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Adequação da ingestão proteica em doentes com fenilcetonúria de acordo com as *guidelines* europeias para a PKU

Adequacy of protein intake in patients with Phenylketonuria according to European PKU guidelines

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Resumo

Introdução: A ingestão correta de proteína descreve-se como um fator crítico para doentes com Fenilcetonúria (PKU). Pretende-se avaliar a ingestão proteica e o padrão alimentar na PKU, um ano após a publicação das guidelines europeias para a PKU (EPG).

Métodos: Foram estudados, em 2018, 99 doentes precocemente tratados (48 PKU moderada e 27 PKU clássica) aquando da avaliação nutricional anual. Foram recolhidos dados sobre ingestão nutricional [proteína total (TP, g/kg/dia), proteína natural (NP, g/kg/dia), equivalente proteico (PE, g/kg/dia) derivado de substitutos proteicos (PS). A Adequação Total Proteica (TPA) foi calculada como percentagem da recomendação da Organização Mundial de Saúde (WHO) *safe levels* e comparada com EPG (WHO+20% para compensar as perdas da digestibilidade dos suplementos de L-aminoácidos (L-AA) e +20% para melhorar o controlo metabólico). Foram recolhidos dados sobre as fontes de NP, tipos de PS e a sua distribuição ao longo do dia.

Resultados: A mediana de TPA foi de 171%. Cerca de 77 doentes apresentaram TPA ≥140%. Apenas 6 doentes revelaram TPA <100%: 2 pediátricos e 4 adultos. TPA entre 100-140% foi encontrada em n=16 (5 pediátricos). Mediana de NP (%) derivada de TP foi de 53%. 64 doentes tomavam SP, 83% L-AA. Neste grupo, NP contribuiu em 34% para TP.

Discussão: Este estudo revela que 78% dos doentes estão acima da EPG e salienta a importância da monitorização da prescrição proteica de forma a melhorar os *outcomes,* especialmente no grupo pediátrico. Mais estudos são necessários de forma a clarificar a melhor fonte proteica e o seu impacto no estado nutricional.

Palavras-chave: Fenilcetonúria; Adequação Proteica; Guidelines Europeias para a PKU e Padrão Alimentar

Abstract

Background: Correct protein intake is critical in the management of PKU. We evaluated protein intake and analysed the food pattern in PKU, one-year post publication of the European PKU Guidelines (EPG).

Methods: 99 early treated PKU (24 HPA, 48 mild PKU and 27 classical PKU), an annual nutritional status evaluation in 2018 was studied. Data on nutritional intake [total protein (TP, g/kg/day), natural protein (NP, g/kg/day), protein equivalent (PE, g/kg/day) from protein substitutes (PS)] was collected. TP adequacy (TPA) was calculated as a % of WHO/UNU/FAO 2007 safe levels of protein intake. Results were compared with EPG (WHO+20% to compensate digestibility losses from L-aminoacids supplements (L-AA) and +20% to improve blood phenylalanine metabolic control). Sources of NP, type of PS and its meal distribution were recorded from patient's assessment.

Results: Median TPA was 171%. The majority of patients (n=77) had a TPA above 140%. Only 6 patients had a TPA <100%: 2 children and 4 adults. A TPA of 100-140% was seen in n=16 (5 paediatrics). Median % NP of TP intake was 53%. 64 patients were taking PS; 83% on L-AA. In this group, NP contributed a median of 34% whilst PS contributed 66% of TP intake.

Discussion: Our audit showed that 78% of patients were above EPG. It is essential that adequate protein should be prescribed and intake monitored in order to optimize outcome, specially in children. Further research is needed to clarify the best protein sources and its impact on nutritional status.

