

Dietary intervention in the management of phenylketonuria: current perspectives

Júlio César Rocha¹⁻³
Anita MacDonald⁴

¹Centro de Genética Médica,
Centro Hospitalar do Porto – CHP,

²Faculdade de Ciências da Saúde,
Universidade Fernando Pessoa,

³Center for Health Technology and
Services Research (CINTESIS), Porto,
Portugal;

⁴The Children's Hospital,
Birmingham, UK

Abstract: Phenylketonuria (PKU) is a well-described inborn error of amino acid metabolism that has been treated for >60 years. Enzyme deficiency causes accumulation of phenylalanine (Phe) and if left untreated will lead to profound and irreversible intellectual disability in most children. Traditionally, it has been managed with a low-Phe diet supplemented with a Phe-free protein substitute although newer treatment options mainly in combination with diet are available for some subgroups of patients with PKU, for example, sapropterin, large neutral amino acids, and glycomacropeptide. The diet consists of three parts: 1) severe restriction of dietary Phe; 2) replacement of non-Phe L-amino acids with a protein substitute commonly supplemented with essential fatty acids and other micronutrients; and 3) low-protein foods from fruits, some vegetables, sugars, fats and oil, and special low-protein foods (SLPF). The prescription of diet is challenging for health professionals. The high-carbohydrate diet supplied by a limited range of foods may program food preferences and contribute to obesity in later life. Abnormal tasting and satiety-promoting protein substitutes are administered to coincide with peak appetite times to ensure their consumption, but this practice may impede appetite for other important foods. Intermittent dosing of micronutrients when combined with L-amino acid supplements may lead to their poor bioavailability. Much work is required on the ideal nutritional profiling for special SLPF and Phe-free L-amino acid supplements. Although non-diet treatments are being studied, it is important to continue to fully understand all the consequences of diet therapy as it is likely to remain the foundation of therapy for many years.

Keywords: phenylalanine, PKU, L-amino acids, sapropterin, glycomacropeptide, large neutral amino acids

Introduction

Phenylketonuria (PKU) is a rare (prevalence varies from one in 2,600 to one in 200,000 births), autosomal recessive, inborn error of amino acid metabolism. It was first described by Asbjørn Følling in 1934.¹ It is caused by a deficiency of the hepatic enzyme, phenylalanine hydroxylase (PAH), which catalyzes the hydroxylation of phenylalanine (Phe) to tyrosine. Enzyme deficiency leads to accumulation of Phe resulting in hyperphenylalaninemia, increased phenylketones (hence PKU), and decreased myelin formation, dopamine, norepinephrine, and serotonin production. More than 950 gene variants have been identified and most individuals (~76%) with PKU are compound heterozygotes.² Without treatment most children develop profound and irreversible intellectual disability,³ seizures, hyperactive behavior with autistic features, psychiatric symptoms, eczema, musty body odor, and light pigmentation. However, PKU is identified in almost all countries in Europe by neonatal screening enabling

Correspondence: Júlio César Rocha
Centro de Genética Médica Doutor
Jacinto de Magalhães, Centro Hospitalar
do Porto – CHP, Praça Pedro Nunes, 88,
4099-028 Porto, Portugal
Tel +351 22 607 0339
Fax +351 22 607 0300
Email julio.rocha@chporto.min-saude.pt

treatment to commence in the majority of infants <14 days of age. Early treated patients have normal intellectual quotients although there is close association with lower intellectual quotient and poor quality of blood Phe control.⁴

The main stay of treatment is a lifelong low-Phe diet. The aim of dietary treatment is to prevent excessive Phe accumulation in the blood by strict control of natural protein intake in combination with the administration of a Phe-free protein substitute, usually based on Phe-free L-amino acids. Alternative or complementary treatments include the use of sapropterin dihydrochloride (a synthetic form of tetrahydrobiopterin [BH4]); large neutral amino acids [LNAA], and glycomacropeptide (GMP) (a protein replacement derived from cheese whey). Pegylated recombinant phenylalanine ammonia lyase is an investigational study drug that substitutes the PAH enzyme by breaking down Phe developed in adult patients with PKU. It is undergoing Phase III clinical trials.

Diet construction – dilemmas

Because the liver is unable to metabolize any excess Phe, dietary treatment is restricted in natural protein in order to reduce blood Phe accumulation.⁵ The magnitude of natural protein restriction required to prevent elevated blood Phe concentrations could cause serious nutritional deficits without protein supplementation. Therefore, protein substitutes are prescribed to compensate for natural protein intake restriction providing the main protein source. They are also supplemented with micronutrients.⁶ In addition, the pathophysiology of PKU is mainly located in the brain and protein substitutes may have an important function in balancing the transport of amino acids across the blood–brain barrier.⁷ The resulting effect is mainly to prevent excessive Phe entrance into the brain combined with reduced availability of other LNAA that share the same amino acid transporter-1.⁸

A regular healthy diet is composed of great food diversity and protein needs are usually supplied throughout the day, in every meal. In PKU, low-Phe containing foods (eg, low-protein bread or pasta referred to as SLPF) commonly constitute the main food sources at each meal. The protein substitute is usually given at mealtime at least three times per day⁹ in order to optimize its metabolism.^{9,10} There is some scientific evidence that the rate of absorption of amino acids is more rapid than intact protein sources (eg, casein).¹¹ Some authors suggest that this causes higher urine nitrogen losses because rapid amino acid appearance in the blood will not allow long-term sustained anabolism.^{9,10} The taste and flavors of protein substitutes are also suboptimal, despite important

improvements in recent years.¹² As a consequence, adherence with protein substitute is commonly suboptimal.¹³

It is advised to divide the total daily amount of prescribed protein substitute between several meals, in order to promote anabolism, that is, to move free amino acids toward protein synthesis rather than to the oxidative route.^{9,10} Anabolism promotion requires free amino acids to be given in combination with energy sources (lipids and carbohydrates). Many protein substitutes contain nonprotein energy sources as well as amino acids. As recently highlighted,¹² the composition of different protein substitutes is variable and it is important to consider the full nutritional composition to ensure that it is optimal to help promote anabolism.

