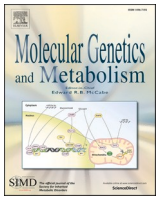
Molecular Genetics and Metabolism 144 (2025) 109068

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme



Research Paper

Protein intake and prevalence of overweight in patients with phenylketonuria: A 10-year longitudinal study

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ARTICLE INFO

Keywords:
Natural protein
Overweight
Phenylalanine
Phenylketonuria
Protein intake

ABSTRACT

Background: Overweight has been identified as a comorbidity associated with phenylketonuria (PKU). A systematic review with meta-analysis found that although patients with PKU had a similar body mass index (BMI) when compared to non-PKU controls, there was a significantly higher BMI when measured in patients described as having classical PKU.
Objective: The aim of this retrospective longitudinal study was to identify the prevalence of overweight in patients with PKU, over 10 years, in a Portuguese Reference Centre, following a phenylalanine-restricted diet.
Methods: Inclusion criteria were diagnosis of PKU and completion of an annual nutritional status evaluation every 2 years. Information on anthropometry, dietary intake and blood phenylalanine levels was collected.
Results: The sample consisted of 94 patients (aged 14.0 ± 7.8y, 46 females). Over the study period, there was a non-statistically significant trend towards an increase in the prevalence of overweight (24.5 vs 33.0 %), as defined by age-appropriate BMI. When compared with normal-weight patients, overweight patients had significantly higher blood phenylalanine levels in the first and fifth biennium. Total and natural protein intake were significantly higher in normal-weight patients, at all timepoints, compared to overweight patients. Univariate analysis showed that a higher protein intake, particularly of natural protein, is a protective factor against the development of overweight. This result remained after adjusting total protein intake for age, gender, and metabolic control.
Conclusions: This study found a trend towards an increase in the prevalence of overweight in patients with PKU. Therefore, the nutritional status of patients with PKU should be regularly monitored, supported by preventive and attentive nutritional support.

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https://doi.org/10.1016/j.ymgme.2025.109068
Received 23 December 2024; Received in revised form 19 February 2025; Accepted 21 February 2025
Available online 23 February 2025

1. Introduction

The World Health Organization (WHO) defines overweight and obesity as abnormal or excessive fat accumulation that presents a health risk [1]. Body mass index (BMI) is commonly used as an indirect marker of body fat, and is defined as weight in kilograms divided by the square of height in meters (kg/m^2). An adult with a BMI above 25.0 kg/m^2 is considered to be overweight, and higher than 30.0 kg/m^2 to be obese [1]. In paediatrics, BMI must be compared to a reference population. Children aged 5 to 19 years are considered overweight and obese when their BMI-for-age is greater than one or two standard deviations (SD) above the WHO Growth Reference median, respectively [2]. In turn, in children under 5 years of age, overweight and obesity are defined by two or three SD above the WHO Child Growth Standards median, respectively [3].

The fundamental cause of overweight is an energy imbalance between the calories consumed and the calories expended. However, changes in dietary and physical activity patterns result from the complex interaction between several biological, and environmental and social factors, such as genetics, gut microbiota, and socioeconomic status [4]. Globally, the prevalence of obesity has nearly tripled since 1975. In 2016, 39 % of adults worldwide were overweight and 13 % were obese. In the same year, the global prevalence of overweight in children and adolescents (aged 5 to 19 years) was 18 %, with 6 % of girls and 8 % of boys being obese [5]. This represents a major public health issue, as overweight is known to increase the risk of type 2 diabetes mellitus, fatty liver disease, cardiovascular diseases, Alzheimer's disease, depression, cancer, and other diseases [6].

Phenylketonuria (PKU) is an inborn error of phenylalanine (Phe) metabolism caused by a deficiency of the enzyme phenylalanine hydroxylase (PAH). Lower residual activity of the enzyme leads to higher Phe concentrations in the blood and a more severe form of the disease [7]. The mainstay of PKU treatment is a diet restricted in natural protein that limits intake of dietary Phe to the amount that maintain blood Phe within target range. Phe-free/Low-Phe protein substitutes are used to meet the majority of requirements, and special low-protein foods (SLPFs) contribute to energy needs as well as dietary variety and satiety [8]. Depending on disease severity, protein substitutes will provide 52 to 80 % of total protein intake, and SLPFs around 50 % of the total energy intake [9,10].

Tetrahydrobiopterin a cofactor for PAH is a synthetic form of BH4 that functions as a pharmaceutical chaperone. Around 25 to 40 % of patients are BH4 responsive and are able to either follow a less restrictive diet, allowing a higher dietary Phe intake or they may have improved blood Phe control [8].

