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Ingestão de micronutrientes pelos substitutos proteicos em doentes com fenilcetonúria

Micronutrient intake from protein substitutes in patients with phenylketonuria

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Abstract and Key-Words in Portuguese

Introdução: Devido à severidade das restrições dietéticas na PKU, os micronutrientes (MN) podem estar em desequilíbrio. Considerando que a maioria dos substitutos proteicos (SP) é uma fonte bem estabelecida de MN, o nosso objetivo foi analisar a % de adequação de micronutrientes (AM) pelos SP de acordo com as recomendações da EFSA. **Metodologia:** Uma amostra de 64 doentes com PKU tratados precocemente, com prescrição de SP, que completaram a avaliação anual do estado nutricional em 2018 foi estudada. A média de idades foi 20.6±9.1 (52% mulheres; 1 HPA; 38 PKU moderados; 25 PKU clássicos). 51 doentes estavam sob uma dieta pobre em Phe enquanto 13 estavam com dieta e tratamento de BH_4. Foram recolhidos o peso (kg), ingestão de SP (g de equivalente proteico (EP)/dia) e de MN pelos SP e comparados com as recomendações da EFSA. AM deficiente ou excessiva foi considerada quando a ingestão <90% ou> 110%. **Resultados:** O peso médio dos doentes foi de 54.8±19.4 kg e a mediana da ingestão de EP pelos SP 0.86 g/kg/dia (45.3±18.2 g/dia). Mais de 50% dos doentes excederam a AM para Ca, Fe, Zn, P, I, colina, vitamina A, B1, B2 e B7. Pelo menos 50% dos doentes apresentaram uma AM deficiente para Cu, Se, K, Mg, F, Mn, Mo, vitamina D, E, K, C e B12. **Discussão:** Uma vez que os SP são prescritos de acordo com as necessidades proteicas, peso, composição dos SP e severidade da doença, vai alterar a ingestão de EP e comprometer a AM. São necessários trabalhos mais robustos de ingestão nutricional na PKU de modo a melhor interpretar o estado de MN nestes doentes.

Palavras-chave: Micronutrientes, substitutos proteicos, adequação, fenilcetonúria

Abstract and Key-Words in English

Background: Due to the severe dietary restrictions used in PKU treatment, micronutrient intake may be imbalanced. Considering that most protein substitutes (PS) are a well established source of micronutrients, we aimed to analyse the micronutrient adequacy (MA) % from PS according to EFSA recommendations. **Methods:** A sample of 64 early treated PKU patients, taking prescribed PS, who completed the annual nutritional status evaluation (ANSE) in 2018 was studied. They were aged 20.6±9.1y (52% females; 1 HPA, 38 mild PKU, 25 classical PKU). 51 patients were on a low Phe diet treatment only whilst 13 were on BH_4 and diet treatment. Patient weight (kg), PS intake (protein equivalent g/day) and micronutrient intake from PS was collected and compared with EFSA micronutrient recommendations. Deficient or excessive MA was considered when intakes were <90% or >110%. **Results:** Mean weight of patients was 54.8±19.4 kg and median intake of protein equivalent from PS was 0.86 g/kg/day (45.3±18.2 g/day). More than 50% of patients exceeded the MA for Ca, Fe, Zn, P, I, choline, vitamin A, B1, B2 and B7. At least 50% of patients had a deficient MA for Cu, Se, K, Mg, F, Mn, Mo, vitamin D, E, K, C and B12. **Discussion:** Since PS is prescribed according to protein needs, age, weight, PS composition and disease severity, it will alter protein equivalent intake and compromise MA. More robust data on full dietary/nutrient intake in PKU is needed in order to better interpret micronutrient status in PKU.

Key Words: Micronutrients; phenylketonuria protein substitutes; adequacy;