After neonatal diagnosis, dietary treatment is implemented immediately. Breast-feeding (directly from the breast) is combined with infant Phe-free infant formula.⁵ In practice, a measured volume of an infant Phe-free infant formula is given before each breast-feed. The amount of infant Phe-free infant formula is titrated according to blood Phe levels; if blood Phe levels are high, the infant is given more infant Phe-free infant formula; if blood levels are low, the volume of infant Phe-free infant formula is reduced. If the mother is unable to breast feed, measured volumes of standard infant replace breast feeds to provide essential Phe requirements. The Phe intake is restricted/controlled to maintain the blood Phe control within target ranges, but it is essential that dietary Phe is given, in order to prevent Phe deficiency, resulting in poor growth and other negative outcomes.¹⁴ Weaning should progress as for other infants but eliminating high-protein foods and providing essential Phe requirements only.¹⁵ In practice, dependence on fruits and vegetables is mandatory to construct the diet.⁵ However, Phe intake is also derived from foods such as potatoes, sweetcorn, spinach, and peas, so an exchange list of protein-containing foods is provided by dietitians in many countries. This not only helps families to calculate the Phe content of their diet but also allows them to vary their diet from day to day.¹⁶

Theoretically, the typical diet used in PKU treatment should be considered healthy but there is limited information about actual feeding patterns. There is some evidence that “free” intake of fruits and vegetables does not have a negative impact on metabolic control,^{17,18} so this should improve eating habits. More research is needed to understand if relying on rigid exchange lists across all age groups will significantly affect dietary patterns. There is an urgent need for validated tools to be used for describing food and nutritional intake in patients with PKU. Food neophobia appears common in young children with PKU and there is a need to understand

if a low intake of fruit and vegetables may be associated with development of later overweight in individuals with PKU.¹⁹

For nutritional education, short- and long-term goals should be considered. In the past, the message to adult patients was very clear that they could stop treatment in early adulthood but then treatment guidelines recommended “diet for life”. As a consequence, there are many unhappy adult patients who struggle to adhere to their dietary treatment as they have become accustomed to eating higher protein foods. These and other issues illustrate the challenges in PKU adherence.¹³ The picture can differ in females because the fear of maternal PKU syndrome may contribute to higher adherence rates.²⁰ The patient’s outcome will also be influenced by the availability of new treatments, changes in treatment strategies, new foods, and different presentations of protein substitutes.²¹ Therefore, educational messages given to patients may significantly influence their motivation and persistence in being able to adhere to their diet.²²

Nutritional challenges: feeding issues and nutritional imbalance

The main objective in PKU treatment is to maintain blood Phe under rigorous control, especially during the first years of life.²³ Diet is calculated to meet all energy and other nutrient requirements and should compensate for any inefficiency associated with Phe-free L-amino acids.⁵ Growth (one of the most important pediatric outcomes) has improved since early PKU treatment.^{24,25} For the majority of patients, dietary adherence is not an issue in the first year of life.¹³ However, teething and childhood illnesses such as fever, gastrointestinal infections, and colic can disturb feeding and consequently adversely affect blood Phe control. When protein substitute is introduced in the neonatal period, it is usually well tolerated. Nevertheless, caution is needed when formula volume or type is adjusted. Energy needs should always be respected, but care is required regarding choice of protein substitute as changes in osmolality, flavor, odor and consistency can lead to its refusal. It is also important for the clinician to differentiate between diet refusal caused by illness, flavor/odor changes of the formulation, and refusals merely as a consequence of usual child tantrums.¹⁶

There are other important considerations with protein substitute administration. Nitrogen sources are known to promote satiety and giving protein substitute as close to the main meals may result in food refusal. In children or adolescent at risk of overweight or obesity, this approach may be advantageous in order to reduce daily food intake. However, in children particularly at risk of undernutrition,

giving protein substitute just before main meals may reduce energy intakes from low-protein foods. Therefore, it is also important to consider the immediate metabolic effects.¹² It is unknown if long-term ingestion of Phe-free L-amino acids will significantly alter metabolism but it deserves further investigation.²⁶

One of the most important messages in general nutrition for healthy individuals is that when energy needs are satisfied using a variety of foods from all food groups, nutritional adequacy and optimal nutrient balance are easily achieved. In PKU the scenario is different.⁵ Out of the three dietary components (natural low-protein foods, protein substitutes, and SLPF), only one is natural. In fact, most natural foods are limited or controlled in variable amounts depending on individual Phe tolerance. Fruits, vegetables, rice, and potatoes are good examples of regular natural foods included in the daily meals in PKU. Some fruits and vegetables (except potatoes) are considered exchange-free containing Phe ≤ 75 mg/100 g (A MacDonald, European PKU Guidelines, personal communication, 2016); others are exchange foods depending on their Phe content.^{17,18} In addition, sugars, fats, and oils can be ingested without any impact on blood Phe control. Even so, classifying their intake as completely exchange-free may not be appropriate, considering the recognized health implications resulting from chronic excessive fat and sugar consumption. Also, referring to “sugar as exchange-free” may eventually modulate/promote food behaviors where sugar-rich foods are consumed on a daily basis, being then challenging to moderate its consumption in later years. Very little is known about the long-term metabolic impact of diet therapy in PKU.^{27,28} There is an urgent need to accurately describe food patterns in PKU, although it is known that it is usually rich in carbohydrate.²⁹

The spectrum of natural foods allowed in a PKU diet is unable to meet full micronutrient requirements.⁶ In addition, while SLPF are mainly responsible to provide energy intake, their micronutrient supply is often very poor in contrast to regular foods,²⁸ and as a consequence, there is a reliance on protein substitutes to provide micronutrient requirements.⁶ They are usually well fortified with vitamins and minerals, and some evidence suggests that they may even contain excessive amounts of some nutrients.²⁹ Vitamin B₁₂ and folic acid are good examples of high amounts being added to protein substitutes and resulting in high serum concentrations. While vitamin B₁₂ toxicity does not appear significant, high serum folate is a concern.⁵ Achieving a good balance between protein intake, energy and micronutrient supply is not always easy because protein substitutes are prescribed according to

