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Original Article

Conventional Phenylketonuria Treatment

Journal of Inborn Errors of Metabolism & Screening
2016, Volume 4: 1–7
© The Author(s) 2016
DOI: 10.1177/2326409816685733
journals.sagepub.com/home/iem
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Abstract

Phenylketonuria (PKU) is caused by a deficient activity of enzyme phenylalanine (Phe) hydroxylase, which results in high Phe blood concentration, which is toxic to the central nervous system. The fundamental purpose of nutritional treatment is to reduce and maintain blood Phe between 2 mg/dL (120 μmol/L) and 6 mg/dL (360 μmol/L) in order to prevent neuropathogenic complications. At the same time, nutrition support must provide enough energy and nutrients to promote normal growth and development and also to avoid vitamin and mineral deficiencies. Phenylketonuria treatment must be maintained long-life and its adherence must be frequently assessed. The amount of Phe required by patients with PKU varies throughout life and must be adjusted according to individual tolerance, residual phenylalanine hydroxylase enzymatic activity, age, sex, growth rate, protein intake, and nutritional and biochemical status among others. Treatment must be done by trained personnel. It is necessary to unify treatment criteria and further research must be done.

Keywords

phenylketonuria, phenylalanine hydroxylase deficiency, nutritional treatment, conventional treatment, Latin America

Introduction

Phenylketonuria (PKU; Online Mendelian Inheritance in Man (OMIM) 261600; *International Classification of Diseases, Thenth Revision (ICD-10)* E70.0) is an inborn error of amino acid metabolism caused by a deficient activity of phenylalanine hydroxylase (PAH), an enzyme that converts essential amino acid phenylalanine (Phe) into tyrosine (Tyr), a nonessential amino acid that becomes essential in PKU. This enzymatic failure results in high blood concentration of Phe, which is toxic to the central nervous system. The natural history of PKU consists in a progressive irreversible encephalopathy, the most common outcome is severe mental retardation and other neurological complications, such as epilepsy, tremor, hyperactivity, autistic features, and behavioral problems, such as aggressiveness, stereotypy, anxiety, and phobias, among others. "Mousy odor," eczema, and hypopigmentation of hair, skin, and iris are also characteristic. From the early 60s in developed countries, this disease is routinely detected through newborn screening (NBS), which allows early treatment and prevention of neurological damage.[1]

Phenylketonuria was the first genetic disease that had specific treatment, becoming a model for the management of other metabolic disorders.[2] The idea that PKU should be treated with a low Phe diet arose in the 1930s. The first attempt was made by Lionel Penrose, with a diet composed solely on fruit, sugar, olive oil, and vitamins, but it led to malnutrition and had to be abandoned because it was nutritionally inadequate.[3] Phenylalanine is an ubiquitous amino acid, present in almost all proteins, so the design of a low Phe diet meant that normal protein sources had to be eliminated from diet and replaced by an artificial amino acid source containing a low amount of this amino acid. This strategy was developed for the first time by Woolf in the late 1940s, removing Phe from casein hydrolysates using columns with activated charcoal and adding Tyr and tryptophan.[4] Woolf suggested this nutritional approach to Bickel and colleagues that proved it for the first time in 1951 at the Birmingham Children's Hospital on an Irish descendent, a 26-month-old patient called Sheila, with positive biochemical and clinical results.[5,6] Initially,

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Received June 17, 2016, and in revised form September 19, 2016. Accepted for publication November 15, 2016.

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Woolf's formula was used along with vegetables, fruit, and gluten-free bread and flour; later on, researchers underline that it had to be supplemented with Tyr, tryptophan, methionine, minerals, vitamins, polyunsaturated fatty acids, and carbohydrates in appropriate amounts.[7] Since then, dietary treatment of PKU has continuously evolved and is still the cornerstone of management in almost all the world. The aim of this work is to describe the fundamental basis of conventional nutritional treatment for PKU.