List of abbreviations, initials and acronyms

AAM: L-amino acid mixture

AI: Adequate intake

ANSE: Annual Nutritional Status Evaluation

AR: Average requirement

Ca: Calcium

Cu: Copper

F: Flouride

Fe: Iron

GMP: Glycomacropeptide-based PS

I: Iodine

K: Potassium

MA: Micronutrient adequacy

Mg: Magnesium

Mn: Manganese

Mo:Molybdenum

P: Phosphorus

PAH: Phenylalanine hydroxilase enzyme

PE: Protein Equivalent

PHE: Phenylalanine

PKU: Phenylketonuria

PS: Protein substitutes

Se: Selenium

Zn: Zinc

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Introduction

Phenylketonuria (PKU) is a rare inborn error of protein metabolism with autososomal recessive transmission [1]. The global frequency of PKU is about 1 to 10000 newborn children in Europe [2] and it should ideally be screened between the 3rd and 6st day of life [3]. It is characterized by a deficient activity or inactivity of the hepatic phenylalanine hidroxylase enzyme (PAH), or a decrease activity of the co-factor tetrahydrobiopterin (BH_4) in a minority of patients [2, 4, 5]. PAH promotes the conversion of phenylalanine (Phe) into tyrosine (Tyr), in the presence of BH4 [4, 5]. When in deficit, Phe cannot be converted into Tyr increasing blood [Phe] and leading to toxicity mainly in the brain [2, 4, 5]. Diagnosis is made when blood [Phe] is higher than 3 mg/dL [6] and treatment should start as early as possible, preferably before the 10th day of life, in order to prevent neurologic damage [4]. Treatment management includes a severe restriction of natural protein and phenylalanine, supplemented with a free-Phe L-amino acid mixture (AAM) and special low protein foods in order to meet nutritional requirements [1, 2, 6]. If untreated PKU can lead to mental retardation, physical impairment, seizures and autistic-like behavior [1, 2, 4]. Other symptoms include “musty” odor (urine and body), hypopigmentation of the skin, hair, and iris, eczema and psychological health problems (depression, anxiety and agoraphobia) [1, 4]. More recently a protein substitute based on glycomacopeptide (GMP), a biopetptide extracted from cheese whey, very low in Phe, has been used as an alternative to the traditional L-amino acid supplements (MAA) [7, 8]. Additionally, a pharmacological formulation of BH_4 (sapropterin dihydrochloride; *Kuvan*®) is now available in several countries, showing an

effective impact for a sub-group of PKU patients showing to be responsive [4, 9]. It shows to be effective in reducing blood [Phe], improving [Phe] tolerance and/or providing some diet relaxation, consequently improving compliance to PKU restricted diet [9].

Protein substitutes (PS) are an essential part of PKU treatment [6] and prescribed according to, Phe tolerance, protein needs (age, gender and weight) and disease severity [4, 6]; moreover they provide a major source of micronutrients [4, 7]. PS are mainly age specific and aim to meet nutritional requirements for protein, vitamins and minerals [10]. Micronutrient composition of PS are calculated to theoretically meet nutritional requirements; however this is rarely validated by rigorous investigation [10]. Though, many adult patients following a vegan-like diet pattern that do not take full prescribed PS may be at risk of micronutrient deficiencies [4].

Micronutrients are essential for health and disease prevention, normal growth and development [5, 10, 11]. Diet is the main source of most vitamins, minerals and trace-elements and excessive or deficient ingestion of micronutrients is associated to adverse health effects [11]. Prevention of this unbalanced intake is very important for health maintenance [11]. Unfortunately, the severe restriction pattern imposed to PKU patients creates significant nutritional imbalances [10]. Micronutrient deficiencies in these patients have been reported for iron, zinc, selenium, and vitamin B12 [4, 12] as well as lower intakes of calcium, zinc, vitamin B12, folate and selenium in a group of patients with irregular AAM intake compared with a regular AAM intake of a group of patients [13]. Bioavailability of micronutrients in PKU is not well studied [10]. Taylor *et al.* had reported already in 1984 significant lower hair zinc and calcium concentrations and higher copper levels in PKU children [14].

Aims/Objectives

The aim of this study was to assess micronutrient adequacy % (MA) from PS according to the European Food Safety Authority (EFSA) micronutrient recommendations.

Methods

Participants

The study was conducted at Centro Hospitalar Universitário do Porto (CHUP), analyzing a sample of 105 PKU patients who were admitted to the Annual Nutritional Status Evaluation (ANSE) during 2018. Some patients (N=41) were excluded from the study: 6 patients were of late diagnosed and 35 did not have a PS prescribed.

The final sample was composed by 64 patients (3-36 years; 20.6 ± 9.1 years; 52% females). Patients included in this study were exclusively early diagnosed by newborn screening, and disease severity was defined according to neonatal blood [Phe], following the Portuguese Consensus for nutritional treatment of PKU [6] (Table 1).

Table 1 – Gender, Age, and Disease Severity of Patients Studied

PKU classification	Neonatal blood [Phe]	Patients				
		Adults (≥19 y)		Paediatrics (<19 y)		N Total
		Female	Male	Female	Male	
Classical PKU	≥20 mg/dL	12	7	1	5	25
Mild PKU	6-20 mg/dL	10	9	9	10	38
HFA	3-6 mg/dL	0	0	1	0	1
Mean age (y)		26,9 (± 4,6)		11,4 (± 5,2)		64

Out of the 64 patients included, 55 were prescribed an AAM, 7 on GMP and 2 on both AAM and GMP prescription. It was also accounted the number of patients under BH_4 treatment (6 paediatrics; 8 adults).

Study design

This was a cross-sectional, retrospective, descriptive and observational study (figure 1). Demographics [gender, birth date, age, PKU diagnosis, 2018 Annual Nutritional Status Evaluation (ANSE) date], anthropometry [weight, height, body mass index (BMI)], metabolic control (median blood [Phe] in the previous year before ANSE completed in 2018) and prescribed PS were collected from patient's records. Micronutrient intake from PS was assessed for each patient.

The ANSE consists of assessments including anthropometry, body composition analysis (BIA), biochemical analysis, blood pressure measurement and dietary intake. All patients are evaluated in fasting conditions.