Phe tolerance and weight. Therefore, micronutrient intake is influenced by the dose of protein substitute intake and the total protein equivalent from a combination of natural protein sources and protein substitute. In BH4-treated patients, an increase in natural protein tolerance helps contribute to a better micronutrient intake in order to compensate for protein substitute reduction. Caution is needed when new foods are introduced in BH4-treated patients; it is important to encourage healthier food choices so that extra dietary Phe is not only provided by low-nutrient density foods.³⁰

Extra micronutrient supplementation beyond the amounts already contained in the protein substitute is not advisable as a standard recommendation in PKU, although evidence demonstrates that micronutrient imbalances continue to occur^{31–33} and the long-term importance of this fact remains to be clarified.³⁴

One of the reasons for nutritional imbalances (deficiencies or excesses) even in patients with good dietary adherence is probably related to micronutrients delivered in large doses at a single time point possibly compromising their bioavailability in the gut, reduced absorption through enhanced competition for absorption, or increased fecal losses. While blood Phe control is the main driver for promoting changes in the diet, it is essential to carefully calculate and provide all the other nutrients. Beyond Phe control, achieving a good nutritional balance should constitute an important objective, especially when other treatment strategies such as BH4 are being used.^{35–37}

Role of special dietary products

With PKU dietary treatment, natural protein and Phe restriction usually result in reduced energy intake. Natural protein sources such as regular cereals, bread, cookies, and pasta usually contain Phe in amounts that will adversely affect metabolic control, so such foods have to be avoided or severely restricted.⁵ Without adequate energy, free amino acids are used in anabolic processes in order to ensure that energy demands are satisfied.⁵ The definitive amount of nonprotein energy to be given with free amino acids is unknown. However, if this was established it would help optimize dietary treatment without increasing overweight risk.¹⁹ In the post meal period, carbohydrate rather than fat can reduce protein catabolism.³⁸ Correct distribution of free amino acids throughout the day has been shown to improve protein synthesis with benefits for metabolic control.³⁹

SLPF are deliberately low in protein and Phe but contribute higher amounts of carbohydrate and fat. An examination of their nutritional profile was recently published.²⁸ Beyond

the biochemical function of SLPF, hunger and hedonic purposes justify their use to compensate for dietary restrictions imposed by the nutritional treatment. They have a role in improving quality of life, allowing patients to eat pleasant foods, and ultimately aid dietary adherence and metabolic control. These aspects probably justify their government reimbursement in some countries.²⁸ With the first reports of overweight in PKU, there was concern about the impact of excessive energy intake from SLPF¹⁹ but there is no evidence to show this is the case. Overall, SLPF provide energy, aid variety, and improve adherence although their long-term impact on nutritional status should be evaluated.

Because SLPF are low in protein, their energy content is provided by carbohydrate and fat. However, based on a recent comparison with standard Portuguese foods, the main energy difference was associated with their carbohydrate content.²⁸ Overall, SLPF have a nutritional profile significantly different from regular foods. Excessive consumption will lead to excessive energy intake with a low micronutrient contribution.²⁸ So attention is required when they are consumed in excessive quantities. Health care professionals may need to advise on sensible and moderated food choices with limitation of foods particularly high in sugar and fat.²⁸ Additionally, a careful analysis of the SLPF available in every country/clinic is recommended.⁴⁰ Better knowledge of nutrient composition of SLPF may help reduce prevalence of overweight or related metabolic abnormalities.²⁷

In European countries and Turkey, the availability of SLPF is significantly different.²⁸ Some countries offer patients a financial allowance so they can purchase their SLPF, while in other countries, families have to self-finance all low-protein foods. In Portugal, a centralized distribution has been maintained for >20 years.²⁸ All systems can have their pros and cons but it is important to try to understand the direct influence of different systems on dietary adherence and long-term clinical outcome.

Measurement of nutritional status

In PKU, nutritional status has gained credence and is considered one of the more important aspects of clinical management.⁵ Considering the artificial characteristics of the dietary treatment nutritional status monitoring is essential.

Defining good nutritional status is not straightforward because several components contribute to the definition. Growth is one of the most important outcomes to consider and its optimization constitutes a priority in PKU.²⁴ Anthropometric and body composition evaluations are crucial components of nutritional status evaluation.⁵ In addition to monitoring

for growth impairment, prevention of excessive weight gain with increased adiposity is an important objective in early infancy.⁴¹ In later childhood, information about pubertal stage and bone maturity age should be collected by the physician.

Also, clinical and a dietary history are mandatory.¹⁶ Social and family history is very important. Food patterns are closely linked with lifestyle, economic, cultural, and social family routines. Unfortunately, there are sometimes episodes when social actions should be taken in order to optimize treatment adherence.⁴²

Clinical symptoms of nutritional deficiencies are rare in patients with good dietary adherence although micronutrient imbalance does occur.^{31,33} Nutritional status indicators that require regular monitoring are: anthropometry, body composition, biochemistry, and nutritional intake. World Health Organization growth standard and reference are advocated.^{43,44} While height growth should be optimized,²⁴ excessive weight gain should be prevented⁴⁰ avoiding overweight and obesity.¹⁹ When possible, body composition analysis will help to identify patients where excessive weight occurs simultaneously with increased adiposity.⁴⁵ Biochemical monitoring is also essential. Protein nutritional status should be monitored biochemically and prealbumin may constitute a good parameter to evaluate satisfaction of protein needs.^{46,47} Considering micronutrient status, vitamin B₁₂ and folic acid blood concentrations are commonly high in patients with good adherence, while zinc and selenium concentrations are sometimes below the expected ranges. Continuous monitoring of micronutrient status is important in order to make appropriate dietary adjustments to compensate for individual imbalances.⁴⁸

