

Update on Dietary Compliance, Nutritional Status, and Neuropsychological Functioning in the Chilean Phenylketonuria cohort

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Abstract

Since 1992, Chile has had a Newborn Screening Program for Phenylketonuria (PKU), which currently has an incidence of 1:18,916 newborns. The objective of the current study was to describe the 2020 follow up of the Chilean PKU cohort. The variables analyzed were: nutritional status, dietary compliance and neuropsychological functioning. We conducted a descriptive cross-sectional statistical analysis. The 271 subjects with PKU had an average age of diagnosis of 17±8 days and a phenylalanine (Phe) level of 1122±546 umol/L. Approximately 80% of protein requirement came from a protein substitute. For those <18 years of age, 80% had good dietary compliance with Phe level between 120-360 umol/L and those >18 years had a median of 522 umol/L (95%CI 468 - 636). Forty-four percent of the active PKU cohort had overweight/obesity. Eighty-five percent of the cohort >4 years of age had a normal intelligence quotient (IQ) (score 80-120). We observed a negative correlation ($p < 0.001$; 95% CI: -0.5, -0.2) between IQ score and Phe level. The Chilean protocol and protein substitute subsidy for life, together with the follow-up and continuous education carried out by the clinical team has encouraged compliance.

Keywords

Phenylketonuria, Chilean protocol, Phenylalanine, Newborn Screening.

Introduction

Hyperphenylalaninemia (HPA) is an autosomal-recessive genetic defect produced by a mutation in chromosome 12q22-q24.1.[1] This chromosome codes for the enzyme phenylalanine hydroxylase (PAH), which catalyzes the chemical conversion reaction from the amino acid phenylalanine (Phe) to tyrosine (Tyr). Thus, Phe begins to accumulate in blood and other tissues, decreasing the synthesis of Tyr.[2] Between 2 to 5% of these HFAs are produced by the defect of the cofactor system of this PAH enzyme. The worldwide incidence is 1:10,000 newborns (NB), 1:18,916 NB for PKU and 1:10,198 NB for HPA in Chile.[3]

Increased levels of Phe in the blood caused by this metabolic blockage is neurotoxic, producing irreversible damage to the central nervous system, unless diagnosed in the neonatal period and adequate nutritional treatment is started.[4-6] As diagnosis must be early to prevent neurological sequelae, Newborn Screening (NBS) programs have been implemented, which has made it possible to eliminate the intellectual disability caused by PKU.[7-9] In Chile, the NBS for PKU and Congenital

Hypothyroidism (CH) began in 1992 and, to date, has diagnosed more than 500 children with different type of PKU/HPA. To date, 271 children have been diagnosed with PKU who had a Phe value over 360 umol/L at the time of diagnosis. Children were classified as a classical or variant PKU and entered the follow-up program of the Institute of Nutrition and Food Technology (INTA) of the University of Chile, the national reference center. Treatment is for life and consists of a Phe-restricted diet that eliminates all foods of animal origin (e.g., meat, dairy products and derivatives, fish and shellfish) and legumes.

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To accomplish energy and protein requirements, a special Protein Substitute (PS) without Phe must be consumed and supplemented with Tyr, vitamins and minerals, which allows for growth and development within normal ranges.[6,10] The Chilean government subsidizes the PS without Phe (Phe-free L-AA mixture) and, since 2017, provides this PS for life. Scientific evidence has shown that maintaining a Phe level between 120 to 360 $\mu\text{mol/L}$ prevents neurological sequelae.[11–13]

The objective of this article was to describe the current situation of critical nutrients and supplementation and dietary compliance, as well as the nutritional status, and the neuropsychological functioning in the Chilean PKU cohort diagnosed by NBS.

Methodology

We conducted a cross-sectional analytical study of the Chilean cohort with PKU in two hundred seventy-one subjects with PKU diagnosed by NBS.

Inclusion criteria for the analysis

Subjects diagnosed by NBS (Phe > 360 $\mu\text{mol/L}$ at diagnosis) who were in the follow-up program and adhered to the recommended medical-nutritional and biochemical follow-ups according to the INTA follow-up protocol.[6]

Exclusion criteria for the analysis

Subjects with a diagnosis of another associated pathology, and PKU subjects who, in addition to their genetic condition, presented maternal PKU syndrome (PKUm).

