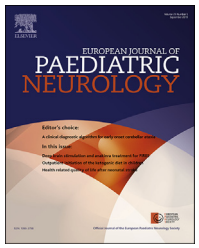
Official Journal of the European Paediatric Neurology Society



Original article

Prevalence of sleep disorders in early-treated phenylketonuric children and adolescents. Correlation with dopamine and serotonin status

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ARTICLE INFO

Article history:
Received 12 May 2019
Received in revised form
31 July 2019
Accepted 14 August 2019

Keywords:
Phenylketonuria
Sleep disorders
Dopamine
Serotonin
Melatonin

ABSTRACT

Phenylketonuric (PKU) patients are a population at risk for sleep disorders due to deficits in neurotransmitter synthesis. We aimed to study the prevalence of sleep disorders in early-treated PKU children and adolescents and assessed correlations with dopamine and serotonin status. We compared 32 PKU patients (16 females, 16 males; mean age 12 years), with a healthy control group of 32 subjects (16 females, 16 males; mean age 11.9 years). 19 PKU patients were under dietary treatment and 13 on tetrahydrobiopterin therapy. Concurrent phenylalanine (Phe), index of dietary control and variability in Phe in the last year, tyrosine, tryptophan, prolactin, and ferritin in plasma, platelet serotonin concentration, and melatonin, homovanillic and 5-hydroxyindoleacetic acid excretion in urine were analyzed. Sleep was assessed using Bruni's Sleep Disturbance Scale for Children. Sleep disorders were similar in both groups, 15.6% in control group and 12.5% in PKU group. In PKU patients, no correlations were found with peripheral biomarkers of neurotransmitter synthesis nor different Phe parameters, 43.3% had low melatonin excretion and 43.8% low platelet serotonin concentrations. Despite melatonin and serotonin deficits in early-treated PKU patients, the prevalence of sleep disorders is similar to that of the general population.

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https://doi.org/10.1016/j.ejpn.2019.08.005

1. Introduction

Phenylketonuria (PKU; OMIM 261600) is an inborn error of metabolism caused by mutations in the phenylalanine hydroxylase (*PAH*) gene (EC 1.14.16.1). These mutations lead to a total or partial decrease of PAH activity, which catalyzes the hydroxylation of Phe into tyrosine (Tyr), using tetrahydrobiopterin (BH4) as a cofactor. The biochemical consequences are Phe accumulation in biological tissues and fluids, as well as a deficit in the availability of Tyr.[1]

It is well-known that untreated PKU causes severe neurological disorders such as intellectual disability, epilepsy, progressive motor disorder, and severe behavioral disorders. Treatment of PKU should begin in the first weeks of life as early as possible and consists of a Phe restricted diet combined with the administration of a special formula, or alternatively with BH4 and total or partial diet liberalization in patients who respond to it.[2–4] Due to the protein requirements for humans it is not feasible to completely remove Phe from the diet and, therefore, early-treated PKU patients maintain blood Phe levels above normal values (40–70 μmol/L), between 120 and 600 μmol/L according to age. Because of this, even early-treated patients may show slight cognitive alterations (a normal intellectual quotient but lower than in control groups, along with executive dysfunction), altered myelination of the cerebral white matter, and a high incidence of anxiety, depression and attention deficit hyperactivity disorder (ADHD).[5–8]

There are several factors involved in the pathophysiology of PKU, including changes in the synthesis of the neurotransmitters dopamine and serotonin[9] (Fig. 1). These deficits are of particular relevance given that dopamine is essential for executive functions and serotonin is necessary for the regulation of mood. Also, both neurotransmitters play an important role in regulating sleep and wakefulness.[10–13] The alterations in dopamine and serotonin synthesis in PKU are the result of several mechanisms: a) lower Tyr synthesis and decreased Tyr and tryptophan (Trp) transport through the blood-brain barrier, since Phe shares the same L-type amino acid carrier (LAT1, SLC7A5) and competes with them, and b) inhibition of tyrosine hydroxylase and tryptophan hydroxylase activity due to elevated Phe levels.[1,9]

One of the compounds derived from serotonin is melatonin, a hormone synthesized mainly in the pineal gland, whose main function is the control of the sleep cycle.[14] Many studies have been published on the consequences of dopamine deficiency in early-treated PKU patients, and ultimately on a higher incidence of mood problems that may be related to serotonin deficiency.[6,15] Regarding the serotoninergic deficit, references to its repercussions on sleep quality have been described in the literature but are scarce,[16] even studies in which sleep is evaluated. In that sense, our group has performed a previous study in early-treated PKU young adult patients in which alterations in sleep quality were not found[17]; however, recent studies have been published in PKU adults[18,19] reporting more sleep disorders than in controls.