Alternative treatments

Glycomacropeptide

Casein GMP (cGMP) is a naturally low-Phe protein source (64-amino acid glycoposphopeptide) that is released from κ -casein by the action of chymosin during cheese making. It has been adapted so that it is suitable as an alternative protein substitute in the treatment of PKU. cGMP is not a complete protein. It is low in some essential amino acids and tyrosine (conditionally essential in PKU) and so is supplemented with essential amino acids and tyrosine to construct a protein substitute suitable for PKU. Usually commercial protein substitutes consist of between 60% and 70% protein equivalent from cGMP and the remaining protein source from conventional amino acids. The combination of cGMP and L-amino acids is referred to as cGMP-AA. Although cGMP is purified by chromatography, ultrafiltration/diafiltration,

and lyophilization, cGMP still contains some residual Phe (~1.8 mg/g of cGMP protein).⁴⁹ Usually a patient taking 60 g/daily protein equivalent from a cGMP-AA will receive at least an extra 90 mg/day Phe from this source.

cGMP has many theoretical advantages: 1) improves protein retention and nitrogen utilization; 2) improves neurophysiology and brain–blood Phe control; 3) improves bone strength; 4) may improve satiety; 5) has prebiotic properties; 6) enhances immunological activities; and 6) improves taste and acceptance. Although cGMP-AA has been widely used in the US, there is no long-term published data about its efficacy in humans. In addition, although there is animal and theoretical data to support potential benefit in patients with PKU, human evidence is generally limited, particularly in children.

cGMP research

Animal studies

There is some evidence that cGMP when given to PKU mice competitively inhibits Phe transport into the brain, and PKU mice show comparable growth and reduced concentrations of plasma and brain Phe when compared to a conventional amino acid sources.⁵⁰ Experiments were conducted in wild-type and PKU mice fed diets containing either 20% protein from casein, Phe-free L-amino acids, or cGMP-AA. The concentrations of isoleucine and threonine in plasma showed a significant two to threefold increase in the cGMP-AA fed mice. They also had decreased concentrations of Phe in the plasma by 11% and in five regions of the brain by 20% when compared to conventional Phe-free L-amino acids.

In a further animal study, PKU mice, fed cGMP-AA or Phe-free L-amino acids, showed growth and lean mass similar to wild-type mice fed GMP or amino acids. However, the cGMP-AA in the PKU mice significantly reduced energy expenditure, food intake, and plasma Phe concentrations,⁵¹ whereas the high-casein diet or Phe-free L-amino acids induced metabolic stress in the PKU mice (associated with increased energy expenditure, intake of food and water, and elevated plasma cytokine concentrations). Also in wild-type and PKU mice, cGMP has been shown to improve bone quality: cGMP and casein diets are associated with larger femoral dimensions whereas diets based on L-amino acid were associated with impaired radial growth of the femur.⁵¹

In other PKU and wild-type mice, cGMP has been shown to act as a prebiotic by reducing proteobacteria, genera *Desulfovibrio*, and PKU mice with genotype-dependent changes in Bacteroidetes or Firmicutes. Caecal concentrations of the short-chain fatty acid acetate, propionate, and butyrate were

increased with lower indexes of inflammation compared with casein and L-amino acid diets.⁵²

Human studies

Human research is more limited and is mainly directed to patients aged ≥ 11 years. There are no data of its use in pregnancy. In a short-term randomized crossover trial in 30 patients aged ≥ 15 years, comparing cGMP-AA with L-amino acid supplements only, cGMP-AA was associated with less gastrointestinal symptoms, less hunger, and improved palatability.⁵³ One study with 11 PKU subjects (aged 11–31 years) compared two 4-day treatments on Phe-free L-amino acids and cGMP-AA. Although there was no difference in postprandial Phe concentrations between the two study groups, fasting Phe concentrations were significantly lower with the cGMP-AA compared with L-amino acid supplements. Blood urea nitrogen was also significantly lower with cGMP-AA. The authors concluded that the cGMP-AA was a safe alternative to L-amino acid-based protein substitutes.⁵⁴

In addition, in the same group of patients, plasma concentrations of insulin and the appetite-stimulating hormone ghrelin was measured before and 180 minutes after breakfast. Postprandial ghrelin concentration was significantly lower with cGMP-AA compared to Phe-free L-amino acids; with no difference in fasting ghrelin. Lower postprandial ghrelin concentrations were associated with greater feelings of fullness after breakfast suggesting greater satiety with cGMP-AA compared to L-amino acids.⁵⁵ cGMP is a peptide that has been shown to stimulate release of cholecystokinin and thereby, may promote satiety.

In the first uncontrolled, short-term study reported in 10 children (aged 4–16 years) with PKU, when 50% of their total protein requirements were supplied by cGMP for 9 weeks, blood Phe concentrations decreased by a median of 114 $\mu\text{mol/L}$.⁵⁶ This difference was nonsignificant and was probably associated with improved palatability and better adherence.

Currently, there is low use of cGMP in Europe⁵⁷ and further research is required in pediatrics before this source of protein substitute can be safely advocated for children.

Large neutral amino acids

LNAAs were first suggested as a treatment for PKU as early as 1948.⁵⁸ Amino acid transporter-1 facilitates the transport of LNAAs across the blood–brain barrier. Peripheral accumulation of Phe is predicted to increase the uptake of Phe to the detriment of other LNAAs, resulting in disruption of brain

amino acid homeostasis.⁵⁹ In PKU, giving large amounts of Phe-free LNAAs competes with Phe and may reduce the influx of Phe into the brain.^{8,60} LNAAs may increase both brain neurotransmitter^{61,62} and brain essential amino acid concentrations.⁶³ In PKU, LNAAs have been shown⁶⁴ to improve executive functions particularly verbal generativity and flexibility.