Active PKU group

Of the total PKU diagnosed by NBS, 205 PKU met the inclusion criteria. Nutritional status, dietary compliance, psychological and biochemical evaluations were evaluated according to the protocol during between January 2019 and July 2020.

Phe and tyr levels

Phe and Tyr levels at diagnosis ($\mu\text{mol/L}$) were determined by tandem mass spectrometry (MS/MS). When Phe level was equal to or above 360 $\mu\text{mol/L}$ and the value of the Phe/Tyr ratio above 3, the patient entered to the monitoring program at INTA, University of Chile.[2]

Dietary compliance

During follow-up, Phe was quantified by fluorometry using a dried blood spot (DBS).[14] Values between 120–240 $\mu\text{mol/L}$ and between 120–360 $\mu\text{mol/L}$ for Phe were considered adequate for children under and over 2 years of age, respectively. Tyr level must be kept between 44 and 100 $\mu\text{mol/L}$.

Nutritional status

Weight (kg) and height (cm) were measured with Seca scale (0.05 kilograms accuracy) and stadiometer (0.01 cm margin of error), respectively. Nutritional status in children under 18 years of age was classified using the technical standard for nutritional evaluation used in our country based on the World Health Organization (WHO)[15–18] standards by age: obesity (> 2 standard deviation, SD); overweight (1 to 2 SD); normal weight (-1 to 1 SD); risk of malnutrition (-1 to -2 SD); malnutrition (< -2SD). In those over 18 years of age, nutritional status classification was based on Body Mass Index (BMI (kg/m^2)) and the standards of the Chilean Ministry of Health supported by the WHO age and sex-specific limits.[19]

Classification of height was only applied to individuals under 18 years of age in SD: tall height (> 2SD); tall normal height (1 to 2 SD); normal height (-1 to 1 SD); short normal height (-1 to -2 SD); short height (< -2SD), according to the technical standards for nutritional status established by the Chilean Ministry of Health.

Diet analysis

A 24-hour dietary recall (24R) was carried out at the nutritional interview and intake was quantified with the amino acid analyzer (AAA) software. Only one 24R was considered, as our protocol establish for the following up registered in the clinical record. Energy intake (kcal/d) and protein (g/d) were estimated. In relation to protein intake, the following were considered: PS without Phe (g/d), Phe intake (mg/d) and Tyr (mg/d). The percentage of nutritional adequacy of: Calcium (Ca; mg/d), Iron (Fe; mg/d), and Zinc (Zn; mg/d) intake was determined, according to the Recommended Dietary Allowance (RDA)[20], considering 90–110% acceptable.

Neuropsychological function

In children under 3 years of age, psychomotor development was determined using the Bayley Scale of infant and toddler development (Bayley II, Second edition)[21,22], which include a mental development index (MDI) and motor development index (PDI). An MDI and PDI score > 80 points was classified as normal development. In those over 4 years of age, the Wechsler Scale was used. The Wechsler Preschool and Primary Scale of Intelligence (WPPSI)[23] test was used in the 4–6 year-old group and, for those between 6 and 18 years of age, the Wechsler Intelligence Scale for Children, Revised Third Edition (WISC-R or WISC-III)[24] was used. For adults, greater than 18 years old, Weschler Adult Intelligence Scale (WISC-IV)[25] was used. The results were expressed as is full scale intellectual quotient (FSIQ) of the total scale according to the score obtained for each individual. Intellectual quotient (IQ) was classified as normal if between 80 and 120 points.

Statistical analysis

A descriptive analysis of the variables was carried out, in which the distribution for each variable was determined using the Shapiro-Wilk test. Depending on the distribution, the mean or median was calculated, and, depending on the statistic used, the SD or the 95% confidence interval (CI) reported. Spearman's correlation was performed to verify the association of variables. A p-value <0.05 was considered statistically significant. Microsoft Excel 16.34 and JMP®14.2.0 were used for analyses.

Ethical committee reports

Medical records review and the use of data from the subjects with active PKU for the analysis of this update was approved by the Ethics Committee of INTA (Approved 20th January).