Overweight is recognised as a comorbidity associated with PKU. The first reports of overweight in PKU were in the late 1970s in children from the USA, after relaxation of dietary Phe restriction [11,12]. This was followed by several studies reporting a higher prevalence of overweight and a higher BMI in PKU compared to the general population, particularly in females [13–17]. Conversely, other studies have found no differences between patients with PKU and healthy individuals regarding BMI and the prevalence of overweight [18–20]. A recent systematic review with meta-analysis [21] found that patients with PKU had a similar BMI when compared to non-PKU controls. Nevertheless, the subgroup of patients with classical PKU had a significantly higher BMI. These results highlight the need for lifelong follow-up by a multidisciplinary team, with systematic and methodical nutritional status monitoring, and personalized nutritional counselling [22].

The aim of this 10-year longitudinal study was to identify the prevalence of overweight in patients with PKU, followed in a single Portuguese Reference Centre, between 2009 and 2018.

2. Methods

2.1. Study design and participants

A retrospective, longitudinal, observational study was performed with patients with PKU followed exclusively at Unidade Local de Saúde de Santo António (ULSSA) - Reference Centre for the treatment of Inherited Metabolic Diseases.

Inclusion criteria were diagnosis of PKU, following a Phe-restricted diet, and completion of at least one annual nutritional status evaluation, every 2 years (biennium), from 2009 to 2018. Patients taking BH4 as an adjunct therapy were also included. BH4 responsiveness was assessed by comparing blood Phe levels before and after a single daily dose of sapropterin (20 mg/kg/day) on two consecutive days. When patients responded to BH4 (defined as a 30 % reduction in blood Phe levels), the BH4 dose was adjusted according to their metabolic control [23]. All patients who had any other medical condition that affected their dietary Phe-restriction or nutritional status were excluded.

Patients with PKU were classified according to their blood Phe concentration at the newborn screening test, according to Portuguese recommendations [24]: hyperphenylalaninemia (HPA) (blood Phe $<$ 360 μmol/L), mild PKU (blood Phe between 360 and 1200 μmol/L) and classical PKU (blood Phe $>$ 1200 μmol/L). This classification describes severity of deficiency of PAH activity respectively, with "classical" patients having the least PAH activity and therefore the least tolerance of dietary Phe. Patients were considered as paediatrics or adult when their age in the last biennium was less than or greater than 19 years, respectively.

2.2. Anthropometry

Weight and height were measured with participants wearing light clothing, and no shoes or accessories. Body weight was measured using a Seca mechanic weight scale (with an accuracy of 0.5 kg), and height was measured with a stadiometer (to the nearest 1 mm), according to standard techniques [25]. Anthro (Version 3.2.2) and Anthro Plus (Version 1.0.4) software were used to calculate BMI z-scores for patients under 19 years, which were interpreted according to WHO growth charts and its guidelines [2,3].

2.3. Dietary intake

All patients were followed up by the same two nutritionists during the study period. Natural protein (g/kg/day), protein equivalent from protein substitute (g/kg/day), total protein (g/kg/day) and Phe intake (mg/day) were assessed using a 24 h dietary-recall. This method of dietary assessment consisted of interviewing the patient about their food consumption on the previous day. During the first 3 bienniums, all patients were exclusively treated on a Phe-restricted diet only. In the fourth biennium 32 patients started treatment with BH4 in combination with dietary Phe restriction, and in the fifth biennium 19 continued with BH4 treatment.

2.4. Metabolic control

Blood Phe concentrations were measured by fasting filter paper blood spots and analysed by tandem mass spectrometry. The median blood Phe level in the year preceding the annual nutritional status evaluation was used to define the metabolic control of patients. During the study period, for the whole group of patients, the median number of blood Phe dried spots analysed varied between 12 in 2009 and 28 in 2015, with a total number of measurements of 19,104 during the 10-year study. According to Portuguese Consensus, good blood Phe control was defined as $\leq$360 or $\leq$ 480 μmol/L, for patients younger or older than 12 years, respectively [24].

2.5. Statistics

Categorical variables were described through absolute (n) and relative (%) frequencies, while continuous variables were described as mean and standard deviation, or median, interquartile (IQR) range, and minimum and maximum, when appropriate.

When testing a hypothesis about continuous variables, T–Test for independent sample or Mann–Whitney test were used as appropriate, considering normality assumptions and the number of groups compared. When testing a hypothesis about categorical variables, a chi-square test and Fisher's exac *t*-test were used, as appropriate.