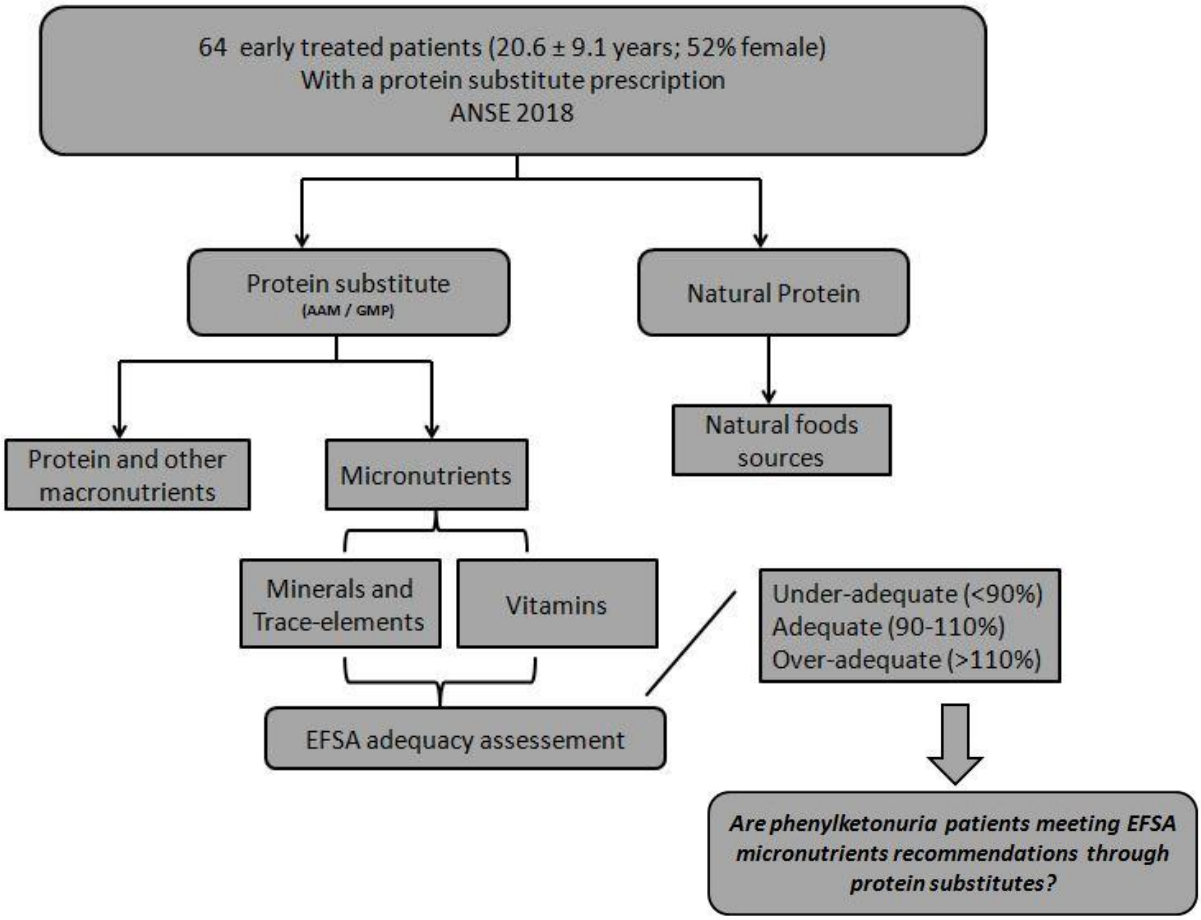

Figure 1 – Study design

To assess micronutrient adequacy (MA), recommendations of the European Food Safety Authority (EFSA) were used [15]. The adequacy cut-point established for all variables was ±10%. This cut-point was already used in studies of dietary compliance [16].

EFSA criteria selection

The "Dietary Reference Values for nutrients – Summary report"[17] of 2017 from EFSA was used to assess patient's micronutrient adequacy intake. Average Requirements (AR) defined as "the level of (nutrient) intake that is adequate for

half of the people in a population group, given a normal distribution of requirement" [17] was used to assess recommendation adequacy. When the AR was not available AI was used. The EFSA report does not present recommendations for sodium, chloride and chromium. Considering that recommendations for sodium and chloride will be only released later this year (2019) their adequacy will not be discussed on this study [17]. Also, chromium adequacy will not be analyzed since EFSA recommendations are not established for this micronutrient [17]. In addition, regarding zinc, recommendation values were presented associated with phytate intake and there were no reference for the adult population (≥18 years) for null phytate content in the diet. As PS do not contain phytate in their composition, the. Zinc recommendation used for adults was the one with the smaller value for phytate intake associated.

Vitamins B1 and B3 units were presented in mg/MJ. In order to convert to mg/day, the AR for energy (MJ/day) for different age groups and gender was used. A physical activity level (PAL) of 1.4 was established when present (due to lifestyle led by our patients) and a PAL of 1.6 used when a 1.4 was unavailable. According to the recommendations for these vitamins, calculations were made to establish mg/day values.

All micronutrient recommendations were presented using the same units proposed by EFSA. It's important to underline that EFSA recommendations were established for healthy individuals / populations and not for patients with PKU.