Results

In the 271 subjects diagnosed with PKU since 1992 who required treatment and entered the follow-up program at INTA, 126 (46%) were female. The average age of diagnosis was at 17 ± 8 days, with Phe levels of 1122 ± 540 $\mu\text{mol/L}$ (95% CI 1056 - 1182) and Tyr of 71.5 ± 55 $\mu\text{mol/L}$ (95% CI 66 - 77). The group was made up of 8 PKU (3%) less than 1 year old, 24 (9%) between 1 and 3 years old, 62 (23%) between 4 and 9 years old, 51 subjects (19%) between 10 and 14 years, 37 (14%) between 15 and 17 years old and 89 subjects (33%) older than 18 years. Because 40% of the Chilean population lives in the Metropolitan Region, which includes the capital, Santiago, 46% of the cohort is concentrated in this region.[26]

In the active PKU group (n=205), 4 subjects (1%) were classified as malnourished, 113 (55%) were classified as normal weight, 48 (24%) as overweight and 40 (20%) as obese. The highest percentage of overweight and obesity was found in participants between 1 and 9 years, reaching up to 50% in these groups. With respect to height, 2% had short stature (2 subjects aged 1-3 years and one aged 4-9 years).

For diet, considering the median intake all age groups complied with the protein recommendation according to the Chilean protocol.[5,6] When quantifying protein contribution from the PS without Phe, it was observed that, in children under 1 year of age, 61% of total proteins came from PS without Phe and, in older age groups, the percentage fluctuated between 75 and 88% (Figure 1). Fifty-one subjects were being supplemented with L-Tyr, due to the fact that they maintained a value of this amino acid under 44 $\mu\text{mol/L}$. The supplementation delivered was between 100 and 1500 mg of L-Tyr; 35% of subjects were between 1-3 years, 38% between 4-9, 31% between 10-14, 19% between 15-17 and 6% were older than 18 years of age. A median value of 55 $\mu\text{mol/L}$ (range: 16.5 $\mu\text{mol/L}$ - 138.6 $\mu\text{mol/L}$) was observed for plasma Tyr value (MS/MS) at the time of supplementation. All PKU patients were observed to have adequate (90 to 110%) intake of Ca, Fe and Zn[20] (Table 1).

According to the Phe values found, it was observed that the cohort maintained Phe levels within levels recommended for their age (Figure 2). We observed that the group between 2 and 14 years of age maintained good metabolic control during the evaluation period (Figure 2). However, for those 15 to 17 years of age, median Phe value was 354 $\mu\text{mol/L}$ (95% CI 336 - 484) and 522 $\mu\text{mol/L}$ (95% CI 468 - 636) in those over 18 years. It is important to point out that 70% and 57%, respectively of these age groups keep their controls up to date (Figure 2).

In this analysis, the metabolic status of the group of women of childbearing age with PKU was also assessed. Those between 10 and 14, 15 to 17, and >18 years of age had median Phe levels of: 306 $\mu\text{mol/L}$ (95% CI 270 - 384), 342 $\mu\text{mol/L}$ (95% CI 270 - 384) and 492 $\mu\text{mol/L}$ (95% CI 390 - 576), respectively. Among women between 10 and 18 years of age (n = 56), 44% had a Phe value above 360 $\mu\text{mol/L}$ and 62% among women over 18 years of age (Figure 3.B). No significant difference was found when comparing median Phe level by sex, even after separating by age group (Figure 3.A, 3.B).

Table 1. Intake analysis for the active PKU group, separated by age group.

	0-1 years	1-3 years	4-9 years	10-14 years	15-17 years	≥ 18 years
Calories per kg of weight (kcal/kg)	110 (87 - 140)	78 (68 - 94)	50 (47 - 56)	34 (32 - 39)	29 (26 - 37)	25 (24 - 29)
Protein intake (g/kg weight)	2.6 (2.2 - 2.8)	2.6 (2.4 - 2.7)	2.0 (1.9 - 2.2)	1.6 (1.6 - 1.7)	1.5 (1.4 - 1.6)	1.4 (1.3 - 1.5)
Phe intake (mg/day)	312 (181 - 431)	274 (245 - 441)	410 (408 - 564)	408 (373 - 569)	491 (370 - 911)	457 (498 - 813)
Calcium, % adequacy	231 (180 - 318)	127 (118 - 144)	143 (130 - 155)	161 (156 - 190)	189 (166 - 201)	217 (213 - 275)
Iron, % adequacy	106 (83 - 129)	132 (130 - 195)	214 (207 - 245)	215 (216 - 296)	215 (185 - 259)	241 (231 - 312)
Zinc, % adequacy	279 (199 - 368)	170 (193 - 348)	188 (201 - 269)	205 (204 - 272)	228 (198 - 269)	211 (190 - 250)

24-hour dietary recall provided in the nutritional consultation for each individual. Calculation performed by Aminoacid Analyzer software (AAA).