To have a more thorough understanding of the factors associated with the overweight univariate and multivariate logistic regression modelling was used. Model goodness-of-fit was assessed using the Hosmer–Lemeshow statistic and discriminative power was evaluated by receiver-operator curve (ROC) curve analysis.

The significance level used was 0.05. Statistical analysis was performed using IBM® SPSS® Statistics v26.0.

2.6. Ethics

The present research and data collection were approved by the ethics committee of Centro Hospitalar Universitário do Porto, on November 7th, 2018, to the investigation project "Trends in Nutritional Status of patients with Phenylketonuria (TNSPKU)", with the reference 2015.101 (092-DEFI/087-CES). Written informed consent was obtained from each participant or caregiver.

3. Results

All patients who met the inclusion criteria were invited to participate in the study. The sample included 94 patients with PKU with mean age of 14.0 ± 7.8 years, and an age range of 3 to 38 years. Twenty-four were adults, 46 were females and 48 were males. Eighteen patients had hyperphenylalaninemia (HPA), 43 mild PKU, and 28 classical PKU. Five patients were described as late diagnosed. All patients were followed for a period of 10 years. Clinical characteristics and nutritional intake of the patients are summarized in Tables 1 and 2. Over the 10 years of follow-up, a trend towards an increase in the prevalence of overweight (24.5 % in the first biennium vs 33.0 % in the fifth biennium), was observed although not statistically significant ($p = 0.197$). At all time-points, females had a higher prevalence of overweight when compared to males, but without statistical significance.

Over the study period, there was a deterioration in the patients' metabolic control, as evidenced by their annual blood Phe levels of 323.3 (220.4–510.3) vs 429.8 (297.2–612.6) μmol/L ($p < 0.001$) and by the decrease in the percentage of patients with good metabolic control (68.1 % vs 46.2 %, $p = 0.030$). There was an increase in the daily intake of Phe of 800.7 ± 486.2 to 2434.6 ± 1778.1 mg/day ($p < 0.001$), which might be related to the initiation of BH4 therapy from the fourth biennium onwards. Natural protein intake increased over the study period, while daily protein equivalent intake from protein substitutes decreased. Most patients were taking Phe-free amino acid (L-AAs) supplements, and by the end of the study 28 patients had stopped taking all protein substitute.

Table 3 provides a comparison between patients with normal weight and patients who were overweight. There were no differences between normal-weight and overweight patients regarding age and height,

Table 2
Dietary intake of patients with PKU.

	1st biennium	2nd biennium	3rd biennium	4th biennium	5th biennium
Natural Protein (g/kg/day)	0.62 (0.51)	0.71 (0.52)	0.73 (0.62)	0.88 (0.69)	0.87 (0.64)
Protein Equivalent (g/kg/day)	1.08 (0.40)	1.05 (0.39)	0.87 (0.32)	0.66 (0.41)	0.56 (0.43)
Total Protein (g/kg/day)	1.70 (0.63)	1.76 (0.58)	1.61 (0.50)	1.54 (0.48)	1.42 (0.41)
Type of PS n (%)					
L-AAs	91 (96.8)	91 (96.8)	88 (93.6)	70 (74.5)	50 (53.2)
CGMP	0 (0.0)	0 (0.0)	0 (0.0)	3 (3.2)	9 (9.6)
L-AAs + CGMP	0 (0.0)	0 (0.0)	1 (1.1)	6 (6.4)	7 (7.5)
LNAA	0 (0.0)	1 (1.1)	3 (3.2)	0 (0.0)	0 (0.0)
No use of PS	3 (3.2)	2 (2.1)	2 (2.1)	15 (16.0)	28 (29.8)
Dietary Phe (mg/day)	800.7 (486.2)	1103.5 (797.5)	1461.5 (1126.5)	2243.4 (1579.3)	2434.6 (1778.1)
Sapropterin treatment	0	0	0	32	19

Data are presented as mean (SD). CGMP, Casein Glycomacropeptide; L-AAs, Phenylalanine Free Amino Acids; LNAA, Large Neutral Amino Acids; PS, Protein Substitutes; Phe, Phenylalanine.

Table 1
Clinical characteristics of patients with PKU.

