Diet therapy and metabolic control among Chilean adults with a neonatal diagnosis of Phenylketonuria

Journal of Inborn Errors of Metabolism & Screening
2024, Volume 12: e20230011
DOI: https://doi.org/10.1590/2326-4594-JIEMS-2023–0011

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Abstract

Phenylketonuria (PKU) is an autosomal recessive defect affecting the metabolic pathway of phenylalanine (Phe), causing hyperphenylalaninemia and neurotoxicity. Diagnosis must occur in the neonatal period and treatment should begin as early as possible. Evidence implies that treatment adherence declines as age advances. The aim was to describe the diet of a subgroup of Chilean adults with PKU currently in follow-up. Fifty-three subjects (49% women) followed up between January 2021 to April 2023 were considered. The concentration of Phe (PheC) in dried blood spots measured by fluorometry and 24-hour dietary recalls were analyzed. The median PheC of the sample was 438µmol/L (interquartile range(IQR):351-585µmol/L). A protein intake of 1.35±0.3 gr/Kg/d was observed of which 87% came from the protein substitute without Phe. Participants had a median Phe intake of 459mg/d (IQR:327-976) and 13.1g/d of fiber intake. Most participants, 51% and 92% reported consuming fruits and vegetables, respectively, and 32% consumed Low-Protein foods. Regarding micronutrients, all participants exceeded 90% adequacy according to recommendations. For vitamin-D and vitamin-B12, 100% is provided by the protein substitute. According to our results, it is mandatory to establish transition programs toward adulthood, to constantly maintain good metabolic control, and to adapt diet therapy to their new lifestyle.

Keywords

Phenylketonuria, Adults treatment, Nutrition.

Introduction

Phenylketonuria (PKU) is a genetic error of metabolism with an autosomal recessive inheritance, in which the hydroxylation pathway of phenylalanine (Phe) to Tyrosine (Tyr) is affected due to the lack of the enzymatic activity of phenylalanine hydroxylase (PHA). PKU causes Phe accumulation and Tyr deficiency [1]. Treatment consists of a Phe-restricted diet, which prohibits the consumption of foods of animal origin and legumes and requires protein substitute (PS) without Phe [2,3]. Due to dietary restrictions, it is necessary to supplement certain critical nutrients during the life cycle.

In Chile, the national newborn screening program for PKU started in 1992, allowing more than 500 children to be diagnosed in time with hyperphenylalaninemia (HPhe) and PKU [4]. In 2010, an incidence of 1:18,916 live births was established [4,5]. In a recent study conducted with a cohort of 271 subjects with PKU, the average age of diagnosis was 17 ± 8 days with a Phe concentration (PheC) of 1122 ± 540 umol/L [6]. In addition, 35% of adults with PKU adhered to the Phe-restricted diet and adequately consumed the PS, maintaining an average PheC of 546 ± 294 umol/L [6].

Evidence from across the world has shown that treatment adherence begins to wane at the transition stage when adults with PKU must change their pediatric treatment team. This change is a barrier for some patients as many treatment centers do not have health professionals who treat adults with PKU [7,8]. In many places around the world, neonatal PKU screening began

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Received September 30, 2023. Accepted for publication January 11, 2024.

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more than 60 years ago. Thus, the adult population of PKU patients continues to grow, as does the evidence on the results of diet therapy that indicates the importance of maintaining good metabolic control during the entire life cycle. This realization has led to the search for adjuvant alternatives to diet therapy to improve adherence, as inadequate metabolic control has been associated with comorbidities [9].

In Chile, the Institute of Nutrition and Food Technology (INTA) is a national reference center for IEMs. To establish the transition to adulthood, an outpatient follow-up clinic was formed made up of neurologists and adult nutritionists. The transition between the pediatric and adult team begins with adolescent patients between 13-15 years old, being evaluated exclusively by the adult team from 15 years old onwards. The metabolic control of PheC is monthly and the nutritional medical control is every 6 or 12 months, frequency that will depend on adherence to treatment. Jointly, constant education is delivered through telemedicine to reinforce maintaining monthly Phe control and evaluate other parameters such as vitamin D level, lipid profile, blood count, biochemical profile, insulin concentrations, and bone densitometry, with the central aim being to promote self-care and prevent neurological, psychological and nutritional complications associated with poor metabolic control such as, attention deficit, osteopenia or cardiovascular diseases [10,11].