In the present work, we study the prevalence of sleep disorders in early-treated PKU children and adolescents and assessed correlations with different biomarkers related to dopamine and serotonin status.

2. Material and methods

2.1. Participants

We recruited 32 early-treated PKU patients with a mean age of 12 years (SD ± 3.36) (16 females and 16 males), and a control group of 32 healthy subjects with a mean age of 11.9 years (SD ± 3.29) (16 females and 16 males) with the same sociodemographic characteristics. The PKU patient group was divided in to three subgroups: 1) 12 patients under dietary

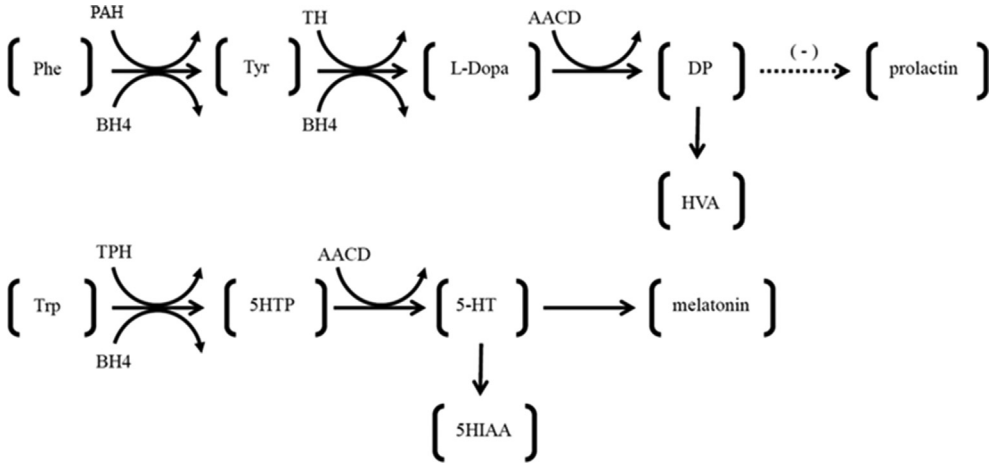

Fig. 1 – Neurotransmitter synthesis. TH: tyrosine hydroxylase. AACD: Aromatic L-amino acid decarboxylase. DP: dopamine. HVA: homovanillic acid. TPH: tryptophan hydroxylase. 5HTP: 5-hydroxytryptophan. 5-HT: serotonin. 5HIAA: 5-hydroxyindoleacetic acid.

treatment and a good metabolic control (last year index of dietary control (IDC) < 360 μmol/L for patients under 12 years old or < 600 μmol/L after 12 years), mean age 10 years (SD ± 2.21); 2) 7 patients under dietary treatment and poor metabolic control (last year IDC > 360 μmol/L for patients under 12 years old or > 600 μmol/L after 12 years), mean age 11.7 years (SD ± 3.09); and 3) 13 patients on prolonged treatment (between 4 and 12.5 years of treatment) with BH4 and good metabolic control, mean age 13.9 years (SD ± 3.48).

Inclusion criteria for PKU patients were: early (started during the first weeks of life) and continuous treatment with restricted diet or BH4, intellectual quotient above 80, absence of medication on the day of the analyses, and absence of associated medical pathology or mood disorders that could condition a sleep disorder. Three patients in the PKU group (one in each subgroup) were diagnosed with ADHD and received pharmacological treatment with prolonged-release methylphenidate; they had good tolerability and therefore were included in the study. Inclusion criteria for the control group were: normal school performance, absence of chronic medication, absence of medical pathology or mood disorders that could lead to a sleep disorder, and not being healthy siblings of PKU patients (to avoid family habits or environmental factors that could have an impact on the quality of sleep).

2.2. *Ethical approval*

Parents and participants older than 12 years of age signed an informed consent agreement in accord with the World Medical Association Declaration of Helsinki adopted in 1964 and amended in 2013. Our hospital ethics committee (CEI - Comité de Ética en Investigación Clínica) approved the study, code PI-47-12.

