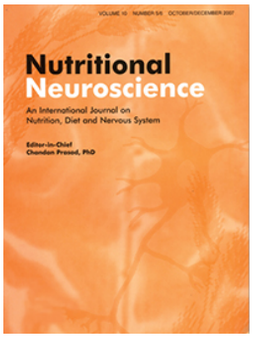

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# The phenylketonuria patient: A recent dietetic therapeutic approach

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Phenylalanine hydroxylase (PAH) deficiency, commonly named phenylketonuria (PKU) is a disorder of phenylalanine (Phe) metabolism inherited with an autosomal recessive trait. It is characterized by high blood and cerebral Phe levels, resulting in intellectual disabilities, seizures, etc. Early diagnosis and treatment of the patients prevent major neuro-cognitive deficits. Treatment consists of a lifelong restriction of Phe intake, combined with the supplementation of special medical foods, such as Amino Acid medical food (AA-mf), enriched in tyrosine (Tyr) and other amino acids and nutrients to avoid nutritional deficits. Developmental and neurocognitive outcomes for patients, however, remain suboptimal, especially when adherence to the demanding diet is poor. Additions to treatment include new, more palatable foods, based on Glycomacropeptide that contains limited amounts of Phe, the administration of large neutral amino acids to prevent phenylalanine entry into the brain and tetrahydrobiopterin cofactor capable of increasing residual PAH activity. Moreover, further efforts are underway to develop an oral therapy containing phenylalanine ammonia-lyase. Nutritional support of PKU future mothers (maternal PKU) is also discussed. This review aims to summarize the current literature on new PKU treatment strategies.

**Keywords:** PAH deficiency, PKU diet, Glycomacropeptide, LNAAs, Sapropterin dihydrochloride

## Introduction

Phenylalanine hydroxylase (PAH) deficiency commonly named phenylketonuria (PKU) is a rare autosomal recessive inborn error of phenylalanine (Phe) metabolism (Figure 1). Deficiency of this hepatic enzyme results in elevated concentrations of Phe levels in the blood and brain, causing complications, most notably severe neurocognitive and neuromotor impairments.[1] Newborn screening for PKU, along with early initiation of dietary treatment, have successfully ameliorated or prevented the above clinical findings.[2]

Dietary management with restriction of Phe intake remains the mainstay therapy of these patients, requiring a decrease in the intake of natural protein and replacing it with a protein source devoid of Phe. Widespread consensus exists regarding the importance of blood Phe control and dietary treatment.[3,4] The recommendations for the appropriate target blood Phe concentrations and treatment duration have evolved over the years as our understanding of the disease has improved.

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Very recently, the need for more standardized management tailored for optimal outcomes in Europe has emerged[5,6] and the European guidelines for the diagnosis and management of patients with PKU were developed.[7] According to these guidelines, untreated blood Phe concentrations determine management of people with PKU, setting an age-dependent target blood Phe threshold. Therefore an upper target Phe blood concentration of 360 μmol/l is recommended for the first 12 years of life, and an upper target Phe blood concentration of 600 μmol/l for all individuals older than 12 years.

American College of Medical Genetics and Genomics (ACMG) guidelines note that although individuals with PAH deficiency are presented by a spectrum of residual enzyme activity, some practitioners find practical utility in referring to the older categories: 'classic/severe', 'moderate', 'mild PKU' and 'mild hyperphenylalaninaemia (HPA)'; the latter does not usually need Phe restriction. According to the Phe blood levels, the following classification is still used: classic PKU presenting with Phe pretreatment levels typically >1200 μmol/l, moderate PKU with Phe pretreatment levels of 900–1200 μmol/l, mild PKU with Phe pretreatment levels of


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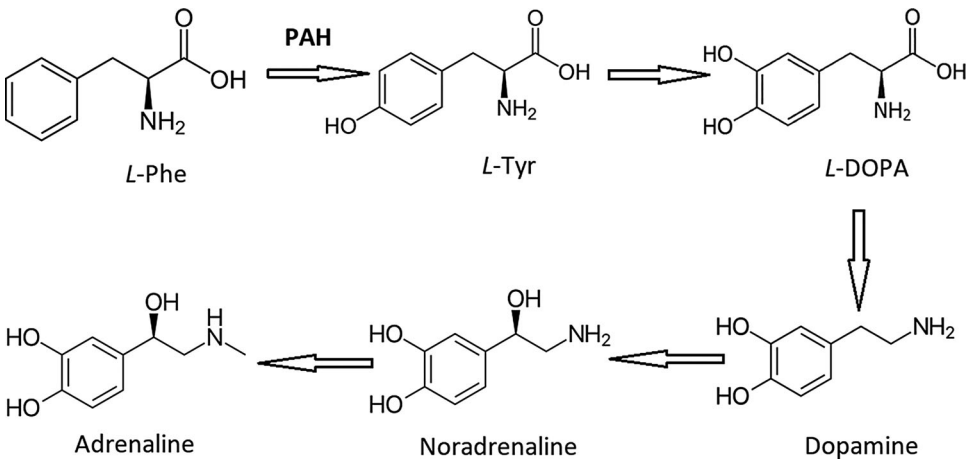