Poor adherence to the Phe-restricted diet in adulthood relates to changes in levels of parental supervision, feeding schedules, and the social environment (study or work schedules), as well as, greater stress and anxiety related to the productive stage (i.e. working) [12]. The use of the PS is the fundamental dietary pillar in our Chilean cohort, covering 85% of the total protein ingested in this age group, allowing PheC to be maintained within recommended ranges (reference value: 120 to 360 umol/L).

In Chile, the PS are granted by state subsidies and every year these formulas are tendered for 3 age groups: infants, children, and adolescents/adults. All PS used are powdered and are mixtures of amino acids, fortified with L-tyrosine and supplemented with vitamins and minerals. The INTA clinical team adapts the requirements of calories, macronutrients, and micronutrients according to age, gender and nutritional status. Since the nutritional status of calcium, iron, and zinc has no direct biomarker, or its biomarker is expensive to measure, these minerals are given by pharmacological supplementation in most of them.

There are other adjuvants to dietary treatment such as large neutral amino acids (LNAA) and glycomacropeptide (GMP), however, these products are not subsidized by the Chilean government.

Low-protein foods (LowP-Food) such as flour, cookies, or pasta are also available, which promote greater satiety and adherence to diet therapy. However, these foods must be paid for by families.

This group of adult patients poses new challenges, especially in countries like ours where neonatal screening is relatively recent. The challenges for patients are related to having a restrictive diet that is different from their peers without affecting their quality of life, making the diet compatible with new environments such as University, work, or family, maintaining a balanced diet that provides adequate nutrition and phenylalanine intake while also achieving greater satiety.

For professionals in charge of these patients, there are also challenges aimed at establishing appropriate dietary strategies adapted to the patient's reality, which do not affect their quality of life and at the same time allow the prevention of long-term complications, both neurological and nutritional. Because of that, this study aimed to describe the diet of a subgroup of adults diagnosed with PKU by neonatal screening, who have maintained diet therapy and continue in follow-up at the Institute of Nutrition and Food Technology (INTA), University of Chile.

Methodology

We conducted a cross-sectional study of the adult Chilean cohort diagnosed with PKU from January 2021 to April 2023. The Chilean cohort of adults with PKU diagnosed in the neonatal period registered in our database is made up of 128 subjects. Subjects who met the following inclusion criteria were considered for this analysis: had a clinical appointment at the medical center at least 1 time during the data analysis period; had at least one PheC result on a filter paper card as part of the clinical appointment; reported compliance with nutritional treatment, defined as maintaining the Phe-restricted diet and using the PS. All participants signed the general informed consent used in the Genetics and Metabolic Diseases Laboratory of INTA, University of Chile, which was approved in May 2022 (Acta n°10/2022).

Variables considered in the current analysis:

- Phe concentration (PheC) at follow-up: Phe concentration was quantified by fluorometry using a drop of dried blood (DBS) on filter paper [13]. A PheC range between 120-360 umol/L was considered a good metabolic control, according to clinical guidelines and Chilean protocol [14].
- Neurocognitive Function: to evaluate neurocognitive performance, the Wechsler Intelligence Scale, revised and third edition (WISC-R, WISC-III) was used. The result was expressed in intelligence quotient (IQ), which classifies as normal between 80 to 110 points [15].
- Weight (kg) and height (cm) were measured on a Seca® scale (0.05 precision) and a stadiometer (0.01 cm margin of error), respectively. Nutritional status was based on the Body Mass Index (BMI (kg/m^2), the standards provided by the Ministry of Health of Chile, and the specific limits for each age and sex according to the World Health Organization (WHO).
- Dietary intake: In the nutritional consultation described in the protocol [14], a 24-hour dietary recall (24R) was carried out. Using this tool, the intake of macronutrients (proteins, carbohydrates, and fats) and

critical micronutrients (Phe, calcium, iron, zinc, vitamin D, vitamin B12) was quantified. Only the 24R recorded in the clinical record corresponding to the last appointment attended during the established analysis period was used. The intake of energy (kcal/d), protein (g/d), protein percentage in the caloric molecule (Prot CM%), protein per kilogram of weight (gr/kg/d), natural protein (g), and protein from the PS (g/kg/d) was estimated. In addition, we quantified: carbohydrate intake (Carb, g/d), carbohydrates percentage in the caloric molecule (Carb CM%), fat intake (Fat, g/d), fat percentage in the caloric molecule (Fat CM%), Phe intake (mg/d). The intake and percentage of nutritional adequacy of Calcium (Ca; mg/d), Iron (Fe; mg/d), Zinc (Zn; mg/d), and Vitamin D (IU/d) were determined; Vitamin B12 (mcg/d) were also considered. The adaptation was carried out according to the Recommended Dietary Allowance (RDA) [16], considering 90-110% acceptable.