	1st biennium	2nd biennium	3rd biennium	4th biennium	5th biennium
Age (y)	14 (7)	16 (8)	18 (8)	20 (8)	22 (8)
Height (cm)	146 (21)	151 (18)	157 (14)	161 (11)	164 (9)
Body mass index (kg/m^2)	19.7 (4.1)	20.4 (4.4)	21.6 (4.6)	22.5 (4.8)	23.3 (4.6)
Prevalence of overweight n (%)	23 (24.5)	28 (29.8)	30 (31.9)	30 (31.9)	31 (33.0)
By gender:					
Females ($n = 46$) n (%)	13 (28.3)	17 (37.0)	16 (34.8)	17 (37.0)	17 (37.0)
Males ($n = 48$) n (%)	10 (20.8)	11 (22.9)	14 (29.2)	13 (27.1)	14 (29.2)
By age *:					
< 19y ($n = 25$) n (%)	3 (12.0)	7 (28.0)	8 (32.0)	7 (28.0)	3 (12.0)
≥ 19y ($n = 69$) n (%)	20 (29.0)	21 (30.4)	22 (31.9)	23 (33.3)	28 (40.6)
By disease severity:					
HPA ($n = 18$) n (%)	2 (11.1)	6 (33.3)	7 (38.9)	5 (27.8)	4 (22.2)
Mild PKU ($n = 43$) n (%)	10 (23.3)	11 (25.6)	11 (25.6)	16 (37.2)	14 (32.6)
Classical PKU ($n = 28$) n (%)	7 (25.0)	7 (25.0)	8 (28.6)	6 (21.4)	9 (32.1)
Late Diagnosed (n = 5) n (%)	4 (80.0)	4 (80.0)	4 (80.0)	3 (60.0)	4 (80.0)
By metabolic control:					
Good n (%)	14 (21.9)	18 (28.1)	17 (29.3)	10 (24.2)	8 (18.6)
Poor n (%)	9 (30.0)	10 (33.3)	13 (36.1)	20 (38.5)	22 (44.0)
Annual blood Phe (μmol/L)	323.3 [220.4–510.3]	305.1 [217.3–525.5]	383.8 [240.3–506.7]	429.8 [280.3–578.1]	429.8 [297.2–612.6]
Good metabolic control n (%) **	64 (68.1)	64 (68.1)	58 (61.7)	41 (44.1)	43 (46.2)

Data are presented as mean (SD) or median [P25-P75]. *Age at the end of 10 years of follow-up. ** Percentage of patients with blood Phe levels within the target range, according to the Portuguese Consensus cut-off points. HPA, Hyperphenylalaninemia; Phe, Phenylalanine; PKU, Phenylketonuria.

Table 3
Comparison of clinical characteristics and nutritional intake between patients with normal weight and overweight.

	1st biennium			2nd biennium			3rd biennium		
	NW ($n = 71$)	OVW ($n = 23$)	P^1	NW ($n = 66$)	OVW ($n = 28$)	P^1	NW ($n = 64$)	OVW ($n = 30$)	P^1
Age (y)	13 (7)	17 (10)	0.054	16 (7)	17 (9)	0.855	18 ± 7	19 ± 10	0.817
Height (cm)	143 (22)	153 (14)	0.133	150 (19)	153 (14)	0.833	156 ± 15	157 ± 12	0.987
Disease severity:									
HPA n (%)	16 (22.5)	2 (8.7)		12 (18.2)	6 (21.4)		11 (17.2)	7 (23.3)	
Mild PKU n (%)	33 (46.5)	10 (43.5)	0.017[3]	32 (48.5)	11 (39.3)	0.079[3]	32 (50.0)	11 (36.7)	0.082[3]
Classical PKU n (%)	21 (29.6)	7 (30.4)		21 (31.8)	7 (25.0)		20 (31.3)	8 (26.7)	
Late Diagnosed	1 (1.4)	4 (17.4)		1 (1.5)	4 (14.3)		1 (1.6)	4 (13.3)	
Annual blood Phe (μmol/L)	281.5 [214.9–477.6]	467.3 [317.8–748.2]	0.026[2]	308.7 [224.0–509.1]	305.1 [210.7–535.1]	0.933[2]	378.4 [236.7–497.0]	422.5 [244.0–529.7]	0.789[2]
Good metabolic control n (%)*	50 (70.4)	14 (60.9)	0.393	46 (69.7)	18 (64.3)	0.607	41 (64.1)	17 (56.7)	0.492
Natural Protein (g/kg/day)	0.71 (0.55)	0.35 (0.12)	<0.001	0.81 (0.56)	0.47 (0.31)	<0.001	0.83 (0.69)	0.53 (0.34)	0.011
Protein Equivalent (g/kg/day)	1.12 (0.44)	0.96 (0.22)	0.040	1.11 (0.42)	0.93 (0.25)	0.020	0.90 (0.36)	0.81 (0.24)	0.032
Total Protein (g/kg/day)	1.83 (0.66)	1.31 (0.28)	<0.001	1.91 (0.61)	1.41 (0.25)	<0.001	1.73 (0.52)	1.34 (0.34)	<0.001
Dietary Phe (mg/day)	828.8 (538.5)	714.1 (257.4)	0.702	1177.2 (901.3)	929.8 (436.7)	0.397	1517.2 (1163.9)	1342.6 (1051.4)	0.465