Data collection

Anthropometry

Data on anthropometry (weight and height) were collected from clinical records of the patients and measured by Seca® (with light clothes and without shoes) (accuracy 0,1Kg) and stadiometer, respectively. BMI and its z-scores were calculated by Anthro® and Anthroplus® programmes and classified according to the World Health Organization (WHO) criteria[18-20].

Nutritional Intake

Data on prescribed amount of PS and their protein equivalent (PE) were collected from the clinical records of each patient. Micronutrient intakes from PS were identified and compared with EFSA recommendations.[17] The micronutrients analysed were: calcium (mg/d), iron (mg/d), zinc (mg/d), copper (µg/d), selenium (µg/d), potassium (g/d), phosphorus (mg), magnesium (mg), iodine (µg), fluoride (mg), manganese (mg), molybdenum (µg), choline (µg), fat-soluble vitamins [A (µg/d), D (µg/d), E (mg/d), K (µg/d)] vitamin C (mg/d) and complex B vitamins [B_1 - thiamin (mg/d), B_2 - riboflavin (mg/d), B_3 - niacin (mg/d), B_5 - pantothenic acid (mg/d), B_6 - pyridoxine (mg/d), B_7 - biotin (µg/d), B_9 - folic acid (µg/d) and B_{12} - cobalamin (µg/d)].

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 obtained for participants and parents/caregivers of peadiatric patients included in this study.

Statistical Analysis

The IBM SPSS Statistics 25 for Windows was used for statistical analysis. In order to verify the variables normality distribution, the Kurtosis and Skewness tests were used and mean ± SD or median [P25-75] were calculated for continuous variables, according to distribution. Statistical significance was found when $p < 0,05$.

Results

Micronutrient intake from PS (AAM and GMP) was analysed for 26 micronutrients (4 minerals; 8 trace-elements; 15 vitamins). Mean weight of patients was 54.8±19.4 kg (41.0±18.4 kg for children/adolescents; 55.0±19.7 kg for adults) and median intake of PE from PS was 0.86 g/kg/day (0.89, [0.77-1.10] g/kg/day for children/adolescents; 0.84, [0.72-1.00] g/kg/day for adults), with a mean intake of 45.3±18.2 g/day for both groups. BMI z-score in the pediatric group (<19 y) varied between -2.6 and 3.3 kg/m^2 (-0.1 ± 1.2 kg/m^2) and in the adult group (≥19 y) BMI varied between 18.1 and 33.4 kg/m^2 (24.2 ± 3.9 kg/m^2). Out of the 64 patients (26 children/adolescents, 38 adults) more than 50% exceeded the micronutrient adequacy for Ca, Fe, Zn, P, I, choline, vitamin A, B1, B2 and B7 and at least 50% of patients had an under adequate micronutrient intake for Cu, Se, K, Mg, F, Mn, Mo, vitamin D, E, K, C and B12 (Table 2).

Table 2 – Patient's overall under-adequacy, adequacy and over-adequacy

Micronutrient	Under-adequate		Adequate		Over-adequate	
	(N)	(%)	(N)	(%)	(N)	(%)
Calcium	10	16%	12	19%	42	**66%**
Iron	6	9%	3	5%	55	**86%**
Zinc	9	14%	7	11%	48	**75%**
Copper	52	**81%**	6	9%	6	9%
Selenium	41	**64%**	13	20%	10	16%
Potassium	64	**100%**	0	0%	0	0%
Phosphorus	14	22%	13	20%	37	**58%**
Magnesium	36	**56%**	21	33%	7	11%
Iodine	12	19%	10	16%	42	**66%**
Fluoride	64	**100%**	0	0%	0	0%
Manganese	54	**84%**	3	5%	7	11%
Molybdenum	43	**67%**	6	9%	15	23%
Choline	17	27%	9	14%	38	**59%**
Vitamin A	20	31%	7	11%	37	**58%**
Vitamin D	42	**66%**	7	11%	15	23%
Vitamin E	37	**58%**	15	23%	12	19%
Vitamin K	32	**50%**	9	14%	23	36%
Vitamin C	32	**50%**	14	22%	18	28%
Vitamin B1	7	11%	3	5%	54	**84%**
Vitamin B2	20	31%	9	14%	35	**55%**
Vitamin B3	22	34%	11	17%	31	48%
Vitamin B5	22	34%	14	22%	28	44%
Vitamin B6	25	39%	8	13%	31	48%
Vitamin B7	17	27%	8	13%	39	**61%**
Vitamin B9	26	41%	10	16%	28	44%
Vitamin B12	41	**54%**	16	25%	7	11%

Out of the 26 paediatric patients, 7 had under-adequacy for Ca (4 under BH4 therapy), 4 for Fe (3 under BH4 therapy), 7 for Zn (4 under BH4 therapy), 16 for Se (5 under BH4 therapy), and 22 for B12 (5 under BH4 therapy). Over adequacy for Zn was seen in 14 patients, 13 patients for vitamin A and in 15 patients for vitamin B9. In adults, of 38 patients, 34 had over adequacy for Zn, 30 for Ca and 24 for vitamin A. Eleven women of child bearing age (all with an over-adequacy above 400µg/d) also showed an over-adequacy for vitamin B9. Regarding under adequacy in adults, 3 presented under-adequacy for Ca (all under BH4 therapy), 2

for Fe under BH4 therapy), 25 for Se (8 under BH4 therapy) and 19 for B12 (7 under BH4 therapy).