– The intake of fruits, vegetables, cereals, and Low-Protein foods (LowP-Food) was quantified in grams per day (g/d).

  - Total dietary fiber: the amount of fiber (g/d) was extrapolated according to the amount of foods ingested with a higher content of:
  - Fruits and vegetables: the amount of fiber in these foods was averaged according to what is recorded by the USDA [17]. We estimated that each 100 grams of fruit or vegetable had an average of 2.3 grams of total fiber.
  - Cereals: the average fiber was obtained considering the cereals registered in the 24R of each patient and the contribution indicated per 100 g of each of these foods according to the USDA [17]. We estimated that for each 100 g of cereal, 3.1 grams of total fiber was estimated.
  - Low-Protein Foods (LowP-Food): the average fiber was obtained considering the LowP-Food recorded in the 24R of each patient per the nutritional information of each of these foods. We estimated per 100 grams of registered LowP-Food they have an average of 4.7 grams of total fiber.

Statistics: A descriptive analysis of the variables of interest was carried out. The distribution of each variable was verified using the Shapiro-Wilk test. Depending on the distribution, the mean ± standard deviation (SD) or the median and interquartile range (IQR 25 - 75) was calculated. When comparing the variables by groups, a model adjustment was made considering sex and continuity of treatment as influential factors in the result. We considered continuity of treatment, the subjects who never suspended the protein substitute intake, and maintained sending samples to verify their metabolic control.

We calculated linear correlations: Pearson or Spearman depending on the distribution of the variables. Statistical significance was considered at a p-value <0.05. Redcap® was used to generate the database and JMP®16.0 for statistical analysis.

Results

Of the 128 adult subjects with a neonatal diagnosis of PKU, 53 subjects (49% female) met the eligibility criteria and were included in the current study. Regarding the nutritional treatment, all the subjects were on a Phe-restricted diet and PS, and no other coadjuvant was observed in the records (BH4, GMP, or pegvaliase). This sample had a median diagnosis age of 15 days (IQR 12.0-19.5) with a PheC of 1026 µmol/L (IQR 780 - 1333) and Tyr of 63 µmol/L (IQR 44 - 83) at diagnosis. The sample had a median age of 23 years (IQR 19 - 26), an average PheC at the last study visit of 438 µmol/L (IQR 351 - 585), and a median number of 7 samples (IQR 3.5 - 16) available during the study period. Of the 53 subjects included, 16 lowered their adherence to treatment due to the lack of the PS government subsidy and then resumed follow-up starting in 2017 when the PS began to be subsidized for life. According to the last cognitive evaluation recorded for each individual, we observed an average IQ score of 90.8 ± 14.5, indicating normal performance. We found a negative correlation between median current PheC and IQ (Spearman test, Rho: -0.45, p-value=0.002, 95%CI -0.6; -0.09), even after adjusting for sex (p-value=0.02) (Figure 1). Regarding educational level, 27% were currently completing high school, 37% had a university or technical degree, and 20% already had some professional degree.

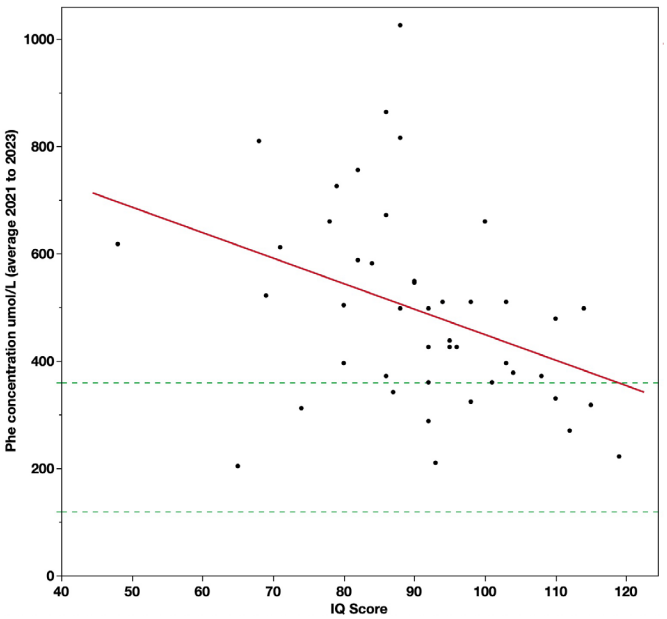

Figure 1. Spearman's correlation between average Phe concentration (µmol/L) calculated for each subject with the score obtained from the last evaluation of neuropsychological functioning. Rho: -0.45; p <0.01; 95% CI: -0.6, -0.09.