2.3. *Sleep disorder evaluation*

All participants in the study were assessed according to Bruni's Sleep Disturbance Scale for Children (SDSC)[20] for the presence of sleep disorders following the recommendations of the Clinical Practice Guidelines on Sleep Disorders in Childhood and Adolescence in Primary Care of the Spanish National Health System.[21]

The scale consists of 26 items and was developed to detect sleep disorders within the last 6 months in children and adolescents based on parental reports. Six factors representing the most common areas of sleep disorders in childhood and adolescence were evaluated: initiating and maintaining sleep disorders, sleep breathing disorder, arousal disorders, sleep wake transition disorders, excessive somnolence and sleep hyperhidrosis disorder. These subscales were considered as abnormal when T-score was >70 (T-score: 50 ± 10).

2.4. *Biochemical analysis*

In the PKU patient group, biochemical markers related to dopaminergic and serotonergic neurotransmission were analyzed. As precursors of synthesis: plasma Tyr and Phe were measured by ion-exchange chromatography with ninhydrin detection using a Biochrom 30 analyzer (Pharmacia-Biotech). Plasma Trp was measured by reversed-phase high-performance liquid chromatography (HPLC) with fluorescence detection (Waters, Milford, MA, USA), according to previously reported procedures.[22] Pituitary prolactin secretion is inhibited by dopamine, and therefore can be used as a parameter of brain dopamine availability. Iron deficiency is involved in some sleep disorders and therefore ferritin blood levels were measured.[23] Serum prolactin and ferritin were measured by a chemiluminescent microparticle immunoassay using an Architect analyzer (Abbott). Due to the presence of similar amine storage granules and identical high-affinity serotonin transporter and receptors, the platelet serotonin concentration has been used as an indirect index of central serotoninergic function. Platelet serotonin concentrations were analyzed by HPLC with fluorescence detection (Waters, Milford, MA, USA) following a modified procedure.[22] Urine excretion of biogenic amine metabolites (homovanillic acid (HVA) for dopamine and 5-hydroxyindoleacetic acid (5HIAA) for serotonin) was analyzed using gas chromatography mass spectrometry. Urinary 6-sulphatoxymelatonin (the final metabolite of melatonin) was analyzed by duplicate using a competitive ELISA kit (IBL: Ref. RE54021).[24] We also evaluated concurrent Phe levels, and variability in Phe (standard deviation of Phe levels) and IDC (median Phe levels in blood) for the last year. The blood tests were performed after fasting in the morning and urine tests were collected in the first sample in the morning. All the biochemical results were compared to the reference values established by our laboratory.

2.5. *Statistical analysis*

The categorical variables were described by their frequency table with percentages, and the numerical ones by means of descriptive statistics (mean, standard deviation). The frequencies of categorical variables were compared between groups using the Chi-squared test, whereas Kruskal–Walllis test was used to compare the means of numerical variables between groups.

SPSS software 19 (Armonk, NY: IBM Corp.) was used to perform the statistical analyses. Statistical tests with a p-value less than 0.05 were considered significant.

3. Results

Biochemical values of metabolic control of PKU and dopamine – serotonin status are shown in Table 1.

The poor metabolic control group is the one that presented significantly higher values of concurrent Phe ($p = 0.021$) and worse metabolic control during the last year (higher IDC) ($p = 0.002$). There are significant differences ($p = 0.023$) in variability in Phe levels for the last year between poor control group (higher SD) and BH4 group.

Mean Trp values were lower in the poor metabolic control group however the differences were not significant ($p = 0.252$).

In the PKU group, 43,3% of patients had low melatonin excretion (Table 2). The group with good metabolic control presented highest melatonin values (Fig. 2), although we did not find significant differences ($p = 0.249$) when comparing the three PKU subgroups (Table 1). Additionally, 43.8% of the PKU group presented low serotonin levels (Table 2), with lower levels in poor metabolic control group (Fig. 3), and we found

Table 1 – Biochemical values of metabolic control of PKU and dopamine – serotonin status.