Keywords: Phenylketonuria; Protein Adequacy; European PKU Guidelines and Food pattern

List of abbreviations

AAM- Aminoacid Mixture

ANSE- Annual Nutritional Status Evaluation

BH4- Tetrahydropterin

BMI- Body Mass Index

CHUP-Centro Hospitalar Universitário do Porto

EPG- European PKU Guidelines

GMP- Glycomacropeptide

HPA- Hyperphenylalaninemia

LAT 1- Large neutral aminoacids transporter 1

LNAA- Large Neutral Aminoacids

NP- Natural protein

PAH- Phenylalanine Hydroxylase

PE- Protein Equivalent

Phe- Phenylalanine

PKU – Phenylketonuria

PS- Protein substitutes

SLPF- Special Low Protein Foods

TP- Total protein

TPA- Total Protein Adequacy

Tyr- Tyrosine

WHO- World Health Organization

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I. Introduction

Phenylketonuria (PKU) is an inborn error of protein metabolism with a frequency of 1 to 10 000 newborn children, in Europe[1]. This autosomal recessive disease belongs to aminoacidopathies defined mostly (98-99% of patients) by the partial or complete enzymatic activity of the hepatic enzyme, phenylalanine hydroxylase (PAH)[1]. In a very small group of patients, PKU may be also caused by a deficiency of the PAH co-factor, tetrahydrobiopterin (BH4)[1], or even by a deficiency of the co-chaperone DNAJC12 responsible for the proper folding of the PAH[2]. With a deficient conversion of phenylalanine (Phe) into tyrosine (Tyr) an increased blood Phe/Tyr ratio is expected[3]. Phe is an essential large neutral amino acid (LNAA) with high affinity for blood-brain barrier large neutral amino acid transporter 1 (LAT-1). Increased blood Phe concentrations in combination with normal/low levels of the other LNAA that share the same LAT-1 will result in increased brain Phe concentrations and low brain concentrations of the non-Phe LNAA [4]. Brain Phe accumulation is one of the mechanisms responsible for the irreversible neurocognitive damage in untreated PKU patients [5]. Thus, the establishment of the newborn screening programmes has been crucial in order to early identify PKU patients, allowing immediate treatment and preventing irreversible mental retardation [5].

The treatment is mainly based on a Phe restricted diet defined by a natural protein (NP) restriction combined with protein substitutes (PS) and special low protein foods (SLPF) to satisfy protein and energy requirements, respectively[6-8]. PS can be Phe-

free amino acid mixtures (AAM), glycomacropeptide (GMP) and LNAA, but their nutritional composition and metabolic efficacy deserve special attention[9, 10].

A pharmacological treatment, sapropterin dihydrochloride (Kuvan®, BH4) may help a sub-group of patients to have lower blood Phe levels, allowing them to consume more natural protein[11, 12].

Protein prescription depends on Phe tolerance, which is influenced by disease severity, age, dosage and adherence to PS[7]. The dietary goal is to optimize Phe tolerance, while maintaining the metabolic control within the target range[8].

Daily total protein (TP) requirements in patients with PKU may be higher when compared to the healthy population[13]. PS, mainly constituted by amino acids, have a different metabolism comparing to natural protein[10]. The fast increase of blood amino acids leads to rapid absorption and quicker oxidation, which suggests lower protein retention when compared with natural protein[14, 15]. PS metabolism might be the explanation for the higher needs imposed on PKU disease. Therefore, European PKU Guidelines (EPG) suggest for PKU 140% of the age-related 2007 World Health Organization (WHO) protein recommendation safe level. Out of the additional 40%, 20% aim to compensate digestibility losses from L-aminoacids and the other 20% aim to optimise phenylalanine metabolic control [7].

The objective of this study was to evaluate protein intake of patients with PKU according to the recommendations proposed by EPG, one-year post publication. As a second goal, we aimed to analyse and characterize patient's food pattern in order to provide a global perception of the main protein sources, description of the NP food sources, type and daily distribution of PS.