Goal of Nutrition Management

The fundamental purpose of nutritional treatment is to reduce and, later on, to maintain blood Phe concentrations between 2 mg/dL (120 μmol/L) to 6 mg/dL (360 μmol/L) in order to prevent neuropathogenic complications. At the same time, nutrition support must provide enough energy and nutrients to promote normal growth and development and also to avoid vitamin and mineral deficiencies.[8,9] Phenylketonuria treatment must be maintained long-life and its adherence must be frequently assessed.[10]

Phenylalanine Requirements and Dietary Sources

There are 20 amino acids that can be found in proteins. Eleven of them are nonessential or dispensable. Phenylalanine is 1 of the 9 essential amino acids, which the body cannot synthesize, and therefore it must be provided by food. Phenylalanine is a part of almost all proteins in the body and is indispensable for brain functioning, as its hydroxylation produces Tyr, which is an important neurotransmitter precursor. That is why Phe intake in patients with PKU should be restricted but not completely erased from diet because it is an indispensable amino acid. The total long-term Phe elimination results in death, and an excessive restriction may cause growth failure, weight loss or poor weight gain, erythema, skin desquamation, alopecia, aminoaciduria, hypoproteinemia, anemia, changes in bone tissue, mental retardation, severe malnutrition, keratomalacia, prolonged diarrhea, immunosuppression, and, in extreme cases, ulcers or corneal perforation, among others.[11,12]

Some treatment protocols suggest, at the beginning of the diagnosis, to eliminate Phe depending on its initial plasma concentration; usually, higher levels will require longer time to reduce, but this should be closely monitored day to day with blood samples in order to achieve and maintain target blood Phe levels (2-6 mg%).[13,14] Other authors suggest that only with initial blood Phe levels of 1500 μmol/L or higher, Phe should be eliminated for 48 hours.[15] Phenylalanine elimination might not be necessary if diagnosis is done by NBS on the first days of life, but each patient should be carefully evaluated.

A Phe dietary source should be given once the blood levels have decreased; the selection of the source depends on the availability, costs, and age of the patients. When initiating PKU treatment in the first year of life, human milk should be used as first choice because of its widely recognized benefits; breast feeding strengthens the emotional bond between mother and child, improves adherence to treatment, and decreases its economic impact.[16] Moreover, in comparison to the amino acid profile of any standard commercial formula, human milk has a lower content of Phe in the same amount of protein.[13] Preferably, human milk can be administrated directly from breast and be complemented with the Phe-free medical formula; when this is not possible, both can be mixed in 1 bottle.[17] To monitor adequate breast milk intake, follow-up blood samples must be collected preferentially at the same hour of day, and if possible, 2 to 4 hours after a meal, not right after eating, because Phe would be higher.[9,18–20]

Table 1. Recommended Daily Phe, Tyr, Protein, and Energy at the Beginning of the Nutritional Therapy in Patients With PKU.

Age	Phe (mg/d)	Tyr (mg/d)	Protein (g/kg/d)	Energy (kcal/kg/d)
0-3 months	130-430	1100-1300	3-3.5	95-145
3-6 months	135-400	1400-2100	3-3.5	95-145
6-9 months	145-370	2500-3000	2.5-3	80-135
9-12 months	135-330	2500-3000	2.5-3 (grams/d)	80-135 kcal/d
1-4 years	200-320	2800-3500	>30	900-1800
4-7 years	200-400	3200-4000	>35	1300-2300
7-11 years	220-500	4000-5000	>40	1650-3300
11-19 years	220-1000	5200-6500	50-65	1500-3900
Adults	220-1100	5600-7000	50-65	1400-3300

Abbreviations: Phe, phenylalanine; PKU, phenylketonuria; Tyr, tyrosine.[9,13]