Table 1 presents the results of the dietary intake. The median caloric intake was 23 kcal/kg body weight (IQR 16 - 28), and an average of 1.35 ± 0.3 gr/kg/day of protein, of which 87% came from the PS. Regarding Phe intake, a median of 459 mg (IQR 327 - 672) per day was observed. The contribution of Tyr from the PS was 5806 mg/d (IQR 5172 - 7023). The distribution of the caloric molecule, considering the 3 macronutrients was: 26.4% protein; 22% fat, and 51% carbohydrates. According to the 24R, 51% reported consuming fruits, and 92% consumed vegetables daily. Only 32% of the subjects consume LowP-Food. Concerning the amount of fruits, vegetables, cereals, and LowP-Food recorded, a median intake of 13.1 g/d (IQR 11 - 19) of fiber was estimated (Table 2).

The intake and supplementation provided are described in Table 3. We observed that 30%, 42%, and 43% are not receiving calcium, zinc, and iron supplementation, respectively. However, 97%, 96%, and 90% of the RDA for calcium, zinc, and iron, respectively, were covered by the PS. About the dietary contribution of vitamin D and vitamin B12, 100% comes from the PS.

Regarding nutritional status, the median BMI was 23.8 kg/m2 (IQR 21.7 - 25.6), 68% were normal weight, 19% were overweight and 13% were obese. When analyzing the group according to nutritional status, no significant difference was observed in PheC, in the intake of fruits and vegetables, nor the intake of LowP-Food (Table 4).

In the sample, 70% (n=37) have maintained diet therapy continuously since diagnosis, with a PheC of 444 ± 150 µmol/L, which, when compared with those who suspended it due to lack of the PS before that was subsidized for life, we observed a PheC of 558 ± 216 µmol/L (p-value=0.038).

When comparing the variables according to metabolic control, 21 subjects had a PheC in the acceptable range (reference value: 120 - 360 µmol/L), with a median value of 330 µmol/L (IQR 279 - 372) and 32 subjects had a concentration above the acceptable range (median PheC of 534 µmol/L; IQR 486 - 660), values that were significantly different (p-value <0.01; models adjusted for sex and continuity of treatment). There was also a significant difference in the number of samples during the data

Table 1. Calories and macronutrient intake analysis (24-hour records).

	Reference value PKU population	Mean	±SD	Median	IQR 25	IQR 75
Kcal*	–	1470	±556	1371	1080	1704
Kcal/Kg*	–	22.7	±9.8	20.9	15.9	27.5
Total protein (g/d)	–	88	±15	88	79	96
Total protein (g/kg/d)	1.5 - 2.1[a]	1.4	±0.3	1.4	1.2	1.5
Total PS (protein g/kg/d)*	–	1.2	±0.2	1.2	1.1	1.3
Prot CM%	20%[b]	26	±8	25	22	31
Phe INTAKE (mg/d)*	200 - 1100[a]	519	±265	459	327	672
Tyrosine intake (mg/d)* (provided by PS)	4000 - 6000[a]	6017	±1490	5806	5172	7023
Carbohydrates (g/d)*	–	1967	±105	165	136	250
Carb CM%	55 - 60%[b]	51	±10	50	47	58
Fat (g/d)*	–	36	±22	37	19	47
Fat CM%	25 - 30%[b]	21	±9	22	14	29

**Non normal distribution variable. Reference value: [a]R.H. Singh et al. / Molecular Genetics and Metabolism 118 (2016) 72–83 (2); [b]FAO/WHO/UNU. «Human energy requirements», 2004. SD: standard deviation; IQR: interquartile range; KCAL: kilocalories; PS: protein substitute; g/d: grams per day; g/kg/d: grams per kilograms of weight per day; mg/d: milligram per day; Prot CM%: protein percentage correspondent to caloric molecule; Phe: Phenylalanine; Carb CM%: carbohydrates percentage correspondent to caloric molecule; Fat CM%: fat percentage correspondent to caloric molecule.*

Table 2. 24 hours record characterization.