Mean/median MA for age groups is presented on figures 2-5 (Attachment A). In paediatric patients, P, Mg, Se, F, Mn, vitamins D, E, K, B3, B9 and B12 mean/median MA were found to be lower (<90%) while the opposite was found for Fe, Zn Cu, I, vitamins A choline and B1 for which an over MA was found (>110%). In adult patients, P, Cu, Se, F, Mn, Mo, vitamins E, C and B12 mean/median MA were found to be lower (<90%) compared with Ca, P, Fe, Zn, I, vitamins A, choline, B1, B2, B3, B6, B7 and B9 for which an over MA was found (>110 %). None of the patients showed a perfect MA (between 90 and 110%) for all analysed micronutrients. Patients under BH_4 treatment seem to have an overall lower MA comparing with non-BH4 treated patients (data not shown).

Discussion

In this study we identified that overall MA from PS was very different between micronutrients, age groups and gender. Paediatric patients had a higher intake of PS in kg/day than adults, however we assessed that generally, adults present more over-adequacy for micronutrients comparing to children. In children, under-adequacy of micronutrients was more frequent in children/adolescents than in adults. This is concerning as an adequate micronutrient intake is critical for children development [5], additionally adults often relax their PS intake to less than the amount prescribed [13], as a result, micronutrient intake is lower jeopardizing nutritional status in these patients. In that way, careful nutritional management is mandatory to guarantee that natural food choices prescribed in addition to PS will help meet micronutrient needs.

Optimal mineral and vitamin supplementation is not well defined in PKU and there is a lack of information about criteria used for PS formulation [12, 21]. Micronutrients are added to PS accordingly to guidelines of the *Foods for Special Purposes Directive* (European Commission Directive number 1999/21/EC and amended by Directive 2006/141/EC) that stipulates minimum and maximum levels of vitamins and minerals per 100 Kcal [10] meaning that vitamins and minerals are added to PS according to energy content and not protein equivalent.

Under-adequacy for Ca in paediatric patients was observed, which can be challenging. Ca adequacy is very important during childhood and adolescence in order to guarantee bone mass acquisition [5]. Studies conducted in this population have reported inadequate bone mass and low bone mineral density in PKU patients and higher incidence of osteoporosis and osteopenia in the future comparing with healthy population [4, 22]. Although the precise etiology of the reduced bone mineral density is not defined in PKU, adequacy of bone nutrients seems obvious.

Over-adequacy for iron is concerning and highly present in both paediatrics and adults. Excessive iron intake promotes Fenton chemistry generating reactive oxygen species causing damage in various organs with high oxidative metabolism [23]. However, the over-adequacy for this trace-element might be due to the fact that the Fe form added to PS is ferrous sulfate of which only 3 to 5% is normally absorbed [24] and so increased amounts of non-heme iron might be added to PS in order to tackle this. Paediatric patients presenting under-adequacy for Fe is distressing as well, as iron needs increase during high growth period, characteristic of paediatric individuals, particularly adolescents [5]. However there

is evidence that the prevalence of anaemia in PKU patients is similar to the general population [12].

It was verified that our patients presented great over-adequacy for Zn (especially adults), comparing to decrease serum levels of this trace-mineral observed in many patients (data not shown). This has been seen in other studies [25, 26]. The chemical form of zinc added to PS is less available than Zn naturally present in foods leading to low serum levels [25] although high intakes of this trace-element from PS are observed. Phytate content of PKU diet, rich in fruits and vegetables has also serious impact on Zn absorption [5] which can impair zinc absorption and consequently lead to decreased serum levels. This might present a reason for the over-adequacy for this trace-element presented by our patients, probably as a result of increased amounts in the formulations to anticipate lower absorption rates.

Regarding Se, in contrast to what we observed, Evans *et al.* reported high levels of intake by their patients [25]. Our study does not confirm these findings as both patient groups presented under-adequacy for this trace-element. However Evans *et al.* gave patients higher doses and different PS which might underline these differences. Se forms added to PS are inorganic and present less-availability than Se in natural food sources, especially high-protein foods, that present a more bioavailable source of Se, seleno-cysteine [5, 12, 25]. This is worrying as our patient's diet already lacks many food sources of high bioavailable Se and the presence of small quantities of this trace-mineral in PS may not permit the full satisfaction of nutrient needs. Additionally, there is evidence of increased oxidative stress in PKU patients with selenium deficiency [27]. However, Sitta *et al.* verified in their study that patients with normal Se levels presented a decreased glutathione peroxidade

activity in two PKU treated groups with different blood Phe control indicating that activity of this enzyme was not related to blood Phe levels in these patients [27].