II. Methodology

A. *Participants*

In 2018, 105 patients with PKU were under follow-up at Centro Hospitalar Universitário do Porto (CHUP). PKU severity was classified according to neonatal screening blood Phe concentrations as stated at Portuguese Consensus: Hyperphenylalaninemia (HPA) (blood [Phe]<6 mg/dL), mild PKU (blood [Phe]≥ 6 and <20mg/dL) and classical PKU (blood [Phe]≥20mg/dL)[16]. Late diagnosed patients (n=6) were excluded from this study. Thus, the final sample was composed of 99 patients, 53 males and 46 females and aged 19,3 ± 8,2 years. There were 24 patients with hyperphenylalaninemia (HPA), 48 Mild PKU and 27 Classical PKU.

B. *Eligibility*

Patients enrolled in this study were those exclusively diagnosed through newborn screening and with an annual nutritional status evaluation (ANSE) protocol scheduled in 2018. ANSE includes anthropometric data, body composition analysis, blood pressure, nutritional intakes and clinical biochemistry.

C. *Study design*

This is a cross-sectional, retrospective, descriptive, observational study (Figure 1). Birthdate, gender, weight, height, body mass index (BMI), disease severity, genotype, TP, NP and PS intake as GMP, AAM and LNAA were compiled from electronic patient clinical records. There is no control group, as all participants had a diagnosis of PKU. It was studied the protein intake in patients with PKU and analysed the percentage of adequacy according to WHO/UNU/FAO (2007) safe

levels of protein intake[17]. Protein intakes were compared with the EPG[7, 17] (WHO+20% and WHO+40%). All patient names were coded to protect their identity and data.

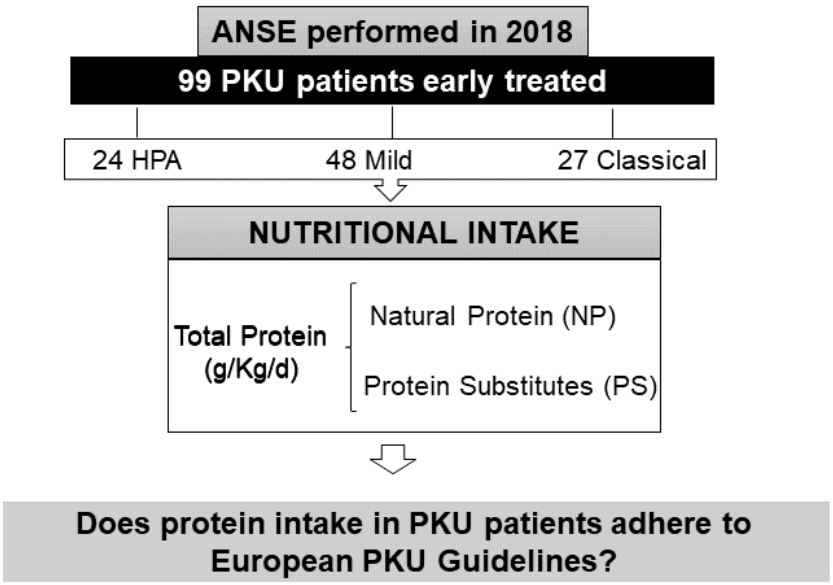

Figure 1- Study design.

ANSE-annual nutritional status evaluation, PKU- Phenylketonuria, HPA- Hyperphenylalaninemia, NP- Natural protein and PS- protein substitutes.

D. Data collection

1) Anthropometry

Patients were weighted by SECA® (accuracy= 0,5 kg) with light-weight clothes, without shoes or jewelry. Height was measured by a stadiometer. BMI was classified by criteria of the WHO. In children patients (<19 years), z-scores were calculated using Anthro® or Antroplus® programmes, and overweight/obesity classified according to recommended cut-offs [18, 19]. For adult patients, BMI was defined by categories as reference the WHO criteria[20].

2) Dietary Patterns

Protein intake data were collected from patient's clinical reports database and reported intake on the day of the ANSE. TP intake (g/kg/day) was measured and described as the sum of NP intake [natural foods, (NP, g/kg/day)] and protein equivalent (PE, g/kg/day) from PS. Median TP adequacy (TPA) was calculated according to WHO/UNU/FAO safe levels as a percentage and compared with EPG recommendation (statement #30, WHO+20%/WHO+40%). Individual patient's records were analysed and the food groups providing natural protein intake were identified. PS were either AAM, GMP or LNAA. Dietary patterns of PS were evaluated as the different forms of intake (liquid/powder), quantity and pattern of intake (meal/s). A sub-group of studied patients (n=17) were prescribed pharmacological treatment with sapropterin dihydrochloride.