In general terms, the amount of Phe required by patients with PKU varies throughout life (Table 1) and must be adjusted according to individual tolerance, residual PAH enzymatic activity, age, sex, growth rate, protein intake, and nutritional status, among others. In the first year of life, a full-term infant requires between 20 and 70 mg of Phe/kg body weight/day, the younger the baby is, the greater requirements will be; also premature babies need more Phe in order to maintain adequate growth. Individual modifications must be done and should include biochemical and clinical data.[15] Phenylalanine constitutes about 4% to 6% of total protein contained in food[21]; thus with a small amount, especially from food with a high content of protein, the requirement for this amino acid is covered. Due to the high content of Phe, protein that comes from animal sources is generally removed from the patients' diet, as well as other high-protein food sources such as legumes and oleaginous. Although cereals, fruits, and vegetables also contain Phe, the amount is lower compared to animal products. Therefore, these food groups are routinely and carefully included in the diet. Calculation of Phe contribution for each food should be made.[22] Almost each country has their own food lists that report the estimated content of Phe, Tyr, and protein. To make dietary prescriptions easier and give more variety, an "equivalent system" has been created, which brings together different food groups that contain the same amount of Phe. A food equivalent is a specific portion that has a similar amount of nutrients, especially Phe, Tyr, protein, and energy. This allows food to be exchanged within a list with multiple options, with

Table 2. Advantages and Disadvantages in Different Dietary Systems Worldwide for PKU Treatment.

System	Definition	Advantages	Disadvantages
Phe exchange list per 15 mg	Food list in which each food in different grams/ portion has 15 mg of Phe, and they are exchangeable	• More control in the amount of food • Easier to establish Phe intake and Phe blood levels • Only 1 food list • Autonomy in selecting their own food • Pick according to availability, preference, costs, and culture	• Patients could only eat 1 or 2 foods to complete Phe requirement and have deficiencies in carbohydrates, fat, vitamins, and minerals because they don't eat different food groups, no variety • Difficult to weight and measure food, arduous, and impractical • Some patients might not understand the exchange concept • Limited information on Phe content of some commercial foods • More restrictive
Phe exchange list per 50 mg	Food list in which each food in different grams has 50 mg of Phe, and they are exchangeable	• More control in the amount of food • Easier to establish Phe intake and Phe blood levels • Only 1 food list • Pick according to availability, preference, costs, and culture • Fruits and vegetables containing less than 75 mg of Phe/100 g are free	• Patients could only eat 1 or 2 foods to complete Phe requirement and have deficiencies in carbohydrates, fat, vitamins, and minerals because they don't eat different food groups, no variety • Difficult to weight and measure food, arduous, and impractical • Some patients might not understand the exchange concept • Patients may eat a significant quantity of extra Phe, protein, or energy from free foods
Phe exchange list by food group	Each food group has their own amount for the exchange, for example, cereals 30 mg, fruit and vegetables 15 mg	• More food group balance/equilibrium • Dietitian will calculate exact amounts of exchanges from vegetables, fruits, and cereals, variety • Pick according to availability, preference, costs, and culture	• Patients could only select 1 option from each food group and have no variety • Difficult to weight and measure food, arduous, and impractical • Some patients might not understand the exchange concept
Counting total protein	Food lists with only protein content no Phe, patients count total grams of protein per day	• More food lists available to count protein • More variety on products that has only the protein content • Less rigid diet	• Amount of Phe per gram of protein depends on the type of food and could have important variations • Difficult to weight and measure food, arduous, and impractical
Food lists with allowed and prohibited	Only one list with allowed and prohibited	• Variety • No measuring • Easier to understand • More freedom	• Difficult to establish relation between Phe intake and Phe blood levels • Not individualization • Inadequate portions

Abbreviations: Phe, phenylalanine, PKU, phenylketonuria.