For vitamin A, our patients had higher intakes from PS than recommended. There is evidence that increased intake of vitamin A can impair bone mineral content and result in increased risk of fracture [28, 29]. Studies with animal models documented hypervitaminosis A associated with bone resorption, hypercalcaemia and bone abnormalities [28]. Sprout *et al.* finds prudent to supplement PS with provitamin A rather than preformed vitamin A given the lack of research in this vitamin field [30].

High levels of folate intake in PKU patients have been reported *[12, 21, 30]* corroborating our findings in PKU adult patients. Andrade *et al.* found altered methylation capacity in PKU patients compared to controls, attributing this to high intake of folic acid and vitamin B12 [26]. There are also studies linking folate intake to cancer risk as folate takes part of replication, repair and methilation of DNA [12, 26]. Robert *et al.* suggest that an intake of 400 µg/day of folic acid from PS should not be toxic [12], however we've seen that 11 of our child bearing age female patients present levels above this threshold. This may be challenging especially if the standard procedure of extra supplementation with folic acid is followed in women known to be pregnant. Sprout *et al.* even highlights that excessive folic acid intake from PS may be considered as a risk for neuropathological symptoms promoting in PKU [30]. In addition, high folic acid intakes can also conceal vitamin B12 pernicious anaemia diagnosis [12] leaving patients eventually more exposed to additional health imbalances.

In this study patients had low intakes of or vitamin B12, for both age groups. Due to the restrictive diet there is a greater risk of functional vitamin B12 deficiency, so

that homocysteine and plama methylmalonic acid could be useful markers [31]. Vitamin B12 deficiency appears to be common in PKU patients with several authors reporting low vitamin B12 status and deficiency [32]. This is distressing as patients usually follow a food pattern that compromises their ability to obtain this vitamin from foods since it is only present in animal origin foods. It would be expected that formulas were highly supplemented in vitamin B12, however according to EFSA recommendations, supplementation seems to be low. Clinical symptoms for vitamin B12 deficiency have been reported in patients who have relaxed their diet and stopped PS intake and in those who choose to follow a vegan pattern [12, 32].

For BH_4 treated patients, Lambruschini *et al.* found no significantly differences between micronutrients serum in patients treated with sapropterin during a year's time, except for selenium [33]. BH_4 patients can relax their diet due to increased Phe tolerance or improved metabolic control [9]. They may be at increased risk for micronutrient deficiency, if not adequately supervised regarding their nutritional intake. Specific formulations of PS for BH_4 patients would be advised in order tackle this problematic as reported by MacDonald *et al.* [34].

In spite of our findings, there were various limitations in our study. A controlled study probably would have provided more conclusive findings as well as a longitudinal and prospective study. PS prescription was collected from patients dietary records upon ANSE, which can create bias as patients may not give an accurate report on PS intake. As for cut-off point for adequacy assessment, it was establishment based on studies on dietary compliance, as EFSA does not define one. For future studies, it would be advantageous to assess global micronutrient intake from diet resourcing to more rigorous methods to evaluate intake (a three

day food journal for example) and compare it with patient's biochemical analysis. More detailed studies on the bioavailability of micronutrients added to PS would also improve knowledge in this area. Moreover, it is still yet to be known if PKU alters nutritional needs of individuals, and if that so, if there is the need to alter recommendations as it happens to protein. Larger studies could also be conducted as well as more impact studies of micronutrients in PKU. More information about how companies formulate the micronutrient content of their PS would also be of advantage.

Conclusions

MA differed greatly within our patients. PS dosage varies according protein requirements, age, gender and weight, PS composition and disease severity, compromising micronutrient adequacy. As so, micronutrient intake is, in some way, hostage to other factors and not considerate a main aspect in PKU patients. Recently, more studies have been developed in order to understand MA and necessities in PKU patients; however more robust data is needed to understand this complex problematic and better interpret micronutrient status in PKU.

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Attachement A– Figures 2 to 5