Group		N	Mean	SD	p-values[a]
Concurrent Phe	BH4	13	387	±173	0.021
μmol/L n.v.: 40–70	Poor control	7	662	±353	
	Good control	12	284	±190	
IDC last year	BH4	13	332	±112	0.002
μmol/L	Poor control	7	534	±169	
	Good control	12	264	±110	
Variability in Phe last year (SD)	BH4	13	98.8	±46.8	0.023
	Poor control	7	183	±50.4	
	Good control	12	135	±64.7	
Tyr	BH4	13	57.2	±13.1	0.231
μmol/L n.v.: 40–87	Poor control	7	60.3	±37.0	
	Good control	12	47.6	±8.23	
Trp	BH4	13	47.2	±7.85	0.252
μmol/L n.v: 30–85	Poor control	6	41.0	±4.69	
	Good control	12	46.9	±10.2	
Platelet Serotonin nmol/10^9 platelets	BH4	13	2.41	±0.75	0.040
n.v.: 1.77–4.46	Poor control	7	1.58	±0.30	
	Good control	12	2.03	±0.74	
Prolactin mU/L	BH4	13	209	±43.3	0.186
n.v.: 108-466	Poor control	7	260	±132	
	Good control	12	201	±160	
Ferritin	BH4	13	52.7	±39.5	0.736
μg/L n.v.: 10–120	Poor control	7	46.2	±26.9	
	Good control	12	37.6	±21.4	
6-sulphatoxymelatonin	BH4	12	13.4	±9.30	0.249
μmol/mol creatinine n.v.[b]	Poor control	6	8.33	±6.03	
	Good control	12	22.8	±19.5	
HVA mmol/mol creatinine	BH4	13	3.25	±1.24	0.416
n.v.: 0.1–9.8	Poor control	7	3.16	±0.95	
	Good control	12	5.34	±5.26	
5HIAA mmol/mol creatinine	BH4	13	3.36	±2.54	0.502
n.v.: 0.3–8.9	Poor control	7	2.27	±1.40	
	Good control	12	3.88	±4.03	

Abbreviations: Phe: phenylalanine; BH4: tetrahydrobiopterin; IDC: index of dietary control; Tyr: tyrosine; Trp: tryptophan; HVA: homovanillic acid; 5HIAA: 5-hydroxyindoleacetic acid.
[a] Kruskal–Walllis test.
[b] 7–14 y: 11.9–66.2; >15 y: 6.3–37.9 μmol/mol creatinine.

significant differences (p = 0.040) between BH4 group and poor metabolic control group (Table 1). Only one patient presented with low levels of Trp and another presented with low excretion of 5HIAA. No correlation was found between the levels of Phe, Trp, serotonin and the excretion of melatonin or 5HIAA.

Table 2 – Melatonin and serotonin status.

Melatonin			
Group	normal	low	*p-value*[a]
BH4	6 (50%)	6 (50%)	0.665
Poor control	3 (50%)	3 (50%)	
Good control	8 (66.7%)	4 (33.3%)	
TOTAL	17 (56.7%)	13 (43.3%)	
Serotonin			
Group	normal	low	*p-value*[a]
BH4	9 (69.2%)	4 (30.8%)	0.213
Poor control	2 (28.6%)	5 (71.4%)	
Good control	7 (58.3%)	5 (41.7%)	
TOTAL	18 (56.2%)	14 (43.8%)	

Abbreviation: BH4: tetrahydrobiopterin.
[a] Chi-squared test.

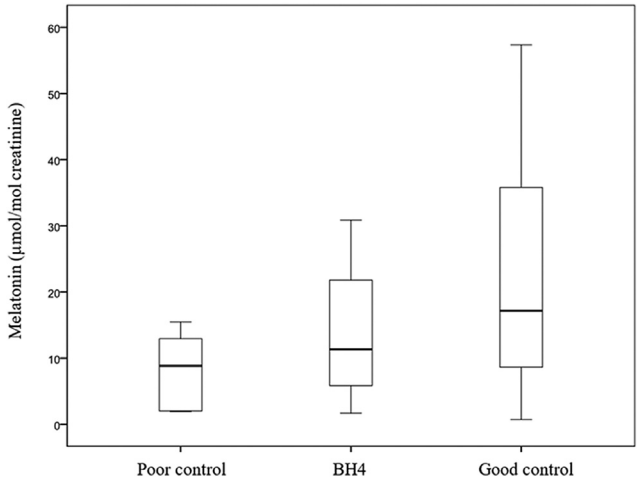

Fig. 2 – Melatonin levels.