In PKU mice, LNAAs (but low threonine) supplementation significantly reduced brain Phe concentrations by 26% compared to normal chow, while alleviating brain deficiencies of some (tryptophan) but not all supplemented LNAAs (tyrosine). However, LNAAs supplementation in PKU mice significantly increased brain serotonin and norepinephrine (the end product of tyrosine-derived neurotransmitter metabolism) concentrations from 35% to 71% and from 57% to 86% of wild-type mice concentrations but not brain dopamine concentrations.⁷

LNAAs supplementation may have a secondary effect by lowering blood Phe concentrations. Matalon et al,^{65,66} in both an open and a randomized controlled trial, gave patients LNAAs supplementation providing 0.5–1 g/kg/day. This resulted in a decrease in blood Phe concentrations by 50% and 39%, respectively. In PKU mice, LNAAs were found to significantly reduce blood Phe concentrations by 33% when taking chow with a regular protein content.⁷

Unfortunately, the optimal composition of LNAAs is unknown. They may not offer any additional benefit to patients already adhering to regular Phe-free L-amino acid supplementation and diet.⁶⁴ Generally, the use of LNAAs supplements is mainly considered in older patients who are unable to maintain dietary adherence.⁶⁷ They are not routinely available to all centers in Europe¹² and they remain untested in children under the age of 11 years. They should not be given during pregnancy as there is no safety data available.

BH4 and diet in PKU

Sapropterin is the dihydrochloride salt of a synthetic preparation of the 6R-diastereomer of BH4, the naturally occurring, essential cofactor for the enzyme PAH. It has been introduced as a supplemental treatment in patients with milder or moderate PKU who are BH4 responsive. It was first reported in 1999 that administration of BH4 reduced blood Phe levels in four patients with PKU after a loading test with BH4.⁶⁸ It is thought that BH4 increases PAH activity in BH4-responsive patients by acting as a chemical chaperone by preventing protein misfolding and providing protection from inactivation.⁶⁹

The prevalence of BH4 responsiveness is variable in different studies, ranging from 20% to 62%. In general,

BH4 is well tolerated, lowers blood Phe, and improves Phe tolerance in responders.^{35,37} It is possible to predict the level of BH4 responsiveness from ~71% of patient genotypes,² and the European PKU Guidelines (FJ van Spronsen et al, personal communication, 2016) suggests that in patients with known genotypes, BH4 responsiveness should not be considered further in patients with two null mutations, while a patient with a genotype with two BH4-responsive mutations may directly proceed to a treatment trial rather than a BH4 loading test.

Sapropterin improves dietary Phe tolerance by a factor of 2–3 and still maintains blood Phe concentrations within target range. Sapropterin may enable a small number of patients to stop diet therapy and protein substitute completely. Patients have reported that diet is easier to manage when taking sapropterin.⁷⁰ Some questions still remain about its effect on long-term efficacy and nutritional status. Some longer term data do suggest that patients on sapropterin eat a lower intake of animal protein, dairy products with a higher intake of potatoes and pasta. As a consequence, intake of nutrients such as calcium and vitamin D are compromised without supplementation.⁷¹

According to the European Summary of Product Characteristics,⁷² sapropterin is indicated for the treatment of PKU in adults and pediatric patients of all ages who have been shown to be responsive to such treatment. It also proposes a reduction in blood Phe of at least 30% to suggest sapropterin responsiveness. The dose of sapropterin is calculated according to body weight. The permitted dose range is 5–20 mg/kg/day, with a usual starting dose of 10 mg/kg. The prescribed number of tablets should be dissolved in water and taken with a meal. Sapropterin should be taken at the same time each day.

Phenylalanine ammonia lyase enzyme substitution therapy

Phenylalanine ammonia lyase (PAL; EC 4.3.1.5) is an enzyme that degrades Phe into trans-cinnamic acid and ammonia.⁷³ The pegylated recombinant phenylalanine ammonia lyase (rAvPAL-PEG) is a genetically modified form of the *Anabaena variabilis* phenylalanine ammonia lyase subsequently pegylated to reduce immunogenicity.⁷⁴ It does not require a cofactor. It is being given by subcutaneous injections in patients aged >16 years with PKU in clinical trials.⁷⁴ It is not yet available for routine clinical use.

In Phase I trials, rAvPAL-PEG was given to 25 adult patients with PKU in escalating doses (0.001–0.100 mg/kg) with the most frequent adverse reactions being injection-site reactions and dizziness. Mean blood Phe concentrations

were reduced by 54% in the five patients who received the highest dose.⁷⁴

In a Phase II extension study, administration of rAvPAL-PEG to 67 subjects with PKU for 1 year lowered blood Phe on average by 65% from pretreatment levels, with patients maintaining blood Phe concentrations <600 µmol/L.⁷⁵ The mean time to achieve two consecutive Phe levels <600 µmol/L was 26 weeks.

In a Phase III, 8-week double blind, placebo-controlled trial, 86 patients were randomized to stay on rAvPAL-PEG or receive placebo. The rAvPAL-PEG-treated group maintained their blood baseline Phe levels (at ~500 µmol/L), whereas the placebo-treated group increased to 1386 µmol/L. Patients in the trial were estimated to be eating 75% of the recommended protein intake for a healthy adult. No benefit in attention or mood scores was noted. rAvPAL-PEG-treated patients had more hypersensitivity adverse events as compared to placebo and the most frequent adverse events were arthralgia, headache, and fatigue.⁷⁶

This treatment has only been studied in adult patients with PKU. It does appear very effective at lowering blood Phe concentrations but is associated with a range of side effects.

Conclusion

Much research is being conducted in PKU in order to identify new and effective treatments for all patients with PKU that are easier to apply than diet therapy only. Although a low-Phe diet is well established, it remains challenging for both health professionals and patients. Some dietary strategies to improve variety or ease administration of dietary components may lead to short- and longer term health problems. Much work remains on the ideal nutritional profiling for SLPF and protein substitute. It is important that rigorous diet therapy research continues as it is likely to remain the foundation of therapy for future years.