	Mean	±SD	Median	IQR 25	IQR 75
Fruits (g/d)*	82	±105	12	0	135
Vegetables (g/d)*	222	±166	200	105	260
Fruits+Vegetables(g/d)*	304	±216	250	135	427
LowP-Food (g/d)*	54	±110	0	0	100
Cereals (g/d)	177	±102	170	100	250
Total Fiber calculated (g/d)*	15	±7	13	11	19

***Non-normal distribution variable. SD: standard deviation; IQR: interquartile range; KCAL: kilocalories. g/d: grams per day*

Table 3. Micronutrient intake analysis and supplementation (24-hour records).

	Mean	±SD	Median	IQR 25	IQR 75	Reference range(ref)
Calcium intake (mg/d)	3424	±100	3433	2926	4061	1000 mg/d
Calcium % (provided by PS)*	96	±4	97	94	98	-
Calcium supplementation (mg/d)*	850	±224	1000	625	1000	-
Zinc intake (mg/d)	39	±8	38	34	45	8 mg/d (woman) 11 mg/d (man) UL 40 mg/d
Zinc % (provided by PS)*	94	±6	96	93	98	-
Zinc supplementation (mg/d)*	16	±4	15	15	15	-
Iron intake (mg/d)*	45	±10	46	41	53	18 mg/d (woman) 8 mg/d (man) UL 45 mg/d
Iron % (provided by PS)*	86	±10	90	83	93	-
Iron supplementation (mg/d)*	16	±2	15	15	15	-
Vitamin D intake (IU)* (mcg/d)	1501	±82	144	112	174	200 IU/d (5 mcg/d)
	(3.8)	(±2.1)	(3.6)	(2.8)	(4.4)	UL 4000 UI (100 mcg/d)
Vitamin B12 intake (mcg)	8.7	±2.6	7.9	7.1	9.9	2.4 mcg/d

***Non normal distribution variable. SD: standard deviation; IQR: interquartile range; %: percentage; PS: protein substitute; mg/d: milligram per day; IU/d: international units per day; mcg/d: micrograms per day. UL: upper limit. (Institute of Medicine of the National Academies Washington, D.C. Dietary Reference Intake 2001.)*

Table 4. Nutritional status characterization.

	Normal (n=36)	Overweight (n=10)	Obese (n=7)	p-value¥
Phe Concentration (µmol/L)	474 ± 186	480 ± 138	528 ± 192	NS
Fru+Veg Intake (g/d)*	326 ± 219	298 ± 262	203 ± 86	NS
LowP-Food (g/d)*	55 ± 123	70 ± 92	26 ± 56	NS

Kruskal Wallis comparison and Mann Whitney test to each group. Adjusted comparison model by sex. Significance p-value < 0.05. NS: no significance.

analysis period. The group with PheC in the acceptable range had a median of 12 samples (IQR 6-18) whereas the group with a PheC above the range had a total of 5 samples (IQR 2-12). A significant difference was also observed in BMI. The group within the acceptable range of PheC had a median BMI of 22.4 kg/m2 (IQR 21-25) versus 24.3 kg/m2 (IQR 22-28) in the group with higher PheC.

Differences in dietary intake by metabolic control are shown in Table 5. In the group with a PheC within an acceptable range, a median Phe intake of 556 mg/d (IQR 389 - 768) was observed compared to 404 mg/d (IQR 291 - 619) in subjects with a PheC greater than the optimal range. The group with good metabolic control had a higher caloric intake and a better distribution of macronutrients, with no differences observed for Carb CM% and Fat CM%. Subjects with good metabolic control had a higher intake of fruits/vegetables and calculated total fiber, variables adjusted by sex and continuity of treatment explained in methods.

The median intake of LowP-Food was 150 gr/d (IQR 100-200) (Table 6). The subjects who consumed LowP-Food had a significantly lower BMI ($p<0.01$), but higher caloric intake (p-value <0.01), covering 57% of Carb CM% compared to 49% in subjects who did not consume LowP-Food (p-value < 0.01). The group that consumed LowP-Food also had a higher intake of total fiber compared to the group that did not (p-value=0.01).

Table 5. Subjects comparison by metabolic control.