Records of 185 subjects were obtained.

All variables had a non-normal distribution. Values represented by the median (95% CI).

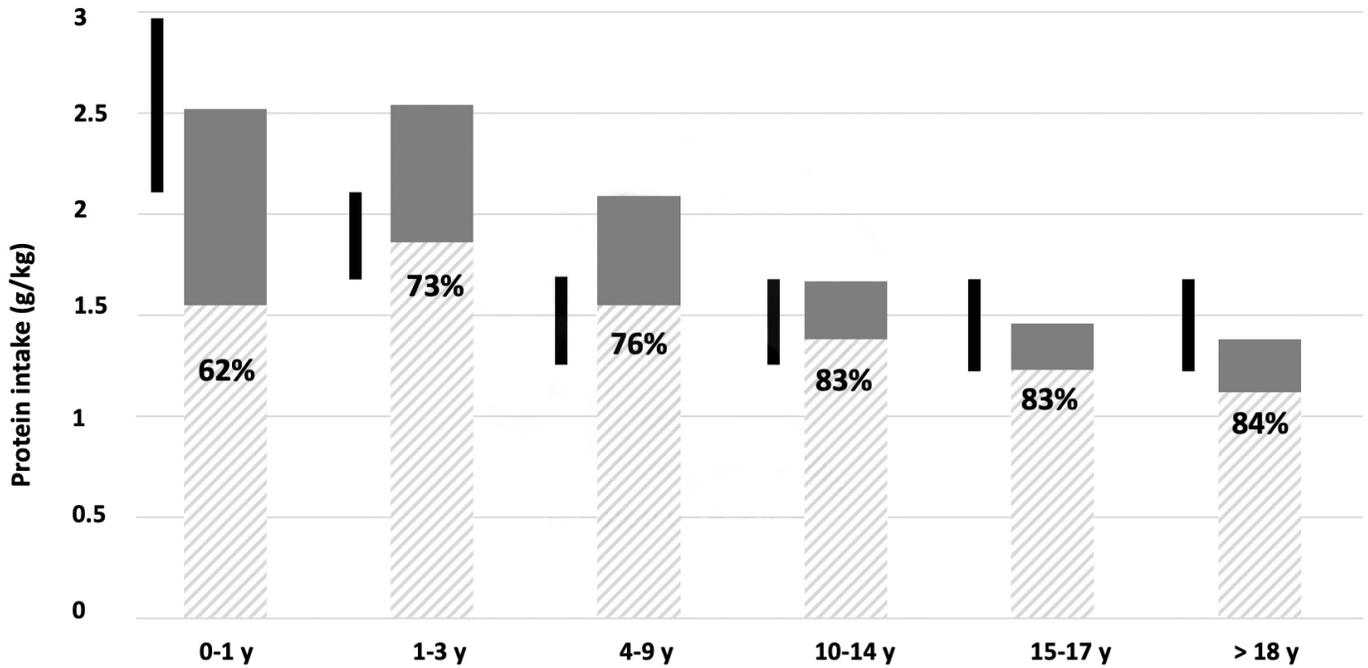


Figure 1. Protein intake by group. Black lines represent protein recommendations per protocol by age group. Each column represents 100% of average protein intake group; shaded regions represent the percentage of the total intake from the protein substitute.