Disclosure

Júlio César Rocha has received research grants from Cambrooke. He is a member of the European Nutritionist Expert Panel in PKU (ENEP), for which he has received consultancy fees from Merck Serono and BioMarin. He is also a member of the Advisory Board for Applied Pharma Research SA (APR). Anita MacDonald has received research grants from Nutricia, Vitaflo, Merck Serono, BioMarin, Metax, and Taranis. She is a member of the European Nutritionist Expert Panel in PKU (ENEP), for which she has received consultancy fees from Merck Serono and BioMarin and is a member of the European Phenylketonuria Group Advisory Board (EPG; formally Merck Serono and now BioMarin), and the Advisory Boards for Element

(Nutricia), Arla Foods and APR. The authors report no other conflicts of interest in this work.

References

- Følling I. The discovery of phenylketonuria. *Acta Paediatr Suppl.* 1994;407:4–10.
- Blau N. Genetics of Phenylketonuria: then and now. *Hum Mutat.* 2016;37(6):508–515.
- Meli C, Bianca S. Dietary control of phenylketonuria. *Lancet.* 2002;360(9350):2075–2076.
- Huijbregts SC, de Sonneville LM, Licht R, van Spronsen FJ, Verkerk PH, Sergeant JA. Sustained attention and inhibition of cognitive interference in treated phenylketonuria: associations with concurrent and lifetime phenylalanine concentrations. *Neuropsychologia.* 2002;40(1):7–15.
- MacDonald A, Rocha JC, van Rijn M, Feillet F. Nutrition in phenylketonuria. *Mol Genet Metab.* 2011;104(Suppl):S10–S18.
- Lammardo AM, Robert M, Rocha JC, et al. Main issues in micronutrient supplementation in phenylketonuria. *Mol Genet Metab.* 2013;110(Suppl):S1–S5.
- van Vliet D, Bruinenberg VM, Mazzola PN, et al. Large neutral amino acid supplementation exerts its effect through three synergistic mechanisms: proof of principle in phenylketonuria mice. *PLoS One.* 2015;10(12):e0143833.
- Pietz J, Kreis R, Rupp A, Mayatepek E, Rating D, Boesch C, Bremer HJ. Large neutral amino acids block phenylalanine transport into brain tissue in patients with phenylketonuria. *J Clin Invest.* 1999;103(8):1169–1178.
- Przyrembel H, Bremer HJ. Nutrition, physical growth, and bone density in treated phenylketonuria. *Eur J Pediatr.* 2000;159(Suppl 2):S129–S135.
- Monch E, Herrmann ME, Brosicke H, Schoffer A, Keller M. Utilisation of amino acid mixtures in adolescents with phenylketonuria. *Eur J Pediatr.* 1996;155(Suppl 1):S115–S120.
- Lacroix M, Bos C, Leonil J, et al. Compared with casein or total milk protein, digestion of milk soluble proteins is too rapid to sustain the anabolic postprandial amino acid requirement. *Am J Clin Nutr.* 2006;84(5):1070–1079.
- Pena MJ, de Almeida MF, van Dam E, et al. Protein substitutes for phenylketonuria in Europe: access and nutritional composition. *Eur J Clin Nutr.* 2016;70(7):785–789.
- MacDonald A, Gokmen-Ozel H, van Rijn M, Burgard P. The reality of dietary compliance in the management of phenylketonuria. *J Inherit Metab Dis.* 2010;33(6):665–670.
- Hoeksma M, Van Rijn M, Verkerk PH, et al. The intake of total protein, natural protein and protein substitute and growth of height and head circumference in Dutch infants with phenylketonuria. *J Inherit Metab Dis.* 2005;28(6):845–854.
- MacDonald A, Evans S, Cochrane B, Wildgoose J. Weaning infants with phenylketonuria: a review. *J Hum Nutr Diet.* 2012;25(2):103–110.
- Dixon M, MacDonald A, White F, Stafford J. Disorders of amino acid metabolism, organic acidaemias and urea cycle disorders. In: Shaw V, editor. *Clinical Paediatric Dietetics*. 4th ed. Chichester: Wiley Blackwell; 2015:381–525.
- Rohde C, Mutze U, Weigel JF, Ceglarek U, Thiery J, Kiess W, Beblo S. Unrestricted consumption of fruits and vegetables in phenylketonuria: no major impact on metabolic control. *Eur J Clin Nutr.* 2012;66(5):633–638.
- Rohde C, Mutze U, Schulz S, et al. Unrestricted fruits and vegetables in the PKU diet: a 1-year follow-up. *Eur J Clin Nutr.* 2014;68(3):401–403.
- Rocha JC, MacDonald A, Trefz F. Is overweight an issue in phenylketonuria? *Mol Genet Metab.* 2013;110(Suppl):S18–S24.