**Figure 1 Schematic presentation of the metabolism of Phe**

600–900 μmol/l and mild HPA with Phe pretreatment level <600 μmol/l.[8]

In ACMG guideline, individuals with PAH deficiency have been also categorized by phenotype and genotype. Phe tolerance is defined as the amount of dietary Phe per kg/body weight per day that an individual with PAH deficiency can tolerate in order to achieve plasma Phe concentrations within a target range.[3,9] Four different phenotypes may be classified by using Phe tolerance: classic PKU with a Phe tolerance <20 mg/kg/day (250–300 mg/day), moderate PKU with a Phe tolerance of 20–25 mg/kg/day (350–400 mg/day), mild HPA with a Phe tolerance 25–50 mg/kg/day (400–600 mg/day), and HPA with patients off diet.[8]

According to the latest European guidelines, a new classification is proposed on the basis of whether patients with PAH deficiency, in order to maintain blood Phe concentrations in the recommended range, do not need treatment or require dietary intervention/tetrahydrobiopterin (BH4) or both. For women preconception and during pregnancy (maternal PKU), untreated Phe blood concentrations of more than 360 μmol/l must be reduced.[7] The dietary management of PKU is based on four parts: (a) natural protein restriction limiting intake of Phe, (b) considering individual Phe tolerance, (c) Phe-free *L*-Amino Acid (AA) supplements and (d) special low protein foods (SLPFs).

**Table 1 Guidelines for Phe, Tyr, and protein intake for individuals with PKU (Adapted from Singh *et al.*[4])**

| AGE | Phe (mg/day)[a] | Tyr (mg/day)[a] | Protein (g/kg/day)[b] |
|---|---|---|---|
| Infants to <4years[a] | | | |
| 0 to 3 months[c] | 130–430 | 1100–1300 | 2.5–3.0 |
| 3 to 6 months | 135–400 | 1400–2100 | 2.0–3.0 |
| 6 to 9 months | 145–370 | 2500–3000 | 2.0–2.5 |
| 9 to <12months | 135–330 | 2500–3000 | 2.0–2.5 |
| 1 to <4 years[d] | 200–320 | 2800–3500 | 1.5–2.1 |
| After early childhood[e] | | | |
| >4years to adult | 200–1100 | 4000–6000 | 120–140% RDA for age[f] |
| Pregnancy/lactation[g] | | | |
| Trimester 1 | 265–770 | 6000–7600 | ≥70 |
| Trimester 2 | 400–1650 | | |
| Trimester 3 | 700–2275 | | |
| Lactation[h] | 700–2275 | | |

[a]Recommendations for Phe and Tyr intake for infants and children <4 years with more severe PKU and treated with Phe-restricted diet alone. Tyr intake recommendations may require adjustment based on blood Tyr monitoring.
[b]Phe recommendations for premature infants may be higher.
[c]Phe tolerance usually stabilized by 2–5 years of age. Recommendations are based on size and growth rate. Individual Phe intake recommendations should be adjusted based on frequent blood Phe monitoring.
[d]Range of recommended Phe intake applies to spectrum of PKU severity.
[e]Recommended protein intake greater than the DRI is necessary to support normal growth in PKU.
[f]Recommendations are slightly higher for pregnant women ≤19years of age.
[g]Recommended nutrient intake during lactation is same as the for third trimester of pregnancy for all women.
[h]Protein recommendations for individuals consuming medical foods as the major protein source.

A) The restriction of dietary Phe remains the mainstay of PAH deficient management and usually begins immediately after confirmation of the diagnosis during the perinatal period. Phe is an essential AA for protein synthesis and tyrosine (Tyr) production. Adequate intake of Phe, protein and energy must be provided to prevent breakdown of body tissues which can lead to elevated plasma Phe concentrations. The wide ranges given for Phe intake[4] (Table 1) reflect the influence of many factors on Phe requirements, including residual PAH activity, patient's age, growth rate, genotype, sapropterin-responsiveness, etc. When *L*-amino acids provide the primary source of protein equivalents in the PKU diet, total protein intake should provide approximately 40% above the age-related safe levels of protein intake recommended by the FAO/WHO (FAO/WHO/UNU 2007) because of inefficient utilization of amino acids to support protein synthesis.