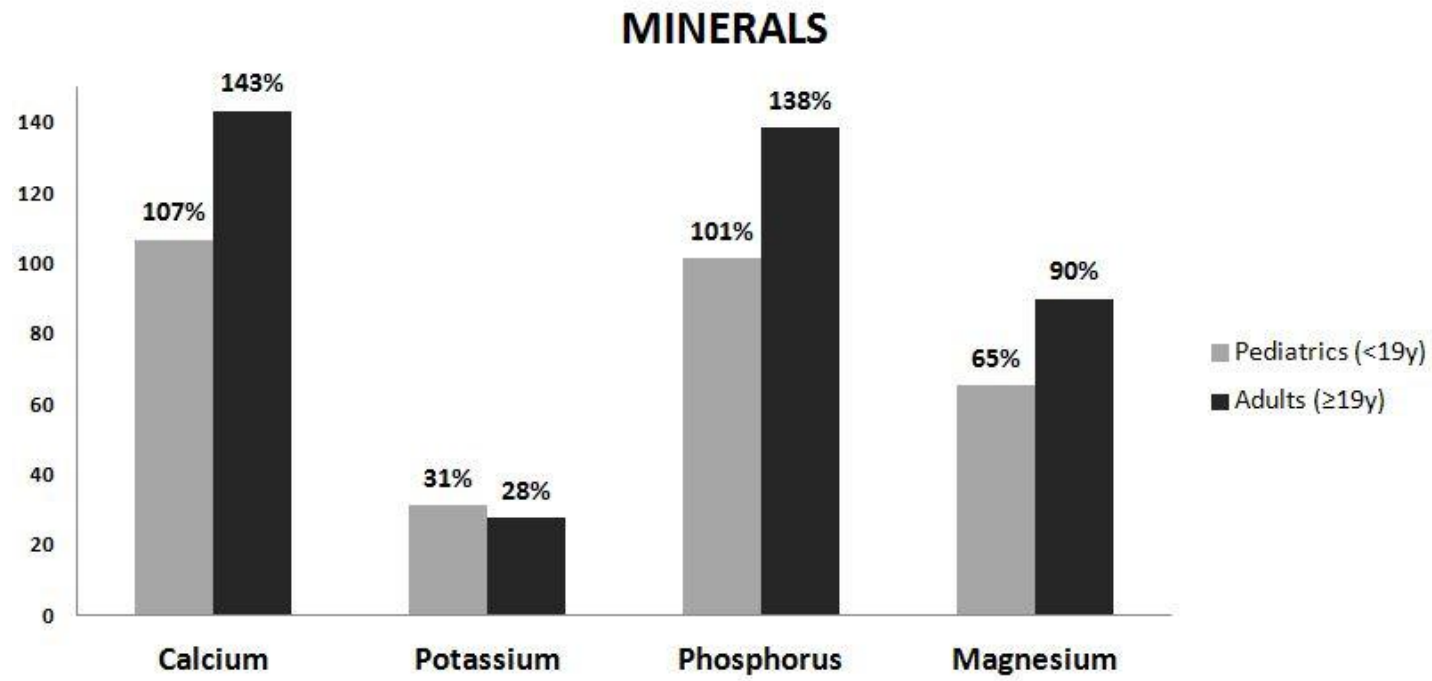

Figure 2 – Mineral adequacy for paediatric and adult patients

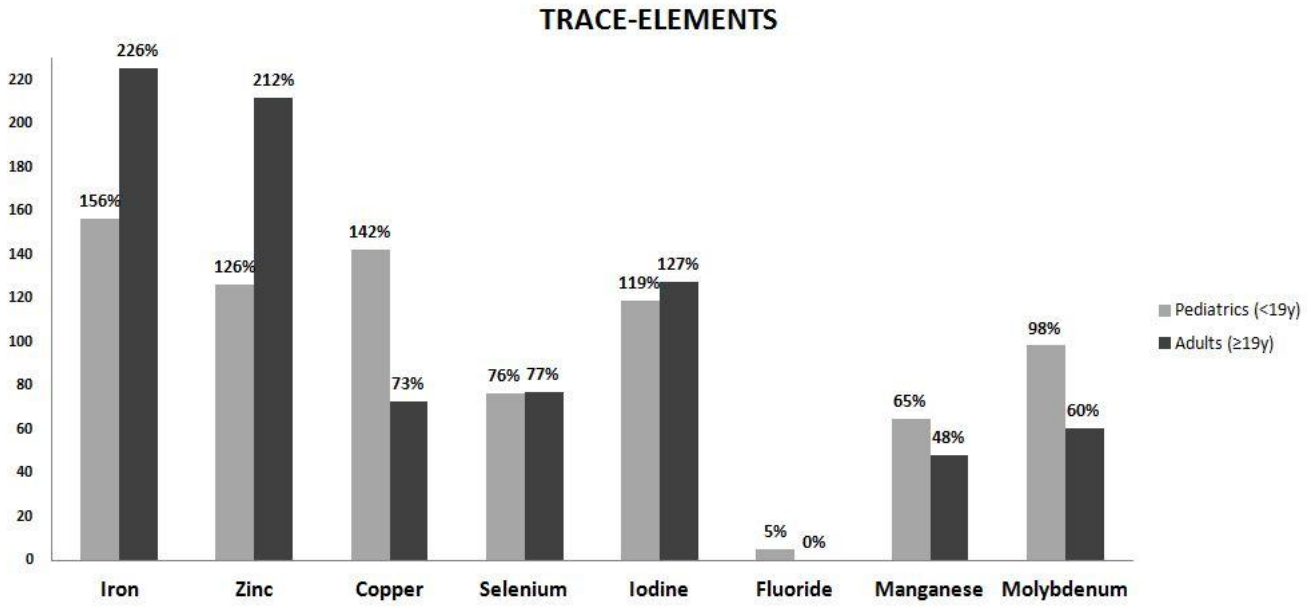

Figure 3 – Trace-Elements adequacy for paediatric and adult patients