- Prick BW, Hop WC, Duvekot JJ. Maternal phenylketonuria and hyperphenylalaninemia in pregnancy: pregnancy complications and neonatal sequelae in untreated and treated pregnancies. *Am J Clin Nutr.* 2012;95(2):374–382.
- Giovannini M, Verduci E, Salvatici E, Paci S, Riva E. Phenylketonuria: nutritional advances and challenges. *Nutr Metab (Lond).* 2012;9(1):7–13.
- Bernstein LE, Helm JR, Rocha JC, Almeida MF, Feillet F, Link RM, Gizewska M. Nutrition education tools used in phenylketonuria: clinician, parent and patient perspectives from three international surveys. *J Hum Nutr Diet.* 2014;27(Suppl 2):4–11.
- Blau N, van Spronsen FJ, Levy HL. Phenylketonuria. *Lancet.* 2010;376(9750):1417–1427.
- Dokoupil K, Gokmen-Ozel H, Lammardo AM, et al. Optimising growth in phenylketonuria: current state of the clinical evidence base. *Clin Nutr.* 2012;31(1):16–21.
- Rocha JC, van Spronsen FJ, Almeida MF, Ramos E, Guimaraes JT, Borges N. Early dietary treated patients with phenylketonuria can achieve normal growth and body composition. *Mol Genet Metab.* 2013;110(Suppl):S40–S43.
- Pena MJ, Rocha JC, Borges N. Amino acids, glucose metabolism and clinical relevance for phenylketonuria management. *Ann Nutr Disord Ther.* 2015;2(3):1026.
- Rocha JC, van Spronsen FJ, Almeida MF, et al. Dietary treatment in phenylketonuria does not lead to increased risk of obesity or metabolic syndrome. *Mol Genet Metab.* 2012;107(4):659–663.
- Pena MJ, Almeida MF, van Dam E, et al. Special low protein foods for phenylketonuria: availability in Europe and an examination of their nutritional profile. *Orphanet J Rare Dis.* 2015;10:162.
- Robert M, Rocha JC, van Rijn M, et al. Micronutrient status in phenylketonuria. *Mol Genet Metab.* 2013;110(Suppl):S6–S17.
- MacDonald A, Ahring K, Dokoupil K, et al. Adjusting diet with sapropterin in phenylketonuria: what factors should be considered? *Br J Nutr.* 2011;106(2):175–182.
- Evans S, Daly A, MacDonald J, et al. The micronutrient status of patients with phenylketonuria on dietary treatment: an ongoing challenge. *Ann Nutr Metab.* 2014;65(1):42–48.
- Crujeiras V, Aldamiz-Echevarria L, Dalmau J, et al. Micronutrient in hyperphenylalaninemia. *Data Brief.* 2015;4:614–621.
- Crujeiras V, Aldamiz-Echevarria L, Dalmau J, et al. Vitamin and mineral status in patients with hyperphenylalaninemia. *Mol Genet Metab.* 2015;115(4):145–150.
- Rocha JC, Martins MJ. Oxidative stress in phenylketonuria: future directions. *J Inherit Metab Dis.* 2012;35(3):381–398.
- Belanger-Quintana A, Burlina A, Harding CO, Muntau AC. Up to date knowledge on different treatment strategies for phenylketonuria. *Mol Genet Metab.* 2011;104(Suppl):S19–S25.
- Strisciuglio P, Concolino D. New strategies for the treatment of phenylketonuria (PKU). *Metabolites.* 2014;4(4):1007–1017.
- Blau N, Longo N. Alternative therapies to address the unmet medical needs of patients with phenylketonuria. *Expert Opin Pharmacother.* 2015;16(6):791–800.
- Mariotti F, Mahe S, Luengo C, Benamouzig R, Tome D. Postprandial modulation of dietary and whole-body nitrogen utilization by carbohydrates in humans. *Am J Clin Nutr.* 2000;72(4):954–962.
- MacDonald A, Rylance G, Hall SK, Asplin D, Booth IW. Factors affecting the variation in plasma phenylalanine in patients with phenylketonuria on diet. *Arch Dis Child.* 1996;74(5):412–417.
- Rocha JC, van Rijn M, van Dam E, et al. Weight management in phenylketonuria: what should be monitored. *Ann Nutr Metab.* 2016;68(1):60–65.
- Scaglioni S, Verduci E, Fiori L, et al. Body mass index rebound and overweight at 8 years of age in hyperphenylalaninaemic children. *Acta Paediatr.* 2004;93(12):1596–1600.
- van Rijn M, Ahring K, Belanger-Quintana A, et al. When should social service referral be considered in phenylketonuria? *Mol Genet Metab Rep.* 2015(2):85–88.
- WHO Multicentre Growth Reference Study Group. WHO child growth standards based on length/height, weight and age. *Acta Paediatr Suppl.* 2006;450:76–85.