	Metabolic control less than 360 µmol/L N=21					Metabolic control more than 360 µmol/L N=32					
	Mean	±SD	Median	IQR 25	IQR 75	Mean	±SD	Median	IQR 25	IQR 75	**P-value**¥
Kcal/d*	1631	±554	1652	1174	2023	1365	±540	1312	1064	1427	**<0.01**¥ **(r^2: 0.27)**
Total Protein (g/d)	93	±18	90	81	110	85	±12	82	77	95	**0.026**¥ **(r^2: 0.17)**
Total Protein (g/kg/d)	1.5	±0.3	1.5	1.3	1.6	1.3	±0.2	1.4	1.1	1.5	**0.0046**¥ **(r^2: 0.23)**
Prot CM%	25	±9	23	19	31	27	±8	27	23	31	**0.038**¥ **(r^2: 0.16)**
Total PS (protein g/kg/d)*	1.2	±0.2	1.3	1.2	1.3	1.1	±0.2	1.2	1.0	1.3	**0.04**¥ **(r^2: 0.15)**
Phe Intake (mg/d)*	624	±306	556	389	768	449	±213	404	291	619	**0.044**¥ **(r^2: 0.15)**
Tyr Intake (mg/d)* (provided by PS)	6373	±1857	6166	4936	7255	5782	±1164	5655	5202	6342	**0.011 (r^2: 0.20)**
Carb CM%	53	±10	52	48	59	50	±11	49	45	58	NS
Fat CM%	21	±12	22	10	30	22	±8	21	16	28	NS
Total Fiber calculated (g/d)*	18	±6	17	13	23	13	±6	12	10	15	**<0.01**¥ **(r^2: 0.36)**
Fru+Veg (g/d)*	410	±262	310	208	670	234	±146	240	114	315	**0.003**¥ **(r^2: 0.24)**
LowP-Food (g/d)*	66	±82	0	0	125	46	±126	0	0	0	NS
Cereals (g/d)	182	±117	170	90	258	174	±92	168	100	250	NS

*****Non normal distribution variable. SD: standard deviation; IQR: interquartile range; KCAL: kilocalories; PS: protein substitute; g/d: grams per day; g/kg/d: grams per kilograms of weight per day; mg/d: milligram per day; Prot CM%: protein percentage correspondent to caloric molecule; Phe: Phenylalanine; Carb CM%: carbohydrates percentage correspondent to caloric molecule; Fat CM%: fat percentage correspondent to caloric molecule. ¥Comparison model adjusted by sex and continuity of treatment; p-value <0.05. NS: no significance.*

Discussion

We are currently faced with an increase in the adult PKU population around the world, who, in addition to controlling their condition, are beginning to present other needs and concerns. In the Chilean cohort with PKU, of the 128 subjects over 18 years of age registered in our database, 41% attended their medical follow-ups, had measured PheC regularly, and maintained good adherence to diet therapy during the current study period.

The state subsidy from the PS until 2017 benefited PKU people up to 18 years of age, this being the main cause of abandonment of treatment. This subsidy was later extended for life and several PKU adults resumed diet therapy. Furthermore, as part of the inclusion criteria of the study, only patients who comply with the sending of DBS were considered. Finally, patients with other associated pathologies, such as deafness or autism among others, were excluded from this study.

According to what is described in the literature, it is widely known that as age increases, adherence to treatment decreases, and around 33% of patients are adherent to maintaining PheC in the allowed range [18,19]. In the sample evaluated in the current study, we found a similar prevalence (40%).

The Chilean protocol for the management of patients with PKU, like the United States one, considers good metabolic control between 120 umol/L to 360 umol/L PheC [2,14]. In our case series, a median PheC of 478 µmol/L (IQR 351 - 585) was observed, 32% above the maximum indicated. However, if we considered the Phe value of the European protocol, where the acceptable range is 120 to 600 µmol/L [3,20], 75% of our adult PKU cohort would have good metabolic control. Behavioral alterations in adult subjects with PKU have been associated with low adherence to treatment, alterations in executive function, IQ, and attention and emotional deficits [21]. In our study, the majority of subjects with PKU were pursuing higher education and 20% had already obtained a university or technical degree.