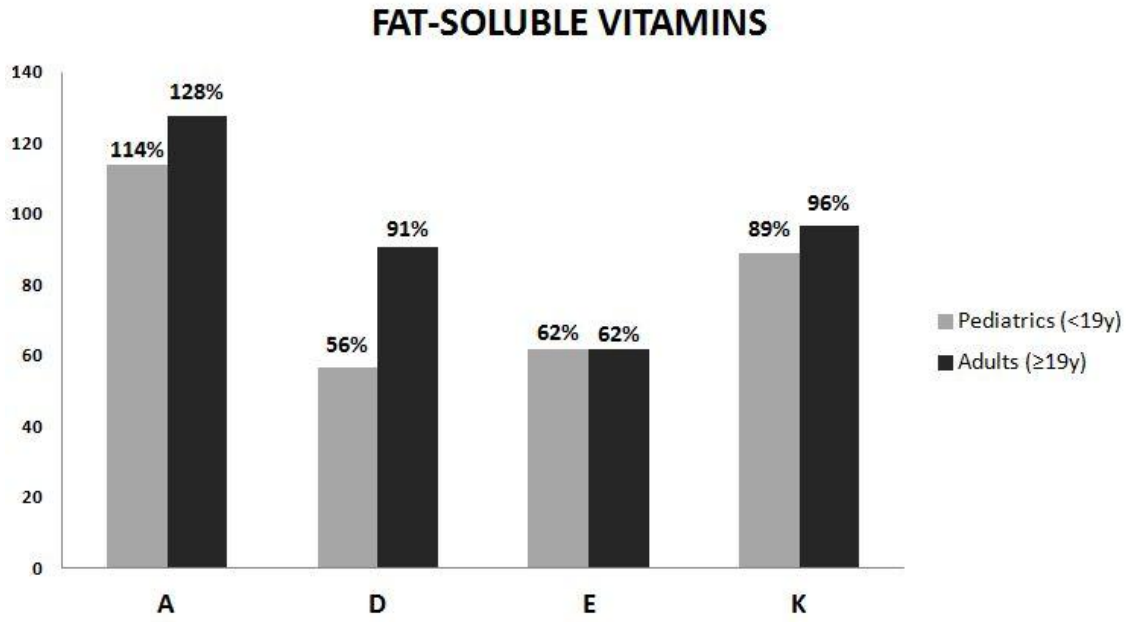

Figure 4 – Fat-Soluble vitamins adequacy for paediatric and adult patients

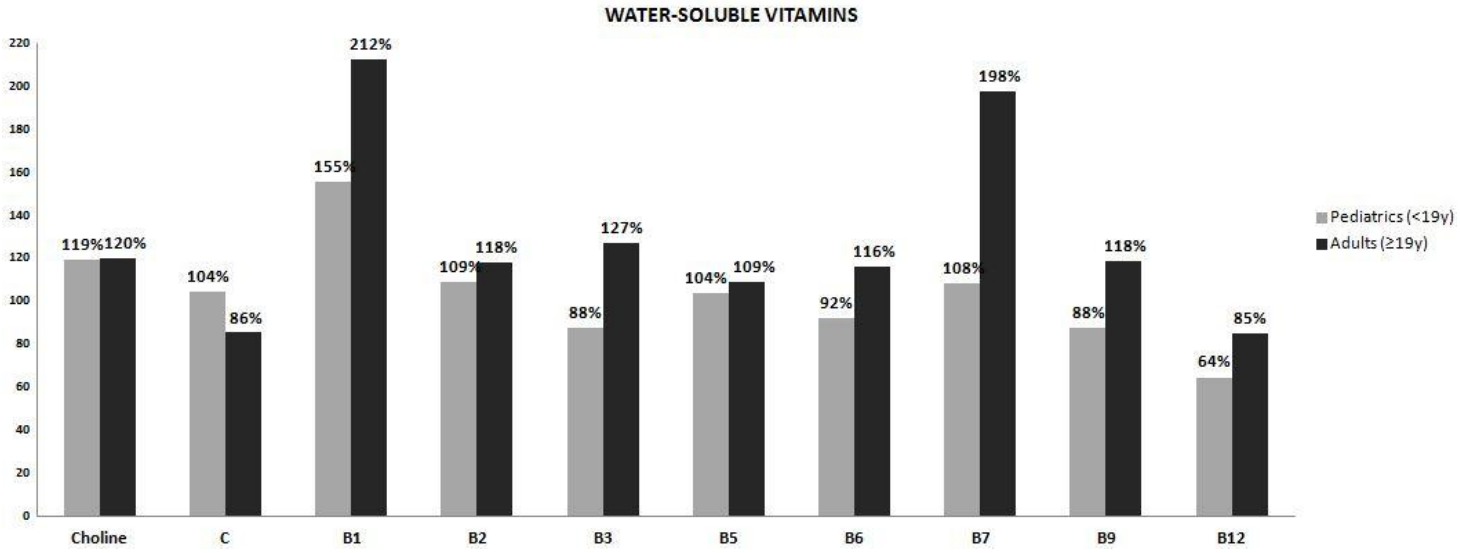

Figure 5 – Water-Soluble vitamins adequacy for paediatric and adult patients