	4th biennium			5th biennium		
	NW ($n = 64$)	OVW ($n = 30$)	P^1	NW ($n = 63$)	OVW ($n = 31$)	P^1
Age (y)	20 ± 7	21 ± 9	0.767	21 ± 7	25 ± 9	0.024
Height (cm)	161 ± 12	161 ± 10	0.798	164 ± 10	164 ± 8	0.705
Disease severity:						
HPA n (%)	13 (20.3)	5 (16.7)		14 (22.2)	4 (12.9)	
Mild PKU n (%)	27 (42.2)	16 (53.3)	0.265[3]	29 (46.0)	14 (45.2)	0.114[3]
Classical PKU n (%)	22 (34.4)	6 (20.0)		19 (30.2)	9 (29.0)	
Late Diagnosed	2 (3.1)	3 (10.0)		1 (1.6)	4 (12.9)	
Annual blood Phe (μmol/L)	418.9 [279.1–584.8]	430.4 [364.4–535.8]	0.840[2]	387.4 [276.7–592.7]	524.9 [389.9–774.9]	0.013[2]
Good metabolic control n (%)*	31 (48.4)	10 (33.3)	0.150	35 (55.6)	8 (25.8)	0.009
Natural Protein (g/kg/day)	1.00 (0.74)	0.63 (0.50)	0.015	1.01 (0.69)	0.58 (0.43)	0.006
Protein Equivalent (g/kg/day)	0.67 (0.45)	0.64 (0.31)	0.280	0.55 (0.47)	0.56 (0.35)	0.839
Total Protein (g/kg/day)	1.66 (0.49)	1.27 (0.33)	<0.001	1.56 (0.40)	1.14 (0.29)	<0.001
Dietary Phe (mg/day)	2381.2 (1648.3)	1949.3 (1401.7)	0.320	2602.6 (1859.7)	2093.3 (1573.1)	0.283

Data are presented as mean (SD) or median [P25-P75]. * Percentage of patients with blood Phe levels within the target range, according to the Portuguese Consensus cut-off points. 1 - t-test for independent sample; 2 - Mann-Whitney Test, 3 - Chi-Square test. HPA, Hyperphenylalaninemia; NW, Normal Weight; OVW, Overweight; Phe, Phenylalanine; PKU, Phenylketonuria.

except in the last biennium when patients with overweight were significantly older (21 vs 25 years, $p = 0.024$).

Patients who were overweight had higher blood Phe levels in 4 bienniums, and these differences were statistically significant in the first and fifth biennium [281.5 vs 467.3 ($p = 0.026$) and 387.4 vs 524.9 ($p = 0.013$), respectively]. Accordingly, the percentage of patients with good metabolic control was systematically higher in patients with normal weight, at all time-points, with statistical significance in the fifth biennium ($p = 0.009$).

Natural protein intake was significantly higher in patients with normal weight, in all bienniums, compared to patients with overweight, and this difference was more pronounced in the first biennium, when the natural protein intake of patients with overweight was half of that ingested by patients with normal weight (0.35 ± 0.12 vs 0.71 ± 0.55 g/kg/day, $p < 0.001$). The same was established for total protein; patients with normal weight had a significantly higher total protein intake when compared to patients who were overweight ($p < 0.001$). Although not statistically significant, Phe intake was also higher in patients with normal weight at all timepoints.

Univariate and multivariate analysis for overweight are provided in Tables 4 and 5, respectively. The results presented in Table 4 show that poor metabolic control (compared to having a good metabolic control) and late-diagnosis (compared to patients with HPA) significantly increased the odds ratio (OR) of being overweight (OR = 3.44, 95 % confidence interval (CI) [1.33; 8.89] and OR = 14.00, 95 % CI [1.20; 163.37], respectively). Nevertheless, when metabolic control was adjusted for age, gender, and total protein intake (Table 5), this effect was no longer seen (OR = 0.56, 95 % CI [0.18; 1.75]). Conversely, a higher intake of protein (OR = 0.02, 95 % CI [0.00; 0.15], Table 4),

Table 4
OR and respective 95 % CI from univariate analysis with logistic regression model.