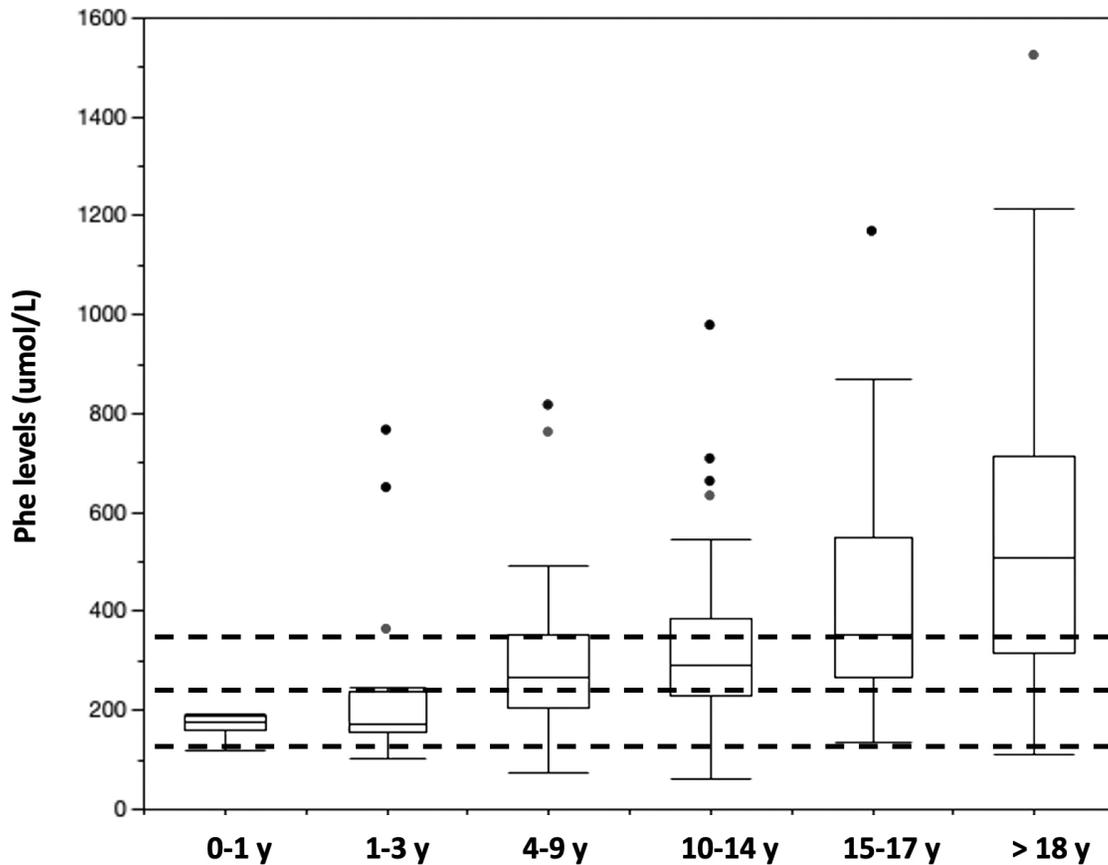


Figure 2. Boxplots of Phe values in blood by age group. Each boxplot represent the 25, 50 and 75 quartiles of Phe level for each group. Dotted lines represent permitted upper and lower Phe levels permitted according to the protocol (<2y: 120-240 umol/L; >2y: 120-360 umol/L).

Considering that the evaluation of neuropsychological function is one of the most important variables in long-term follow-up among people with PKU, it was observed that children aged 1 to 3 years had an average MDI of 82 ± 16 points. For IQ, the 4-9-year-old group had an average an FSIQ of 93.9 ± 12.3 points; 96.4 ± 13.5 points among subjects aged 10-14

years; 88.9 ± 17.6 for those between 15-17 years; and 88.6 ± 13.2 points for those over 18 years. The FSIQ obtained in the last psychometric evaluation was negatively and significantly correlated with average Phe level for the period considered for the analysis (Rho = -0.311; p-value <0.001; 95% CI: -0.5; -0.2; n = 154) (Figure 4).