3) Metabolic Control

Metabolic control was measured and considered satisfying according to blood [Phe] targets: ≤ 6mg/dL or ≤ 8mg/dL in patients below and above 12 years, respectively[16]. Blood [Phe] were measured at overnight fasting and analysed by tandem mass spectrometry (MS/MS). Blood [Phe] were recorded from the patient's electronic clinical record database. Blood Phe measurements conducted over the previous 12 months to the ANSE were used to calculate median of blood [Phe]. Medians were compared with target blood Phe levels according to the Portuguese Nutritional Consensus[16].

4) Ethical statement

This study and its data collection were approved by the ethics committee of Centro Hospitalar do Porto, 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 applied for participants and parents/caregivers of children included in the study.

5) Statistical Analysis

SPSS 25 for windows was performed to statistical analysis. To verify the normality of variables was used Kolmogorov-Smirnov test. Concerning the distribution of continuous variables, mean ±SD or median [P_{25}-$_{75}$] was measured. Comparison of continuous variables was performed by the Mann-Whitney test. It was considered significant difference statistical when $p<0,05$.

III. Results

Descriptive data of patients are shown in Table 1.

Table 1- Descriptive sample data: sample size, age, gender, weight and PKU severity.

SAMPLE SIZE	**N=99, 50 on paediatric age**
AGE	**19.3±8.2y** <19y: **12.6±4.7y** ≥19y:**26.1±4.5y**
GENDER	N=**53 Male** N=46 Female
DISEASE SEVERITY	HPA PKU: **24** Mild PKU: **48** Classical PKU: **27**

The median NP intake represented 53% [31-100] of TP intake whilst PE from PS intake contributed with the rest 47% [0-69] to TP intake. Although in respect of g/kg, NP was 0.69 g/kg [0.43-1.4] and 0.7 g/kg [0-0.91] from PS. Nevertheless, only one HPA patient was prescribed PS. Patients median metabolic control showed adequacy as established on Portuguese Consensus (<12 years: 4.9 mg/dL [3.9-5.9] and ≥12 years: 7.5 mg/dL [4.5-10]). Out of 64 patients prescribed PS, NP provided 34% [25-51] of TP intake. Thereby, PS patients provided a median PE intake of 0.86 g/kg [0.71-1.04]. Children under PS prescription had a median PE intake of 0.89 g/kg [0.77-1.10] in relation to 0.84 g/kg [0.72-1.00] in adults.

The majority of patients (n=77) had a TPA over EPG (WHO+40%). 43 of the 77 patients were children/adolescents and 34 were adults. Percentage of TPA is shown on table 2 according to disease severity and BH4 treatment.

Median TPA was of 171% [146-203]. NP represented 79% [51-165] and PS 84% [0-109] of the TPA. Figure 2 represents NP and PS contribution to median TPA according to PKU severities and BH4 therapy. TPA below 140% was found on 22 patients, one patient below 12 years showed metabolic control within blood [Phe] target (100%) comparing with 33% of the patients above 12 years (n=21, 7 with metabolic control within blood [Phe] target range).

In the BH4 sub-group almost every patient presented TPA over 140% (n=14, 82%). Only 3 adults had a TPA lower than 140% of intake, 2 of them between 120-140% TPA, and one patient did not meet WHO/UNU/FAO (2007) cut-point (TPA 100%). TPA was not different when comparing patients on BH4 therapy and patients on diet treatment: 182% [155-206] vs.168% [144-202]; p=0.55.

Table 2- Percentage of patients with different grades of TPA, according to disease severity.