	Overweight		
	OR crude	95 % CI	*P*
Age (at the 1st biennium)			
2–4	ref		
5–10	1.39	[0.14; 13.75]	0.781
11–18	5.29	[0.57; 49.13]	0.143
>18	3.86	[0.40; 37.58]	0.245
Gender			
Female	ref		
Male	0.70	[0.30; 1.67]	0.423
Metabolic control*			
Good	ref		
Poor	3.44	[1.33; 8.89]	0.011
Natural protein (g/kg/day)	0.27	[0.11; 0.66]	0.005
Protein equivalent (g/kg/day)	1.06	[0.39; 2.88]	0.910
Total protein (g/kg/day)	0.02	[0.00; 0.15]	<0.001
Disease severity			
HPA	ref		
Mild PKU	1.69	[0.47; 6.09]	0.422
Classical PKU	1.66	[0.42; 6.49]	0.468
Late Diagnosed	14.00	[1.20; 163.37]	0.035
Sapropterin treatment			
No	ref		
Yes	1.407	[0.37; 5.40]	0.619

* According to the Portuguese Consensus cut-off points. CI, Confidence Intervals; HPA, Hyperphenylalaninemia; OR, Odds Ratio; PKU, Phenylketonuria.

Table 5
OR and respective 95 % CI from multivariate analysis with logistic regression model.

	Overweight		
	OR adjusted	95 % CI	*P*
Age (at the 1st biennium)			
2–4	ref		
5–10	0.83	[0.07; 9.71]	0.878
11–18	1.95	[0.17; 21.80]	0.588
>18	0.90	[0.07; 11.71]	0.938
Gender			
Female	ref		
Male	0.66	[0.21; 2.06]	0.478
Metabolic control*			
Good	ref		
Poor	0.561	[0.18; 1.75]	0.320
Total protein (g/kg/day)	0.04	[0.01; 0.30]	0.002

* According to the Portuguese Consensus cut-off points. CI, Confidence Intervals; OR, Odds Ratio.

particularly natural protein (OR = 0.27, 95 % CI [0.11; 0.66], Table 4), appears a protective factor against the development of overweight. This result remained after adjusting total protein intake for age, gender, and metabolic control (OR = 0.04, 95 % CI [0.01; 0.30], Table 5). This means that an increase in protein of 1 g/kg body weight, decreases the odds of being overweight by 96 %. That is, a protein increase of 0.1 g/kg body weight, decreases the odds of being overweight by 9.6 %.

In the multivariate model, the receiver operating characteristic (ROC) curve achieved an area under the ROC curve = 0.85 (95 % CI [0.76; 0.94]) which indicates a good discriminative ability, and the Hosmer–Lemeshow goodness-of-fit test showed a good calibration (p = 0.211).

4. Discussion

Our study shows a trend towards an increase in the prevalence of overweight in patients with PKU with increasing age. Over a 10-year period, the prevalence of overweight gradually increased from 24.5 % to 33.0 %, although with no statistically significant differences. In 2016, according to the National Food, Nutrition and Physical Activity Survey of the Portuguese general population [26], 57.1 % were overweight, with 22.3 % being obese. While this increase in the prevalence of overweight in patients with PKU is important, it remains below the general population. Results from this study reflect the importance of the annual nutritional status evaluation and continuous follow-up by a nutritionist in patients with PKU. All patients received personalized nutritional counselling to make healthier food choices and were encouraged to practice regular physical activity.

A higher trend towards overweight was found in females comparatively to males, which is in accordance with previous studies with patients with PKU [16,27]. This trend was also observed in children under the age of 2 years. Evans and colleagues [28] found that girls with PKU had higher weight z-scores and BMI compared to boys with PKU. These gender disparities also occur in the general population, with women having a higher prevalence of overweight (40 % vs 39 %) and obesity (15 % vs 10 %) when compared to men [5].

Patients with overweight had higher annual blood Phe levels and poorer metabolic control. Although poor metabolic control significantly increased the odds of being overweight (OR = 3.44, 95 % confidence interval (CI) [1.33; 8.89], when the variable was adjusted for age, gender, and total protein intake, the effect was no longer seen. Nevertheless, several studies have found a positive correlation between mean blood Phe levels and BMI or the prevalence of overweight [16,18,29,30], indicating that good metabolic control is associated with a lower risk of developing overweight. As high blood Phe levels may affect patients' organisational skills and executive functioning, patients with poor metabolic control may be less successful in planning healthy meals. As a result, they may eat more ready-made convenience snack foods with a higher fat and energy content, predisposing them to overweight [30]. Moreover, high blood Phe levels are associated with a negative effect on mood, which may negatively affect patient's food choices [31,32].