the advantage that the patient and family can choose different food groups according to their preference, economic status, culture, availability, and diet prescription.[15] In other countries, counting grams of intact protein is done as indirect control of the amount of Phe.[23] In some other clinics, only recommendations of foods list are given to avoid high contents of Phe, and also free food lists are available, which includes fruits and vegetables that are not considered important sources of Phe.[21,24] All these approaches have advantages and disadvantages, and some of them are shown in Table 2. Ahring et al reported the experience of 10 centers treating PKU in Europe analyzing 1927 patients with different systems of counting Phe, and no differences were found between methods regarding PKU control; it is important to notice that each center had different dietary managements and also their own Phe blood targets.[25] Similar results were found in Europe by comparing 5 different dietary regimes to treat PKU; all of the patients had good metabolic control, which was defined as 240 µmol/L for patients younger than 10 years and 900 µmol/L for older patients. The group that followed the exact calculation of the Phe content in all of their food was the one with a median Phe concentration of 360 µmol/L; in other groups, Phe concentrations were higher than 532 µmol/L.[26] In Australia, Sweeney et al compare different methods of counting Phe, counting 1 gram of protein = 50 mg (food with less than 50 mg were considered free) with 1 unit = 15 mg of Phe; no significant changes in Phe among groups were found, and in their clinic, patients preferred to use grams of protein.[27] Different cutoff points are considered in our clinic, 360 µmol/L regardless of age,[2,9,14,15] which are lower than the ones described above;

evidence is still necessary in Latin America where no surveys in different countries have been done on this matter.

Tyrosine in PKU

Tyrosine is a nonessential amino acid as it comes from Phe hydroxylation; however, in patients with PKU, it becomes essential because it cannot be synthesized. It is important to provide an adequate supply of Tyr, as either a Phe-free supplemented formula or Tyr supplement to maintain blood levels in normal ranges. The Tyr deficiency can decrease dopamine, noradrenaline, and melanin synthesis. To ensure an adequate supply, about 8% to 10% of total protein calculated in the diet must come from Tyr.[15] There are also age-specific Tyr recommendations, and it is suggested to calculate the contribution from medical Phe-free formula plus the contribution of food used as Phe source and most importantly to have Tyr blood concentrations frequently monitored. It is pertinent to take into account that water solubility of this amino acid is poor, so it could precipitate and form a waste residue at the bottom of the container in which medical formula was prepared, so parents and patients must shake well the mixture to ensure the calculated Tyr intake.

Protein Recommendations in PKU

There are recommended daily intakes (RDIs) for total protein in patients with PKU (Table 1); however, optimal dosage depends on individual needs. Achieving optimal protein intake to maintain adequate levels of lean body mass is possible only with close biochemical and nutritional monitoring.

The main source of protein and nitrogen in patients with PKU must be the Phe-free formula, and it is suggested that formula should represent between 70% and 85% of total protein requirements in patients with a severe form of the disease; hence, their use is indispensable and essential for an adequate control, growth, and development. MacDonald et al studied 2 groups of patients with PKU with different amounts of Phe-free protein substitute, with and 1.2 g/kg/d, respectively. The higher dosage of protein was associated with lower blood Phe concentrations; however, variations were observed depending on its carbohydrate content.[28] The Phe-free formula must be carefully selected because variations are wide in terms of carbohydrates, protein, fats, and Tyr contents.[29]

Formula distribution throughout the day is important and a minimum intake of 3 servings is recommended; when it is given as a single dose, urinary nitrogen excretion, protein catabolism, and amino acids oxidation could increase with a concomitant protein synthesis decline.[15,30]

Regarding the natural protein intake, this one accounts for 15% to 30% of the total protein recommendation and must come from different food groups to provide variety; titration to give the maximum amount is needed because it is related to benefits such as better muscle mass, vitamin, and mineral status, among others.[31]