Regarding nutritional status, 32% were classified as overweight or obese, which is lower than what was reported in 2021 [6]. There is no substantive evidence linking a Phe-restricted diet to overweight or obesity and similar BMIs between patients with PKU and control subjects have been reported [22]. On the other hand, subjects with classic PKU with a high PheC, due to poor adherence to dietary treatment, tend to have a higher BMI compared to those with lower PheC. In our study, there was no significant difference between groups according to nutritional status (Table 3).

Table 6. Subjects comparison by low-protein food intake.

	LowP-Food intake (n=17)					**Non LowP-Food intake (n=36)**					
	Mean	±SD	Median	IQR 25	IQR 75	Mean	±SD	Median	IQR 25	IQR 75	**P-value¥**
BMI (kg/m^2)	24.6	±4.3	23.8	22.2	25.2	25.6	±6.5	23.7	21.3	27.5	**<0.01¥ (r2:0.27)**
Phe Concentration (µmol/L)	417	±124	372	144	510	508	±191	467	382	617	NS
KCAL/d*	1828	±733	1690	1234	2489	1302	±351	1333	1064	1549	**<0.01¥ (r2:0.36)**
Total Protein (g/d)	90	±18	88	77	102	87	±14	85	79	95	NS
Total Protein (g/kg/d)	1.4	±0.3	1.4	1.3	1.5	1.3	±0.3	1.4	1.2	1.5	**<0.05¥ (r2:0.2)**
Prot CM%	22	±7	22	15	30	28	±8	27	23	34	**<0.01¥ (r2:0.24)**
Total PS (protein g/kg/d)*	1.2	±0.1	1.2	1.1	1.3	1.2	±0.3	1.2	1.0	1.4	**<0.05¥ (r2:0.2)**
Phe Intake (mg/d)*	435	±230	375	286	544	558	±275	512	353	694	NS
Carb CM%	57	±11	57	50	66	49	±9	48	45	53	**<0.01¥ (r2:0.21)**
Fat CM%	19	±9	17	12	26	23	±9	24	16	30	NS
Total Fiber calculated (g/d)*	19	±8	19	12	26	13	±5	13	11	15	**<0.01¥ (r2:0.35)**
Fru+Veg (g/d)*	338	±257	280	125	495	288	±196	250	143	429	NS
Cereals (g/d)*	103	±87	100	15	160	212	±90	206	150	265	**<0.01¥ (r2:0.27)**

**Non normal distribution variable. SD: standard deviation; IQR: interquartile range; KCAL: kilocalories; PS: protein substitute; g/d: grams per day; g/kg/d: grams per kilograms of weight per day; mg/d: milligram per day; Prot CM%: protein percentage correspondent to caloric molecule; Phe: Phenylalanine; Carb CM%: carbohydrates percentage correspondent to caloric molecule; Fat CM%: fat percentage correspondent to caloric molecule. ¥Comparison model adjusted by sex and continuity of treatment; p-value <0.05. NS: no significance.*

Analyzing caloric intake and considering that the majority of the sample had a normal nutritional status, a median intake of 21 kcal/kg/d was observed, covering 70% adequacy by what is expected for a normal-weight population (30 kcal/kg/d) [23] (Table 1).

According to the distribution of the caloric molecule, we observed what was suggested by the RDI-FAO/UNU/WHO [16]. Concerning protein intake, the percentage of adequacy was 30% over the amounts indicated for the general population. This is likely because the PS has a lower bioavailability than a natural protein and should be prescribed between 20 to 40% above the RDA to ensure adequate absorption of this macronutrient [14]. In our study, 87% of the total protein came from the PS, which also provides enough Tyr to avoid deficiencies, being that in PKU it is an essential amino acid.

Regarding Phe intake, we observed that subjects who maintained PheC less than 360 µmol/L had a median Phe intake of 556 mg/d. The majority of subjects with classic PKU tolerate less than 500 mg of Phe per day [24,25], defining tolerance as the amount of Phe ingested that allows maintaining a Phe concentration within the recommended range according to clinical protocol. In our cohort, we observed that the group with a good Phe concentration (330 µmol/L; IQR 279 - 372) had a higher Phe intake (556 mg/d (IQR 389 - 768) compared to subjects with a PheC out of range (534 µmol/L; IQR 486 - 660) who ingested a lower amount of Phe (404 mg/d (IQR 291 - 619). It is noteworthy that the group with better metabolic control had a higher caloric intake, consumption of LowP-Food, fruits, and vegetables, and better nutritional status than the group with metabolic control above the recommended range. We acknowledge that the 24R can be subjective and may be undervalued by patients, in particular, sporadic dietary transgressions may be underreported. It has been reported that in all self-report dietary assessment tools, 24 hours, an average of 4 registered days, or frequency food consumption (FFQs), exists as an underestimation. Still, both mentioned in the first place provide the best estimates of absolute dietary intakes [26]. In addition, many adult patients use Phe exchange lists and do not maintain as rigorous of control of their intake as the pediatric population.