		Children/adolescents n=50 (0-18y)	Adults n=49 (>18y)
HPA (n=24)	TPA **<100%**	**2/24 (8%)**	1/24 (4%)
	TPA **≥100, <120%**	1/24 (4%)	-
	TPA**≥120, <140%**	-	-
	TPA **≥140%**	16/24 (67%)	4/24 (17%)
Mild (n=48)	TPA **<100%**	-	1/48 (2%)
	TPA **≥100, <120%**	-	3/48 (6%)
	TPA **≥120, <140%**	2/48 (4%)	2/48 (4%)
	TPA **≥140%**	23/48 (48%)	17/48 (35%)
Classical (n=27)	TPA **<100%**	-	2/27 (7%)
	TPA **≥100, <120%**	-	1/27 (4%)
	TPA **≥120, <140%**	2/27 (7%)	5/27 (19%)
	TPA **≥140%**	4/27 (15%)	13/27 (48%)

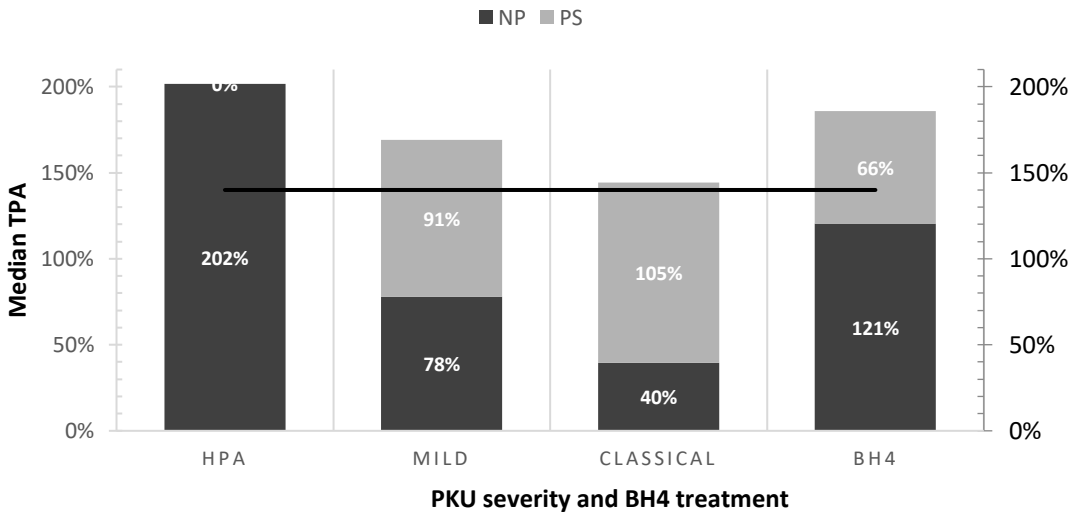

Figure 2- Median TPA from NP and PS on different PKU severity and under BH4 treatment. Black line represents the 140% TPA (EPG recommendation).

Of the 64 patients prescribed PS prescription, AAM contributed the major source of PS for most of the patients (n=53). Only 10 patients were on GMP±AAM (8 exclusively GMP) and 1 prescribed LNAA pharmacology. The majority of patients was taking PS divided in 3 daily meals (57 patients): breakfast (97%), afternoon snack (84%) and supper (78%).

A powdered PS was used by 48% of patients, while 50% combined liquid and powdered PS. Liquid PS was used only by 8% of the patients. NP food pattern according to different PKU severity is stated in Figure 3. Food groups with a lower protein contribution are not shown on figure 3 as they were consumed by the of most patients (potato-99%, fruit-98%, rice-95% and vegetables-90%). Excluding milk/yoghurt (consumed by 70%), pulses and high biological protein were the least ingested food groups (44% meat/fish/eggs, 33% cheese/ham and 30% pulses).

Patients on BH4 therapy (n=17) had a NP intake of 1 g/kg [0.79-1.2], while non-BH4 treated patients had 0.6 g/kg [0.35-1.45] of NP intake. BH4 treated group had an increased intake of all food-groups, specially of high biological protein as meat, fish and eggs, cheese/ham, dairy products and pulses.