Lipids and PKU

Several studies have reported lower levels of total cholesterol in untreated patients with PKU, and there are different hypotheses for this; the first one is a low intake through diet because it is devoid of animal foods, which are the primary sources of cholesterol. Another explanation is that high levels of Phe are associated with impairment of cholesterol synthesis due to downregulated expression of 3-hydroxy-3-methylglutaryl-CoA reductase and inhibition of mevalonate 5-pyrophosphate decarboxylase, besides the high consumption of acetyl CoA to synthesize phenylacetylglutamine.[15,32] Recent data support that high Phe levels rather than an effect of a low protein diet lead to hypocholesterolemia in patients with PKU.[33]

Low plasma total concentrations of linolenic acid, arachidonic acid (AA), and significantly reduced docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) have been demonstrated in patients with PKU.[34] Phenylketonuria diet is deficient in essential fatty acids; AA, DHA, and EPA supplementation and observance are necessary since these fatty acids play an important role in the brain and retina. Koletzko et al conducted a study supplementing 36 children with PKU with fish oil, providing a daily dose of 15 mg DHA/kg/d, founding a significantly faster visual-evoked potential latencies and an improvement in motor function and coordination.[35] Increased DHA and AA concentrations have been reported after administration of 200 mg of AA and 100 mg of DHA in a powdered blend to patients with PKU.[36]

Bosdet et al found high levels of linolenic acid and low DHA in PKU adults.[37] A systematic review of literature showed that supplementation with DHA may be an effective way to increase the omega-3 long chain polyunsaturated fatty acid status.[34] Even though the number of commercial Phe-free formulas supplemented with fatty acids has increased, currently some of them contain linoleic and linolenic acid but they do not have DHA, so it is important that health-care personnel treating PKU be aware of this situation to consider an optimal supplementation dosage.

Carbohydrate Recommendation in PKU Diet

Carbohydrate intake stimulates insulin secretion; this hormone increases protein synthesis by increasing amino acid transport into cells, so dietary inclusion of carbohydrate is essential for patients with PKU. The same percentage as in children without PKU is recommended, between 55% and 60% of total energy intake. A group of French researchers concluded that carbohydrates in the diet significantly decreased the postprandial catabolism of proteins to urea. Carbohydrates halved the oxidative peak of dietary nitrogen during the first 2 hours after a meal. The net postprandial protein utilization improved by 5% and nitrogen retention by 14% when carbohydrates, not fat, are ingested in combination with protein.[38] This suggests the importance of a specific macronutrient calculation and the utility of providing medical formula along with carbohydrates. The quality of carbohydrate food sources should be supervised, avoiding sugar, high glycemic index foods, or low fiber content, in order to maintain adequate nutritional status.[39,40]

Vitamin and Mineral Supplementation in PKU

Research in micronutrients has always been studied since the first formula appeared.[4] Intake of minerals and vitamins in patients with PKU must be greater than Recommended Dietary Allowances (RDAs) by age, in order to prevent deficiencies, which are mainly caused by restriction of natural food protein sources, lack of adherence to nutritional treatment, low bioavailability, lack of micronutrients added to medical formulas, or dietary supplements, among others.[15,22] It is essential to take into consideration the important differences in nutrients and also vitamins and minerals that different Phe-free formulas have. Recommended daily intakes for patients ingesting elemental diets have been published; as mentioned above, nutritional requirements of minerals and vitamins are higher than the RDAs for normal population, hence the importance of frequent biochemical and nutritional assessment, because even with a 100% RDI ingestion of minerals and vitamins, individual deficiencies could still come up. There are evidences of a greater fecal loss than dietary intake of copper, iron, and zinc in patients with PKU, who received elemental diets. Serum levels below the normal range of inorganic nutrients such as copper, iron, selenium, and zinc in patients with PKU have been reported.[15,41] There is evidence that high Phe concentrations can modify the metabolism of iron, copper, and zinc, as well as negatively affect bone status in PKU rats.[42,43]