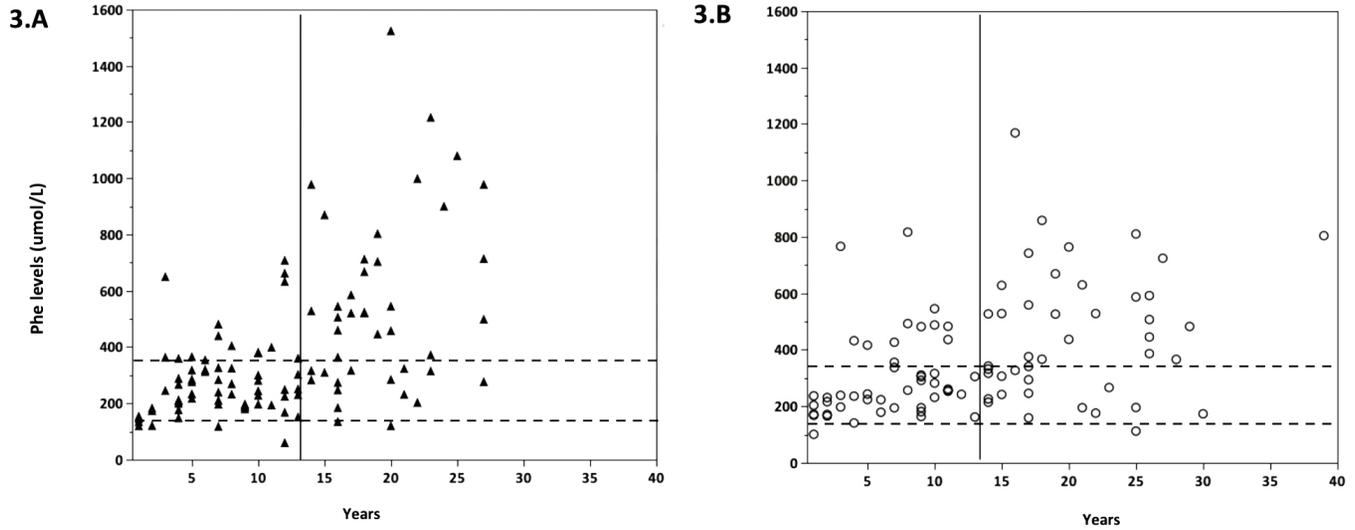


Figure 3. Dispersion graph of Phe level by sex. 3A. Black triangles represent male subjects. 3B. White circles represent female subjects. The vertical line separates < 14 years of age and ≥ 14 years. Permitted levels of Phe according to the protocol (<2y: 120-240 umol/L; >2y: 120-360 umol/L) is represented between the dotted lines.

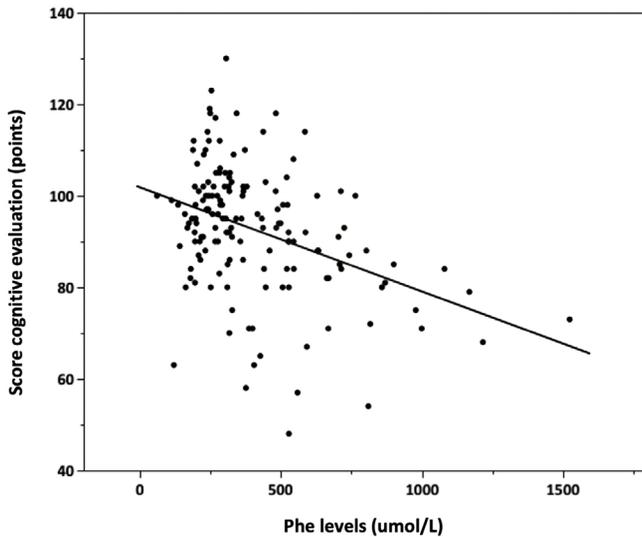


Figure 4. Spearman's correlation between average Phe level (mg/dL) calculated for each subject with the score obtained from the last evaluation of neuropsychological functioning, considering subjects over 4 years of age and who had both values (n= 154). Rho: -0,31; p <0.001; 95% CI: -0.5, -0.2.

Discussion

The national NBS programs for PKU and CH in Chile has been a pioneer, jointly with Cuba, Costa Rica and Uruguay have pioneered the implementation of this type of programs in Latin America for the last 28 years.[27] Currently there are 9 countries in the region with a similar program underway, who together developed of the Latin American Consensus Guide for Newborn Screening of PKU in 2016.[28]

Since the last published update of the Chilean PKU cohort in 2010[3], we observed that the age of diagnosis has decreased from 20 ± 8 days to 17 ± 8 days of age, with treatment starting before one month of life. In relation to Phe levels at the time of diagnosis, in 2010, Phe level was on average 1194 ± 540 umol/L. In the current study, we observed levels of 1122 ± 540 umol/L (95% CI: 1056 - 1182), which allows us to confirm that after 10 years of development, the NBS maintains stable and a good coverage in Chile.

The current population was distributed across age groups. We observed that the highest percentage of children is found in the group between 4 and 9 years of age (23%) and in the group over 18 (33%).