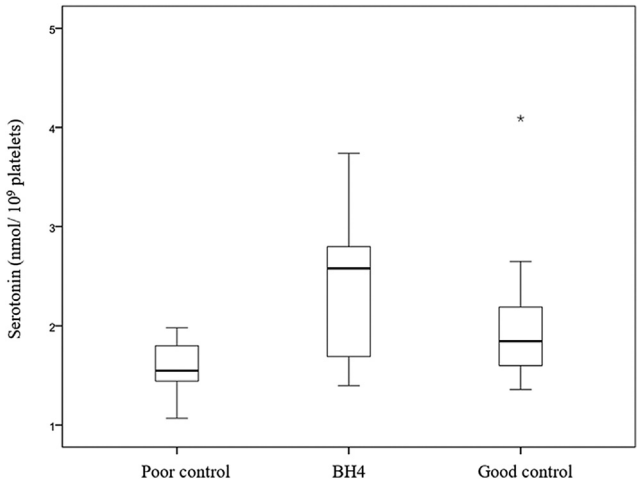

Fig. 3 – Serotonin levels.

We did not find statistically significant differences when comparing the presence of sleep disorders in the whole group of PKU patients and the control group (Table 3). Sleep disorders were slightly more frequent in the control group than in the PKU group (15.6% vs 12.5%), but the difference was not significant ($p = 0.719$). Only 4 subjects in PKU group and 5 in control group presented sleep disorders (one or more than one SDSC subscale abnormal). We did not find significant differences between the different SDSC subscales, or specific questions related to the alteration in the synthesis of dopamine/serotonin and melatonin such as: "How long after going to bed does your child usually fall asleep", "The child has difficulty getting to sleep at night", "The child has frequent twitching or jerking of legs while asleep or often changes position during the night or kicks the covers off the bed".

No differences were found when comparing the four groups for the presence of sleep disorder, the subscales or specific questions. Regarding the nine subjects with sleep disorders, five were in the control group, one in the poor metabolic control group and three in the good metabolic control group. No subjects in the group receiving BH_4 presented sleep disorders.

Biochemically, no correlation was found between the presence of a sleep disorder and the rest of the studied parameters (concurrent Phe, last year IDC and variability in Phe, Tyr, Trp, prolactin, serotonin and ferritin, excretion of melatonin, HVA and 5HIAA).

We did not find any correlations between low melatonin or serotonin levels and the presence of a sleep disorder.

4. Discussion

The prevalence of sleep disorders reported in different countries is between 13% and 27% in children aged 4–12 years.[21] According to the International Classification of Sleep Disorders, seven major categories can be described: insomnia disorders, sleep-related breathing disorders, central disorders of hypersomnolence, circadian rhythm sleep-wake disorders, sleep-related movement disorders, parasomnias and other sleep disorders.[25]

Our study is the first that analyze sleep disorders in early-treated PKU children and adolescents; 12.5% had sleep disorders, similar than in the control group (15.6%) and prevalence reported in normal children.[21] Therefore, our PKU patients presented sleep disorders as expected.

Cognitive functions have been extensively evaluated in PKU patients, but there are hardly any references as to how PKU patients sleep, even though they are theoretically a population at risk of presenting sleep disorders given the alterations in the synthesis of neurotransmitters and melatonin.

Initially, published studies address questions related to EEG sleep patterns rather than sleep quality. Behbehani[26] studied EEG sleep patterns in 22 early- and late-treated PKU patients (8–10 years), detecting minor EEG changes in early-treated children and pathological EEG changes in late-treated PKU patients; however, no significant changes were found during diet therapy and after diet termination. De Giorgis et al.,[27] found a delay in the maturation of patterns of

Table 3 – Bruni's Sleep Disturbance Scale for Children (SDSC) in PKU patients compared control group.

	Initiating and maintaining sleep disorders	Sleep breathing disorders	Arousal disorders	Sleep wake transition disorders	Excessive somnolence disorders	Sleep hyperhidrosis
PKU patients						
BH4 (0/13)[a]	normal	normal	normal	normal	normal	normal
Poor control (1/7)[b]	**abnormal (P_1)**	**abnormal (P_1)**	normal	**abnormal (P_1)**	**abnormal (P_1)**	normal
Good control (3/12)[b]	**abnormal (P_2)**	normal	**abnormal (P_2)**	**abnormal (P_2)**	**abnormal (P_2)**	normal
	normal	**abnormal (P_3)**	normal	normal	normal	normal
	normal	normal	normal	**abnormal (P_4)**	normal	normal
Control group (5/32)[b]	normal	normal	**abnormal (C_1)**	normal	**abnormal (C_1)**	**abnormal (C_1)**
	normal	normal	normal	normal	normal	**abnormal (C_2)**
	normal	normal	normal	normal	**abnormal (C_3)**	normal
	normal	**abnormal (C_4)**	normal	normal	normal	**abnormal (C_4)**
	normal	normal	normal	**abnormal (C_5)**	normal	normal

P: patient; C: control.
[a] In brackets altered cases versus total subgroup.
[b] The same case may have more than one abnormal subscale.

