and protein requirements of each patient, while decreasing the PKU formula. When Phe tolerance could not be improved, BH4 therapy was considered unsuccessful and therefore it was interrupted. The combination of BH4

with Phe restricted diet and special formula would worsen the quality of life and increase the cost of treatment in PKU patients. Since Phe restricted diet lacks foods rich in mineral salts, trace elements, and vitamins, the reduction of special formula may lead to decreased levels of several essential nutrients such as selenium and carnitine, as has consistently been reported [26–29]. This would be especially important in children who are growing up and who require high levels of micronutrients for satisfactory growth and the avoidance of deficiencies [4].

Our results confirm that the combined 24-h-long Phe/BH4 loading test [20] is a useful tool to select PKU patients who will respond to long-term treatment with the cofactor. Nevertheless, even a decrease in plasma Phe concentration higher than 45% after a single 20 mg/kg BH4 dose does not predict long-term response to BH4 treatment in all cases (Table 1). Probably, a one-week BH4 loading would have been a better option [18], but it would also be more difficult and expensive to apply in clinical practice. In our experience, patients with good BH4 response and final Phe levels lower than 500 μ mol/L after 21 h of the BH4 loading (Table 1) responded well to long-term therapy with BH4 (Table 2). Interestingly, three patients (with moderate and classic PKU phenotypes) who had a poor metabolic control on BH4 therapy showed the highest Phe levels (>500 μ mol/L) 21 h after the BH4 loading test, in spite of good BH4 response (Table 1). Our results confirm the benefit of long-term BH4 therapy in 9 mild PKU and 2 moderate PKU phenotypes.

These data provide evidence that only selected subgroups of mild and moderate PKU patients are candidates for BH4 monotherapy.

Different BH4 doses have been reported ranging from 5 to 20 mg/kg/day [13,18,19]. In our experience, doses of 5 mg/kg/day of BH4 in monotherapy were effective to maintain a good metabolic control (evaluated as IDC) in most of our BH4 responsive PKU patients. In patient 12, the increase of BH4 dose up to 7 mg/kg/day maintained blood Phe concentrations within the safe range, whereas in patients 5, 10, and 14 doses of BH4 10 mg/kg/day increased mean Phe concentrations beyond 400 μ mol/L. In them, BH4 malabsorption was excluded by monitoring urine biopterin concentrations. These patients showed good compliance with the Phe restricted diet, so further increases in daily BH4 doses were ruled out, according to the parents. We observed frequent acute metabolic decompensations caused by febrile infections in our infants (patients 2, 6, and 11) despite BH4 therapy. These data suggest that further increases in BH4 doses may be considered in these cases [19]. Optimal doses of BH4 may be difficult to establish and protocols for BH4 should be individualized depending on genotype and clinical status.

As regard patients' genotype, we confirmed the long-term BH4 responsiveness of the combined mutations carried by the 11 treated patients. In the whole group of treatment, two patients carried mutations not previously associated with BH4 responsiveness (P275S in patient 12 and P362T in sibs 10 and 14) [20]. The P275S mutation was indeed associated with long-term BH4 responsiveness, while the P362T in combination with a responsive mutation (V388M) did not result in good metabolic control with the long-term treatment. The R243Q, which was also associated with BH4 responsiveness [20] in combination with a null mutation (IVS10-11G > A) in patient 5, resulted in poor response to long-term BH4 therapy.

To our knowledge, there have been no reports about nutritional status in PKU patients under BH4 therapy. As commented above, classical dietary treatment of PKU may cause several micronutrient deficiencies [26–29], and therefore special formula is supplemented with several vitamins and oligoelements. Among these deficiencies, decreased plasma selenium concentrations have been reported in PKU under dietary treatment [26], which may lead to an impairment of selenoprotein functions [29]. In our patients, the monotherapy with BH4 (and consequently, the replacement of the special formula with a free diet) resulted in normal levels of all micronutrients analysed (Table 4). Furthermore, plasma selenium concentrations significantly increased after BH4 therapy, whereas selenium daily intake was not significantly different before and after BH4 therapy. These data would suggest an increased selenium bioavailability of natural diet compared with that of the special formula. The increase in selenium concentrations after BH4

therapy would probably enhance selenoprotein activities, although this subject will deserve further investigations.

Anthropometric measurements in BH4 treated patients remained within the same percentile in most patients, with a tendency to increase in brachial muscular area (4 patients), and in adipose area (6 patients). These data suggest that BH4 treatment does not impair the normal development of children, at least in terms of anthropometric and nutritional examination.

As expected, data from 12 month follow-up in 8 patients indicated that intelligence scores persisted within normal limits. No alterations were observed in attention, and executive function tests. Sleep disorders, hyperkinesia or behavioral problems were not reported by the families during BH4 treatment. No other adverse effects were documented over the duration of the treatment. Long-term follow-up and larger series of patients seem necessary to evaluate neurological outcome and cognitive functions in PKU patients treated with BH4, although these preliminary data would support the safety and the positive effects of BH4 therapy in the clinical parameters studied by our group.

In summary, our data confirm that BH4 is a safe and effective therapy in a selected group of mild and moderate PKU patients who respond to the BH4 loading test. The long-term response to BH4 may be different from that observed in BH4 loading test, and the initial doses might have to be modified according to the metabolic control, suggesting the need to apply individualized protocols of treatment. Low doses of BH4 in monotherapy allow for withdrawal of the special formula and guarantee a good clinical and nutritional outcome with no adverse side effects in PKU patients.

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