Bone mineral density (BMD) is determined by different factors such as calcium and phosphorous intake, vitamin D levels, physical activity, and medical conditions, among others. The relationship between BMD and PKU has been described in several meta-analysis and systematic reviews.[44–46]

Some data suggest that bone health in children with PKU is affected by the disease itself.[47] But criteria for osteoporosis and osteopenia diagnosis in patients with PKU are needed, in order to make studies comparable and reach to more reliable conclusions. It has been found that BMD is lower in patients with PKU than in control subjects; however, many studies do not adjust measurements for height, and therefore, BMD may be underestimated.[46] They all agree that there is no evidence that suggests an association between plasma Phe concentrations and BMD[44–46]; nevertheless, according to Geiger et al, diet plays a crucial role in bone mineralization.[44] A systematic review of the literature found a fracture rate of 20% in patients with late diagnosed PKU.[46] Demirdas et al found that although BMD in patients with early diagnosed and treated PKU is lower compared to healthy controls, it remains in normal ranges.[45]

A recent study from Geiger et al found no evidence of low serum vitamin D or BMD in well-controlled patients with PKU with adequate intake of metabolic formula and compared with a control group had the same risk of vitamin D deficiency. The Phe-free formula constitutes the main source of vitamins and minerals for patients with PKU including calcium, phosphorus, and vitamin D (essential nutrients for bone health). An adequate compliance with nutritional treatment ensures an optimal supply of these nutrients, and hence, BMD is not affected.[44]

It is common to find vitamin B_{12} deficiency in patients with PKU. Low blood levels of vitamin B_{12} have been reported, especially in those who were not compliant with the nutritional treatment and do not include in their diet a Phe-free medical formula or animal protein, which are the main sources of this vitamin. Also, low serum levels of ferritin and transferrin receptors, which suggest iron deficiency, have been found.[48,49] As medical Phe-free formulas have been supplemented over the years, metabolism of niacin, biotin, and vitamin A has not shown biochemical or clinical deficiencies.

In order to calculate the contribution of inorganic nutrients and vitamins in the diet, it is necessary to collect information through a 24-hour diet recall of 3 consecutive days. Patients must write everything that they ate and drank and give the information to the metabolic dietitian that will evaluate the quantity and quality of the diet.

Energy Requirements

To achieve appropriate energy requirements, it is important to know that having PKU does not cause a reduction in resting energy expenditure (REE). There is a direct correlation between lean mass and REE, and no difference between a healthy control group and a PKU group regarding REE has been found.[50]

The use of free amino acids given as Phe-free medical formula in PKU as a main source of protein equivalent increases the total energy requirements in approximately 20% more than RDAs.[15] During the first year of life, most of the energy comes from the metabolic formula and this will decrease with the introduction of solid food, so energy requirements should be personalized. Anthropometric measurements and nutrition counseling must be done repeatedly in order to prevent overweight or obesity, which has been reported in different countries as a health malnutrition issue, especially in women with PKU.[39,40]

An observational, cross-sectional study was done with 36 female adolescents with PKU aged 14.2 ± 1.9 years, to evaluate the agreement of REE measured with indirect calorimetry with 6 predictive REE equations; conclusion was that most of the equations underestimate REE and Schofield equation had the highest level of agreement, within $\pm 10\%$ in 14 of the 36 participants.[51] Careful calculations and use of equations must be used with caution in order to predict accurate energy requirements in patients with PKU. It is also important to consider that weight loss rises plasma Phe levels, so this situation must be considered in obese patients with PKU.

Conclusion

Even when PKU was the first metabolic disease that had specific treatment, it is still a challenge for every metabolic clinic. Treatment must be done by expert personnel that takes into account energy, protein, minerals, vitamins, and requirements, among others. Individualized management and permanent surveillance are needed in order to achieve an optimal Phe blood

level and an adequate nutritional status, long life. It is necessary to unify treatment criteria, and further research must be done.

Declaration of Conflicting Interests

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

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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