"tracé alternant" and sleep spindles in 16 early-treated PKU infants during their first months of life, during plasma Phe normalization. In 1973, Schulte et al.,[16] hypothesized that PKU patients may present sleep behavior alterations due to Trp metabolism disturbances. They studied 22 untreated PKU infants and young children (16 days and 3.75 years of age) and found no differences in the distribution between rapid eye movement or active, non-REM or quiet, and undifferentiated sleep compared to the control groups (healthy normal subjects and early-treated PKU patients). They concluded that, under chronic reduced conditions and not abruptly decreased, a normal or near normal sequence of quiet and active sleep could be maintained despite a severe lack of blood and cerebrospinal fluid serotonin as it occurs in PKU.

In recent years, interest in evaluating sleep in PKU patients has increased, but studies are scarce and focused on the adult population. In a previous study by our group,[17] 25 early-treated PKU young adults (range 18–31 years, mean age: 23.66 years; 12 females, 13 males) were assessed regarding sleep quality using the Pittsburg Sleep Quality Index. Thirteen out of 25 patients presented with good metabolic control (IDC < 600 μmol/L). All patients showed subjectively good sleep quality, 15 very good and 10 fairly good. By contrast, Bruinenberg et al.,[19] found more sleep disorders (specially insomnia and circadian rhythm sleep disorders) and reduced sleep quality in 25 treated adult PKU patients (mean age: 30 ± 9) compared to controls, but they did not assess metabolic control or provide the age when treatment was started in these patients. Bilder et al.,[18] reported a sleep disorder prevalence of 14.4% in 3714 adult PKU patients (aged 20 to >80 years, mean age: 38.5 years) versus 6.9% in general population controls, which fell to 9% in the age range of 20–39 years (n = 2247) (the authors supposed that this adult cohort were most likely to have had early and continuous treatment). Regard metabolic control, Huijbregts et al.,[28] in a study of health-related quality of life in early-treated PKU patients (7–40.8 years) found, only in adult patients, association between lifetime Phe levels and poorer functioning in the domain sleep (problems/limitations concerning sleeping). We did not find correlation between metabolic control and the presence of a sleep disorder, but in our case, we evaluated more recent Phe levels (concurrent Phe, last year IDC and variability in Phe) and only 21.8% of the sample had poor metabolic control.

In adult PKU, mood disorders are more common than in children, and should be taken into account that could affect sleep independent of neurotransmitter status.[6,18]

Platelet serotonin concentrations and urinary melatonin excretion have been shown to be good long-term indicators of the amount of circulating plasma serotonin and melatonin secretion, respectively.[22,29] In our sample, 43.3% of PKU patients had low melatonin levels and 43.8% had low serotonin concentrations, but these values were not correlated with sleep disorders. These patients have a chronic serotonin and melatonin deficits, and the hypothesis proposed by Schulte,[16] could explain that there is no correlation with sleep disorders, or perhaps, more exposure over time or a greater deficit of neurotransmitter synthesis is necessary to induce a sleep disorder. Other possibility is that urinary melatonin is an indirect estimation of pineal melatonin excretion, and differences in hepatic metabolism of melatonin would explain interindividual differences.

5. Conclusions

In conclusion, we highlight a prevalence of sleep disorders in early-treated children and adolescent PKU patients similar than in the control group, despite having a deficit in the synthesis of serotonin and melatonin. However, we should not completely rule out the higher incidence of sleep disorders given the small sample size we studied, and the fact that we only used questionnaires as a tool to assess sleep disorders. We think it is important to follow up sleep characteristics in PKU patients, especially those who are older or have poor metabolic control.

Conflict of interest

The authors have stated that they had no interest which might be perceived as posing a conflict or bias.

Acknowledgements

This work was supported by a grant from the Instituto de Salud Carlos III of Spanish Ministry of Economy and Competitiveness, European Regional Development Fund (FIS PI12/01469).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpn.2019.08.005.

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