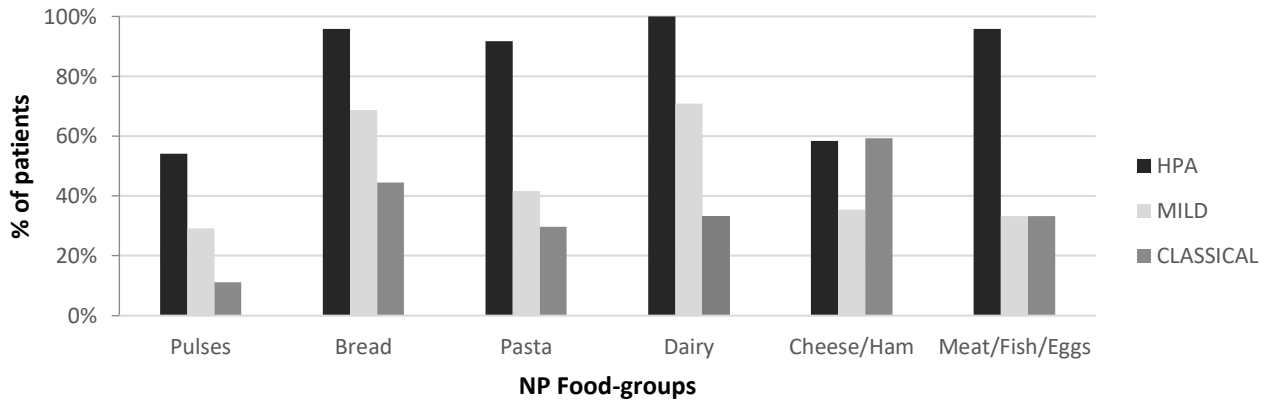

Figure 3- Percentage of patients with NP food-group present in their food pattern according to PKU severity.

HPA patients had a NP intake of 1.8 g/kg [1.42-1.97]. More than 58% of HPA patients were prescribed high biological NP food sources as described on table 3. Only one HPA patient was prescribed BH4 treatment.

Table 3- NP intake on BH4 and HPA patients.

	Pulses	Bread	Pasta	Rice	Dairy	Cheese/Ham	Meat/Fish/Eggs
BH4 (n=17)	47%	94%	76%	100%	82%	59%	47%
HPA (n=24)	54%	96%	92%	96%	100%	58%	96%

IV. Discussion

The main finding of this study is that 78% of patients (77 out of 99) achieved TPA as recommended by the EPG (WHO+140%). According to severe forms of PKU, PS intake was essential to attain TPA. The majority of protein intake (in %) came from NP, although 65% of patients took PS prescription. As shown in the results, only 1 HPA patient was under PS prescription. Patients were mainly prescribed powder PS, followed by the liquid formulations. The PS was taken 3 times per day in the majority of patients and their global food pattern where inserted on breakfast, afternoon snack and supper. Considering PKU severities clear difference are noted according to NP food pattern, specially in high protein sources.

Over WHO/UNU/FAO (2007) protein safe levels were prescribed an extra median of 71% protein. Median metabolic control of patients showed adequacy as stated on Portuguese Consensus, a recommendation stricter than EPG for patients older than 12 years [(16)]. These results are relevant because they revealed controlled patients in relation to metabolic control. In fact, this supports the veracity of this study results to the preconized treatment, protein intake analysis and food pattern reported.

Taking in account that protein prescription is influenced by Phe tolerance, Pinto *et al* showed that 65% of their patients (n=40) tolerated more NP than prescribed[(21)].