In 2017, after more than 30 years of experience in the treatment of PKU, the Chilean protocol for the follow-up for this population was published, establishing 360 $\mu\text{mol/L}$ as the Phe level to start a Phe-restricted diet. Values of Phe permitted during follow-up and the critical nutrient requirements in different age groups, according to international recommendations were also determined.[6]

Of the 271 subjects diagnosed with PKU, 76% (205 subjects) belonged to the active PKU in the period considered for the current analysis. In our active PKU group, 44% were found to be either overweight or obese. In 2015, an abstract was presented at the Latin American Society for Inborn Errors of Metabolism and Neonatal Screening (SLEIMPN) conference, where the nutritional status of the PKU cohort in 2015 was reported. At that time, 35% of the cohort had overweight or obesity.[29] In the current study, conducted 5 years later, we observed that overweight and obesity increased by 9%. This result is in accordance with the current obesity pandemic in Chile. In the most recent reports of the National Health Survey in Chile carried out in 2017 and 2018, it was reported that 74.2% of the adult population over 18 years of age and 52% of schoolchildren under 15 years of age were overweight or obese.[30,31] Scientific evidence has indicated that PKU patients, compared to the non-PKU population, have a prevalence ratio of 2.06 for obesity.[32] Therefore, especially in the pediatric population, it should be a top priority to take these data into account to avoid the development of obesity at all life stages and the risk factors for triggering associated pathologies, such as cardiovascular diseases, insulin resistance, or Diabetes Mellitus, among others. The importance of conducting nutrition education and incorporating healthy lifestyles in people with PKU is emphasized to prevent diseases associated with excess malnutrition, which could affect health and long-term quality of life.[33,34]

Regarding protein, Phe, Tyr and micronutrient intake, the recommendations of the Chilean protocol[6] and the protocol published by Singh *et al.*[5] were followed. We know that meeting protein requirements from natural foods is not possible, and, thus, the PS without Phe is essential to achieve growth and development within the normal range. Prior to 2017, the Chilean government subsidized the PS without Phe until 18 years of age, but now the subsidy is for life. In our cohort, among those over 18 years of age, we found that 85% of the total protein ingested comes from the PS without Phe, becoming an essential product to achieve satiety and maintain good metabolic control. In children with PKU under one year of age, this percentage is 60%, because, during this period of accelerated growth, the Phe requirements are higher, and these requirements are mainly covered by intact protein from breast milk or infant formula.[9,35] The PS without Phe not only provides quality protein, but also covers the recommendations of Tyr, vitamins and minerals.[10,36,37] In our active PKU cohort, when comparing Ca, Fe and Zn intake with international recommendations, we observed that all received contributions over 110% adequacy according to RDA.

The use of adjuvant treatments in PKU with low or no adherence to dietary treatment is recommended. However, we must point out that in Chile this cannot be applied, because the government does not cover the costs of BH₄, Large neutral amino acids (LNAA), Glycomacropeptide (GMP). The Chilean protocol considers good metabolic control when patients under 2 years of age with PKU have Phe values between 120 – 240 $\mu\text{mol/L}$. This more restricted range is due to the fact that this group has a greater metabolic fluctuation due to the mandatory vaccination program in Chile and/or intercurrent infections, which induce catabolism and increase Phe concentration. The period under 2 years of age is considered a critical period for brain development, thus lower concentrations of Phe allows a better control over increases. For PKU patients older than 2 years of age, good metabolic control is defined as Phe values between 120 - 360 $\mu\text{mol/L}$.

Scientific evidence highlights that in adolescence and adulthood, adherence to treatment decreases with age, which signifies a progressive increases in Phe values and risk of damage to central nervous system. Burton *et al.* demonstrated in 2002 that 75% of adolescents had high Phe levels and more than 80% had low adherence or had abandoned treatment in adulthood.[38] In 2017, in a survey carried out among approximately half of the PKU patients seen in clinics in the USA, it was observed that more than 60% of adolescents aged 13 to 17 did not comply with recommendations given, which translated into Phe values over those recommended.[39] In our cohort, considering the group between 10 to 17 years of age, 70% of this group maintained their metabolic control below 360 $\mu\text{mol/L}$.