Additionally, patients with chronic diseases that require lifelong dietary treatment might have a higher risk of developing disordered eating and eating disorders. This has been identified in patients with type 1 diabetes, cystic fibrosis, inflammatory bowel disease, irritable bowel syndrome among others. Disordered eating behaviours may include fasting, restraint eating, emotional eating, binge eating, laxative use, excessive exercising, and body image disturbances [33]. In fact, adults with PKU were more likely to have a diagnosed eating disorder than adults in the general population [34]. Within patients with PKU, those who report having a poor metabolic control are more often concerned about food, according to Luu and colleagues [35]. In this study, the authors observed that patients who self-reported poor metabolic control had more disordered behaviour in dieting and body image. These patients were also more likely to report they have been overweight, to have bingeing behaviour, and to engage in activities to lose weight. Conversely, patients who report a good metabolic control appear to think of dieting as a way to manage their disease.

The practice of physical activity should also be addressed when discussing the prevalence of overweight. Alghamdi and colleagues [36] compared patients with PKU with healthy controls and found no differences in body composition, resting metabolic rate and diet-induced thermogenesis. However, when analysing physical activity, the authors found that patients with PKU had a significantly lower duration of moderate physical activity (MPAD) compared to healthy controls. Children with PKU had a MPAD of 138 ± 52 min/week, while healthy children averaged 264 ± 84 min/week. Adults with PKU engaged in 27 ± 10 min/week of MPAD, whereas healthy adults had 77 ± 26 min/week. The overall PKU group had a MPAD of 73 ± 26 min/week, compared to 152 ± 43 min/week in the overall healthy control group. A similar pattern was observed for moderate physical activity energy expenditure (MPAEE), with PKU patients showing significantly lower values. Children with PKU had a MPAEE of 618 ± 226 kcal/week, while healthy children had 972 ± 187 kcal/week. Adults with PKU expended 221 ± 115 kcal/week, compared to 587 ± 215 kcal/week in healthy adults. The overall PKU group had a MPAEE of 404 ± 127 kcal/week, compared to 741 ± 153 kcal/week in the healthy controls. Additionally, within the PKU group, having a low MPAD and MPAEE was associated with a higher percentage of blood Phe levels above the recommended target. Patients with high blood Phe levels are described as more likely to experience fatigue [37], which may also affect motivation to engage in physical activity. Taken together, these results suggest that patients with PKU are less physically active, which is an important risk factor for developing overweight.

Late-diagnosed patients are usually dependent on full-time caregivers for all their care, as some neurological sequels are evident [38]. This neurological impairment might influence their eating behaviour, which will impact their nutritional status. For this reason, it is important to carry out a careful and thorough assessment of the patient's nutritional status and lifestyle, to ensure that the therapy instituted is in accordance with their needs and familiar social context. Although patients with late diagnosis in our cohort appeared to have a higher risk of developing overweight (OR = 14.00, 95 % CI [1.20; 163.37]), the sample was very small (n = 5). A French study based on health insurance claims data [39] found that patients with a late diagnosis had a higher prevalence of obesity when compared to early-diagnosed patients with PKU and healthy controls (matched for age and gender). Conversely, a German study carried out with similar methodology [14] found that patients with a late diagnosis had a similar prevalence of overweight compared to matched controls, and a significantly higher prevalence of overweight in patients with an early diagnosis. It is important to note that, in both studies, the majority of patients with late diagnosis were

not taking any protein substitutes or pharmacological treatment.

Our results show that total and natural protein intake are protective factors against the development of overweight (OR = 0.02, 95 % CI [0.00; 0.15] and OR = 0.27, 95 % CI [0.11; 0.66], Table 4, respectively). After adjusting total protein intake for age, gender, and metabolic control (OR = 0.04, 95 % CI [0.01; 0.30], Table 5) this variable remained a protective factor with statistical significance. High-protein diets are known to increase energy expenditure by increasing post-prandial thermogenesis and resting metabolism. For instance, while carbohydrates require 5–10 % and dietary fats require 0–3 % of its usable energy to be expended for metabolism and/or storage, dietary protein requires 20 to 30 %. Higher-protein diets also lead to a greater preservation of resting energy expenditure (REE) [40]. Additionally, a higher amount of protein increases circulating levels of peptide YY (PYY) and glucagon-like peptide 1 (GLP-1), and these peptides acutely suppress appetite and reduce food intake [40]. Taken together, this may justify the finding that a protein increase of 1 g/kg body weight, decreases the odds of being overweight by 96 %.