Pinto's *et al* findings were crucial in order to establish the real NP tolerance[21]. Considering that patients were recruited also from our centre, we consider that nowadays, dietary intakes are closer to the real Phe tolerance. Meanwhile, 36% of patients with TPA under the EPG recommendation had a blood [Phe] according to the target range established on Portuguese Consensus[16]. Special attention is needed in these patients in order to prevent overtreatment. Consequences related to overtreatment in PKU is described by growth impairment, specially noted on children/adolescents, anorexia, osteopenia, nutrient deficiencies and also may present lower levels of fat-free mass[4, 22]. In contrast, 22% of patients were above EPG recommendation. In children, in order to achieve good development and growth is essential an adequate intake of protein. Furthermore, our results revealed 4 paediatrics patients below PKU protein recommendation, 2 of them under WHO/UNU/FAO (2007) recommendation. These results emphasize the important role of qualified nutritionists performing a constant review of the patient's diet, within the full nutritional status evaluation in order optimise Phe tolerance and prevent overtreatment consequences. On the other side, within the process of increase natural protein intake, some patients may find it a difficult process, since they were not familiar with this regular protein rich foods [23-25].

Food patterns of patients with PKU is influenced by their disease severity and its nutritional treatment [4]. As recommended, fruits and vegetables with ≤75 mg/100g showed an interesting percentage of NP intake (at least 91% of intake)[26]. We found clear differences according to PKU severity on the dietary pattern, specially in relation to animal sources. In this study, NP from high biological sources (meat, fish, eggs and cheese/ham) were less ingested compared with plant protein in

agreement with higher PKU severities. NP from animal sources has higher digestibility since plant sources present anti-nutritional factors and resistant fibres[27, 28]. This evidence may suggest advantageous in prescribing a higher amount from animal sources. However, Phe intake in PKU patients should always be prescribed according to their tolerance.

HPA and BH4 patients showed a higher percentage of TPA from NP, a consequence of milder forms of the disease, or as a consequence of pharmacological treatment [4, 22]. As shown in literature, BH4 offers an enhance intake of NP intake, so in BH4 responsive patients, Phe tolerance may increase[7]. The HPA patients had a higher NP intake in relation to BH4 group, with a more diverse and variable food pattern. More several differences were found in these patients, mainly on meat/fish and eggs food group. Additionally, Evans *et al* showed that children with PKU have higher neophobia when compared with an age matched controlled group, this supports the food refusal in patients whose diet lead to a liberalization[23, 24].

We recognize that adherence of PS is an issue although its prescription is essential to achieve protein recommendations, mainly in those with lower protein intake[8, 15]. In this study, both in mild and classical phenotypes, PS was crucial to achieve TPA, and its main source was AAM. This audit revealed that nutritional treatment is well established and according to the literature recommendation – PS at least divided in 3 meals per day [8, 10, 29]. PS intake timing may affect diurnal Phe blood variation[30]. Evidence suggest that increased number of AAM dosages per day will improve Phe tolerance[31]. However, the higher the number of meals per day with PS intake, the bigger the challenges, because PS odour and flavour may lead to lower compliance and refusal[10]. GMP, an alternative protein substitute derived from cheese

production, is a peptide that contains a small amount of Phe[4, 10]. Prescription of GMP is low and efficacy evidence is lacking[15]. Thus, further research of GMP prescription is needed as Phe content may affect the patient's metabolic control and lead to a decrease of NP intake.

This study has some limitations. Self-report data from 24h recall method is the main study limitation, dietary assessments might not correspond to real intake of patients concerning to NP and PS. Self-report compliance with dietary treatment tends to be under/overestimate[32]. A more controlled study using 3-day dietary registries would improve data accuracy in order to provide more detailed data on food intake. Despite these limitations, the fact that our sample of patients were reasonably under a good metabolic control, underlines the importance of these results for a detailed analysis of the food patterns in PKU.

V. Conclusion

In conclusion, this study showed that WHO/UNU/FAO (2007) safe levels of protein intake for every age group were clearly met or exceeded by patients with PKU. Also, the EPG recommendation was also achieved in the majority of patients studied. Our study underlines the different food pattern used in PKU patients, with important changes according to biochemical phenotype and the use of BH4. The evidence-based medicine should guide us according to the EPG, although, in clinical practice individualized nutritional treatment should be offered to distinct PKU patients with very different nutrient needs.

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