In the European PKU cohort, those over 18 years of age had an average Phe level of 960 $\mu\text{mol/L}$ with low adherence to the indicated treatment.[40] Among those in our cohort over 18 years of age, we found that 35% maintained diet compliance with Phe values recommended by the protocols.[5,10,41] The average Phe level in blood was 546 ± 294 $\mu\text{mol/L}$, which is close to the maximum level allowed by the European protocol.[10,41] However, it is important to remember that before 2017, the subsidy for PS without Phe ended at 18 years of age. Therefore, many of these adult patients with PKU have now resumed nutritional treatment. Since the changes in the policy, dietary compliance has increased from 35 to 55%.

Another important point to discuss is the adherence and good metabolic control that female PKU patients should have, since Phe levels over 360 $\mu\text{mol/L}$ during pregnancy is strongly associated with congenital malformations, microcephaly, intrauterine growth retardation, low birth weight, congenital heart disease, facial dysmorphism and intellectual disability in the unborn child.[42] In our cohort, 67% of women were of childbearing age and, of them, 44% had a Phe level above 360 $\mu\text{mol/L}$. For this reason, we are implementing new strategies, such as a specialized clinic, a PKU protocol, educational workshops and talks to improve adherence and prevent sequelae.

The main objective of proposing a protocol for the diagnosis and monitoring of PKU is to prevent intellectual disability

in diagnosed individuals and ensure optimal growth and development. In the current analysis, carried out in the active PKU Chilean cohort, we evaluated psychomotor development among children under 4 years of age and obtained the results for 22 subjects between 1 - 3 years and only 1 individual between 6 - 12 months, out of a total of 31 individuals considered in these age groups. We observed an average MDI score of 81.9 ± 15.8 which is close to the lower limit of normality (80 points).[22]

High levels of Phe alter neurotransmission and brain protein synthesis due to the imbalance of LNAA, with a consequent reduction in serotonin and dopamine synthesis. In addition, there is a structural effect on dendritic arborization, synaptic density, as well as alterations in postnatal myelination in specific areas such as the prefrontal cortex. These changes would explain the alteration in executive functions, attention, emotional and behavioral disorders observed in untreated PKU patients.[43]

In the sub-set of active PKU participants older than 4 years of age, 85% had a normal IQ (normal range: 80 - 120 points), 6% were classified as borderline (range: 79 - 70 points) and 8% classified with intellectual disability, mostly in the mild range (range: 69 - 55 points). Supporting this result, when correlating the scores obtained in the cognitive evaluations and blood Phe level we observed a negative association. Therefore, keeping Phe levels under 360 $\mu\text{mol/L}$, avoiding fluctuations, will favor the correct neuropsychological functioning of individuals with PKU.[40,44] More than 80% of the sample had neuropsychological functioning within the normal range, likely a result of good Phe level control.

Governments or institutions considering implementing NBS programs for PKU should include a centralized clinical follow-up program, which uses validated protocols, and conducts evaluations according to age. PKU is a chronic pathology, which will require maintenance of a good lifelong dietary adherence to ensure that patients are actively incorporated into society.[45]

Conclusions

We know that early diagnosis of PKU reduces the risk of Phe toxicity at the brain level, which prevents intellectual disability. Implementation of the NBS program in Chile in 1992, the subsidizing of the PS without Phe, the creation and publication of the Chilean protocol for the management of PKU, based on more than 30 years of experience and on the protocols published by the United States[5] and the European Union[10,41], have allowed for the monitoring of the Chilean cohort.

Analyses carried out for the period 2010-2020 show us that the NBS program has had little variation in relation to age of diagnosis, allowing early diagnostic confirmation and the start of treatment before one month of life. Overweight/obesity must be addressed in advance to avoid obesity later in life and reduce the risks of triggering diseases such as cardiovascular disease, insulin resistance and diabetes mellitus.

With respect to protein intake, approximately 80% of the total protein ingested comes from the PS without Phe, therefore, it is vitally important to promote its use at all stages of life.

Eighty percent of the Chilean cohort maintains their medical/nutritional, metabolic and cognitive follow-up appointments. This is due to the comprehensive and constant education that families and patients with PKU receive by the multidisciplinary team and the commitment acquired by families from the start of treatment.

We emphasize the importance of a reference center, preferably a centralized one, as in Chile, since in this way it is possible to have and apply a protocol in a unified way. In addition, it is fundamental to provide education throughout the life cycle, which is a substantial part of the comprehensive monitoring program carried out by the multidisciplinary team in Chile.

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Declaration of Conflicting Interests

No conflict of interests.

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