It is known that different protein sources can influence protein digestion, metabolism, and homeostasis. During the study period most patients were taking L-AAs supplements. Free amino acids bypass the digestive phase, and their absorption profile is different from that of intact proteins [41]. In fact, when comparing the ingestion of L-amino acids, whole protein (from cottage cheese), and L-amino acids combined with whole protein, increments in total and essential plasma amino acid concentrations were greater and the maximum concentration (Cmax) was faster after the ingestion of L-amino acids alone and L-amino acids combined with whole protein. Additionally, after the ingestion of L-amino acids alone and L-amino acids combined with whole protein the decrease in plasma amino acids was faster than after the ingestion of whole protein [42]. This results in greater amino acid oxidation rates and urine nitrogen losses, and lower protein retention. It also leads to higher blood Phe fluctuations throughout the day [43]. In contrast, slowly digested proteins promote a better nitrogen utilization because amino acids are released at different rates into blood stream [44]. However, in this study, it was not possible to evaluate the influence of the different protein substitutes on the prevalence of overweight in patients with PKU.

Additionally, even though protein is the most satiating nutrient and influences weight regulation, it remains unclear whether the synthetic protein provided by protein substitutes alters satiety. However, it is possible that a diet with a greater amount of natural protein, that is, with less synthetic protein, is more satiating. This could partially explain why natural protein is a protective factor against the development of overweight. Pinto et al. [45] found that 65 % of patients aged ≥12 years were able to tolerate more natural protein than prescribed. Therefore, periodically assessing the maximum tolerance to natural protein would be beneficial, as it would improve the nutritional status of patients with PKU.

The present study has some limitations. Firstly, the information regarding lifestyle and physical activity of the included patients was not evaluated. Secondly, the BMI was used to identify overweight, although this variable may not always identify individuals with increased adiposity [22]. Most patients included in the study had mild PKU or HPA, and results might have been different if more patients with classical PKU were included. Furthermore, this was a cohort of young patients, with the mean age of 14 years at the start of the study. This could have an impact on the results obtained. Finally, this study lacked a control group.

In conclusion, this study found a trend towards an increase in the prevalence of overweight in patients with PKU over a 10-year period. Although this prevalence remains below the general population, it is important to follow-up and mitigate this trend. In addition, a higher protein intake appears to be a protective factor against overweight. Therefore, patients with PKU should be monitored by a multidisciplinary team that systematically assesses their nutritional status and provides personalized nutritional counselling accordingly. In the future, more studies are needed to evaluate the prevalence and risk factors for the development of overweight, actual protein tolerance, and optimal protein intake in patients with PKU.

Funding

This work was supported by an Individual Research Agreement between BioMarin Pharmaceutical Inc. and CINTESIS, R&D Unit (reference UIDB/4255/2020). The supporting source had no such involvement or restrictions regarding publication.

CRediT authorship contribution statement

Catarina Rodrigues: Writing – review & editing, Writing – original draft, Investigation. **Catarina Sousa Barbosa:** Writing – review & editing, Writing – original draft, Investigation. **Manuela Ferreira de Almeida:** Writing – review & editing, Investigation, Data curation. **Anabela Bandeira:** Writing – review & editing, Investigation. **Esmeralda Martins:** Writing – review & editing, Investigation. **Sara Rocha:** Writing – review & editing, Investigation. **Arlindo Guimas:** Writing – review & editing, Investigation. **Rosa Ribeiro:** Writing – review & editing, Investigation. **António Soares:** Writing – review & editing, Investigation. **André Moreira-Rosário:** Writing – review & editing, Investigation. **Cláudia Camila Dias:** Writing – review & editing, Investigation, Formal analysis. **Anita MacDonald:** Writing – review & editing, Investigation. **Nuno Borges:** Writing – review & editing, Investigation. **Júlio César Rocha:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

Anita MacDonald received research funding and honoraria from Nutricia, Vitaflo International, and Merck Serono. She is a member of the Advisory Board entitled ELEMENT (Danone-Nutricia), and member of an Advisory Board for Arla, Applied Pharma Research and Vitaflo International. Catarina Rodrigues received honoraria as a speaker from PIAM. Júlio César Rocha was a member of the European Nutritionist Expert Panel (Biomarin), the Advisory Board for Applied Pharma Research, Vitaflo, Synlogic, Biomarin, PTC Therapeutics and Nutricia, and received honoraria as speaker from APR, Merck Serono, Biomarin, Nutricia, Vitaflo, Cambrooke, PIAM, Lifediet and PTC Therapeutics.

Data availability

Data will be made available on request.

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