44. de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. *Bull World Health Organ.* 2007;85(9):660–667.
45. Wells JC, Fewtrell MS. Measuring body composition. *Arch Dis Child.* 2006;91(7):612–617.
46. Rocha JC, Almeida MF, Carmona C, et al. The use of prealbumin concentration as a biomarker of nutritional status in treated phenylketonuric patients. *Ann Nutr Metab.* 2010;56(3):207–211.
47. Arnold GL, Vladutiu CJ, Kirby RS, Blakely EM, Deluca JM. Protein insufficiency and linear growth restriction in phenylketonuria. *J Pediatr.* 2002;141(2):243–246.
48. Feillet F, Agostoni C. Nutritional issues in treating phenylketonuria. *J Inherit Metab Dis.* 2010;33(6):659–664.
49. Lim K, van Calcar SC, Nelson KL, Gleason ST, Ney DM. Acceptable low-phenylalanine foods and beverages can be made with glycomacropeptide from cheese whey for individuals with PKU. *Mol Genet Metab.* 2007;92(1–2):176–178.
50. Ney DM, Hull AK, van Calcar SC, Liu X, Etzel MR. Dietary glycomacropeptide supports growth and reduces the concentrations of phenylalanine in plasma and brain in a murine model of phenylketonuria. *J Nutr.* 2008;138(2):316–322.
51. Solverson P, Murali SG, Litscher SJ, Blank RD, Ney DM. Low bone strength is a manifestation of phenylketonuria in mice and is attenuated by a glycomacropeptide diet. *PLoS One.* 2012;7(9):e45165.
52. Sawin EA, De Wolfe TJ, Aktas B, Stroup BM, Murali SG, Steele JL, Ney DM. Glycomacropeptide is a prebiotic that reduces *Desulfovibrio* bacteria, increases cecal short-chain fatty acids, and is anti-inflammatory in mice. *Am J Physiol Gastrointest Liver Physiol.* 2015;309(7):G590–G601.
53. Ney DM, Stroup BM, Clayton MK, Murali SG, Rice GM, Rohr F, Levy HL. Glycomacropeptide for nutritional management of phenylketonuria: a randomized, controlled, crossover trial. *Am J Clin Nutr.* 2016;104(2):334–345.
54. van Calcar SC, MacLeod EL, Gleason ST, Etzel MR, Clayton MK, Wolff JA, Ney DM. Improved nutritional management of phenylketonuria by using a diet containing glycomacropeptide compared with amino acids. *Am J Clin Nutr.* 2009;89(4):1068–1077.
55. MacLeod EL, Clayton MK, van Calcar SC, Ney DM. Breakfast with glycomacropeptide compared with amino acids suppresses plasma ghrelin levels in individuals with phenylketonuria. *Mol Genet Metab.* 2010;100(4):303–308.
56. Zaki OK, El-Wakeel L, Ebeid Y, et al. The use of glycomacropeptide in dietary management of phenylketonuria. *J Nutr Metab.* 2016;2016:2453027.
57. Aguiar A, Ahring K, Almeida MF, et al. Practices in prescribing protein substitutes for PKU in Europe: no uniformity of approach. *Mol Genet Metab.* 2015;115(1):17–22.
58. Christensen HN, Streicher JA, Elbinger RL. Effects of feeding individual amino acids upon the distribution of other amino acids between cells and extracellular fluid. *J Biol Chem.* 1948;172(2):515–524.
59. Vogel KR, Arming E, Wasek BL, Bottiglieri T, Gibson KM. Characterization of 2-(methylamino)alkanoic acid capacity to restrict blood-brain phenylalanine transport in *Pah enu2* mice: preliminary findings. *Mol Genet Metab.* 2013;110(Suppl):S71–S78.
60. Rocha JC, Martel F. Large neutral amino acids supplementation in phenylketonuric patients. *J Inherit Metab Dis.* 2009;32(4):472–480.
61. Lou H. Large doses of tryptophan and tyrosine as potential therapeutic alternative to dietary phenylalanine restriction in phenylketonuria. *Lancet.* 1985;2(8447):150–151.
62. Guttler F, Lou H. Dietary problems of phenylketonuria: effect on CNS transmitters and their possible role in behaviour and neuropsychological function. *J Inherit Metab Dis.* 1986;9(Suppl 2):169–177.
63. van Spronsen FJ, de Groot MJ, Hoeksma M, Reijngoud DJ, van Rijn M. Large neutral amino acids in the treatment of PKU: from theory to practice. *J Inherit Metab Dis.* 2010;33(6):671–676.
64. Schindeler S, Ghosh-Jerath S, Thompson S, et al. The effects of large neutral amino acid supplements in PKU: an MRS and neuropsychological study. *Mol Genet Metab.* 2007;91(1):48–54.
65. Matalon R, Michals-Matalon K, Bhatia G, et al. Large neutral amino acids in the treatment of phenylketonuria (PKU). *J Inherit Metab Dis.* 2006;29(6):732–738.
66. Matalon R, Michals-Matalon K, Bhatia G, et al. Double blind placebo control trial of large neutral amino acids in treatment of PKU: effect on blood phenylalanine. *J Inherit Metab Dis.* 2007;30(2):153–158.
67. Ahring KK. Large neutral amino acids in daily practice. *J Inherit Metab Dis.* 2010;33(Suppl 3):S187–S190.
68. Kure S, Hou DC, Ohura T, et al. Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency. *J Pediatr.* 1999;135(3):375–378.
69. Pey AL, Martinez A. The activity of wild-type and mutant phenylalanine hydroxylase and its regulation by phenylalanine and tetrahydrobiopterin at physiological and pathological concentrations: an isothermal titration calorimetry study. *Mol Genet Metab.* 2005;86(Suppl 1):S43–S53.
70. Brown CS, Lichter-Konecki U. Phenylketonuria (PKU): a problem solved? *Mol Genet Metab Rep.* 2016;6:8–12.
71. Thiele AG, Rohde C, Mutze U, et al. The challenge of long-term tetrahydrobiopterin (BH4) therapy in phenylketonuria: effects on metabolic control, nutritional habits and nutrient supply. *Mol Genet Metab Rep.* 2015;4:62–67.
72. Kuvan summary of product characteristics. Available from: www.ema.europa.eu. Accessed July 7, 2016.
73. Sarkissian CN, Gamez A, Wang L, et al. Preclinical evaluation of multiple species of PEGylated recombinant phenylalanine ammonia lyase for the treatment of phenylketonuria. *Proc Natl Acad Sci U S A.* 2008;105(52):20894–20899.
74. Longo N, Harding CO, Burton BK, et al. Single-dose, subcutaneous recombinant phenylalanine ammonia lyase conjugated with polyethylene glycol in adult patients with phenylketonuria: an open-label, multicentre, phase 1 dose-escalation trial. *Lancet.* 2014;384(9937):37–44.
75. Longo N, Thomas JA, Wasserstein M, et al. Evaluation of multiple dosing regimens in phase 2 studies of rAvPAL-PEG for control of blood phenylalanine levels in adults with phenylketonuria. *J Inherit Metab Dis.* 2015;38(Suppl 1):S41–S42.
76. BioMarin. Phase 3 Study of Pegvaliase for Phenylketonuria (PKU) Meets Primary Endpoint of Blood Phenylalanine (Phe) Reduction (p<0.0001) [press release]. San Rafael, CA: BioMarin Pharmaceutical Inc; 2016 [March 21]. Available from: <http://investors.bmrn.com/releasedetail.cfm?ReleaseID=961419>. Accessed July 7, 2016.

Pediatric Health, Medicine and Therapeutics

Publish your work in this journal

Pediatric Health, Medicine and Therapeutics is an international, peer-reviewed, open access journal publishing original research, reports, editorials, reviews and commentaries. All aspects of health maintenance, preventative measures and disease treatment interventions are addressed within the journal. Practitioners from all disciplines are invited to submit

Submit your manuscript here: <http://www.dovepress.com/pediatric-health-medicine-and-therapeutics-journal>

their work as well as healthcare researchers and patient support groups. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.