Regarding critical micronutrients that are decreased due to the restriction of foods of animal origin, such as calcium, zinc, iron, vitamin D, and vitamin B12, among persons with PKU, PS plays a fundamental role. Thus, in adult subjects with good treatment adherence, it is difficult to observe micronutrient deficiency [27]. Collectively, our cohort was supplemented with some critical nutrients such as calcium, iron, and zinc. In this

study, considering both nutrient contributions, we observed that most participants were at the upper limit of the RDA. As there is no scientific evidence that indicates the bioavailability of these micronutrients from the PS [28], we maintain the supplementation of calcium, zinc, and iron is standard clinical practice. However, the evaluation of this clinical protocol warrants further research.

The WHO recommends a dietary intake of 400 grams of fruits and vegetables per day, which ensures the intake of vitamins and minerals. In the Chilean national food survey, carried out in 2007, it was observed that, on average, the Chilean population consumed 395 g/d of fruits and vegetables (227 g/d of vegetables and 168 g/d of fruits). In our adult PKU cohort, we observed a median intake of fruits and vegetables of 250 g/d (IQR 135 - 427), of which 92% of subjects consumed vegetables and only 51% fruits.

Regarding the total fiber ingested per day, one of the limitations of diet calculation software is that information is not obtained for each food. This is why the average fiber delivered per 100 g was extrapolated by food group according to information from the USDA [17] for fruits, vegetables, cereals, and LowP-Food. Inulin is added in large amounts in low protein flour because it provides similar characteristics to gluten, such as increased thickness, emulsify, and jellify, increasing the palatability of these products and helping to grant more fiber to the PKU diet [29]. The WHO recommends a daily intake of 25 g/d; we observed a median consumption of 13 g/d (IQR 11 - 19), which corresponds to 50% of what is recommended. In the general population, this low fiber intake can also be seen, which can impact intestinal problems, in the microbiota, and is also considered a risk factor for cardiovascular diseases [30].

Regarding LowP-Food, in Chile, availability is limited, they are high-cost and are not government-subsidized, which likely explains why only 32% of the sample reported consumption. Because of the cost, the main product consumed in Chile is flour, which allows a greater variety of food since it allows the preparation of a large number of recipes compatible with the diet, such as bread, cakes, puddings, or pizzas. Some suppliers sell prepared products such as cookies, pasta, rice, and sweet desserts such as mousse, pies, or cupcakes, but they have a larger price than the flour, therefore, they are consumed by a small number of patients. When dividing the group by consumption, we observed that subjects who consumed LowP-Food had a normal BMI, better PheC in blood, higher caloric intake, and lower Phe intake. These differences remained statistically significant after adjusting for sex and treatment continuity. Thus, LowP-Food may help maintain better adherence to diet therapy, resulting in better metabolic control by providing greater satiety, diversifying the diet, and indirectly avoiding dietary transgressions.

Conclusion

We can emphasize that neonatal diagnosis and early initiation of diet therapy in subjects with PKU allow for reversing the normal course of the pathology if it is not diagnosed and treated promptly.

Long-term follow-up, and continuous education to maintain adequate metabolic control, allow PKU subjects to have normal growth and cognitive abilities, and to subsequently enter this society as productive adults.

The importance of the Complementary Food Program for Metabolic Diseases, of the Chilean government, must be highlighted, which subsidizes PS to all PKUs universally, favors adherence, and allows the requirement of macro and micronutrients to be covered throughout the entire life cycle.

According to our results, it is mandatory to establish transition programs toward adulthood, to constantly maintain good metabolic control, and to adapt diet therapy to their new lifestyle.

Acknowledgments

The authors would like to acknowledge all the patients and their families for their trust in our staff, as well as the laboratory staff at the Laboratory of Genetics and Metabolic Diseases (LabGEM) of INTA, University of Chile, for their assistance.

Funding

The study was not funded by any grant. The study was supported by LabGEM, INTA, University of Chile.

Declaration of Conflicting Interests

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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