PKU patients have to accept the Phe-free formula and avoid foods rich in protein (e.g. meat, fish, eggs,

standard bread, etc.) and drinks containing aspartame. Phe comprises 4–6% of all dietary protein-containing foods. Practical methods for prescribing Phe intake vary throughout the world and it is either allocated by prescribing total daily allowance, where all Phe-containing foods are calculated in the diet, or by Phe exchange systems that comprises of weighted portions of food all containing the same amount of Phe.[10] Low protein foods in the diet, such as potatoes, some vegetables and most cereals can be eaten but only in severely restricted amounts that should contain 50 mg of phenylalanine or less per 100 g (equivalent to 1 g of protein per 100 g) of dry product. Fruit and vegetables containing less than 75 mg of phenylalanine per 100 g of food product should not adversely affect blood Phe control and can be characterized free.[11]

The optimal amount of Tyr provided in a low-Phe diet is unknown, but regular monitoring is necessary to ensure that supplementation is adequate to maintain blood Tyr concentration in the normal range. Additional supplementation in excess of amounts provided by AA supplements is not associated with a benefit.[12] Tyr is a precursor of the neurotransmitters (dopamine, norepinephrine and epinephrine). Investigations into the biochemical markers associated with executive function (EF) impairment in children with early and continuously treated PKU remain largely Phe-only focused, despite experimental data showing that a high Phe:Tyr ratio is more strongly associated with EF deficit than Phe alone.[13] Although the diet is supplemented with Tyr, it has been demonstrated that blood Tyr concentrations fluctuate throughout the day, and may decrease after prolonged fasting.[14]

B) The individual dietary Phe tolerance is pragmatically determined as mentioned above.

C) To guarantee a sufficient intake of daily protein, the patients, use a designated Phe-free AA-medical food (AA-mf), supplemented with Tyr and containing the right mixture of essential AAs, vitamins, minerals and trace nutrients, with the aim of meeting the nutritional requirements for a specific age targeted population.[15] There is now evidence that the quantity, composition, diurnal distribution, acceptability and administration of the protein substitute could influence blood Phe control. Nutritional composition, labeling and manufacturing of these special formulas, is regulated by the European legislation.[16]

Phe-free protein substitute is essential to prevent protein deficiency, optimize metabolic control,[17] improve growth,[18] prevent micronutrient deficiency.[19] It may block Phe transport across the blood–brain barrier (BBB)[20] and has an important role in helping to prevent neurological disability.[21] Several studies documented differences in gut absorption rate of AAs between medical formulas and natural protein.[22] Efficient utilization of AAs for body protein synthesis is influenced by many factors, including the rate of protein digestion and absorption of AAs into blood, the presence of all essential or indispensable AAs at the same time and adequate intake of energy and total dietary nitrogen to support the high metabolic cost of protein synthesis. A more rapid and elevated increase in AA plasma concentrations, followed by a rapid decline, has been noted after ingestion of free AAs compared to an equivalent amount of AAs provided as intact protein.[23] Thus, a higher dose of Phe-free AAs is required when giving AAs instead of intact protein as the main source of protein intake.[24] Today, AA-mf is presented in several types, as amino acid powders, gels, etc., supplemented with vitamins, minerals, fat and carbohydrates; the latter playing a role in reducing leucine oxidation and increasing net protein synthesis.[24] This elemental mf often provide an osmolality that exceeds physiologic tolerance of the patient. Abdominal cramping, diarrhea, distention, nausea, and vomiting may result from hyperosmolar feeds, especially in young children if protein substitute is diluted with less water than recommended. It is important to divide the daily intake of AA-mf into at least three to four equal parts throughout the day and given each dose with other foods containing natural protein or beverages to try to slow the absorption rate, minimizing in this way fluctuations in blood Phe variability.[17] Infrequent administration of large doses of AA-mf increases nitrogen excretion, as well as oxidative utilization of AAs, so this practice is not advocated.[25] Although the mean nutritional composition of the AA-mf designed for different ages may be similar, the AA-mf developed for specific age ranges of patients vary widely in nutrient composition, and not all are formulated to meet the specific nutritional requirements of patients or the widely varying disorder severity spectrum.[26]

Although these products, with added vitamins and minerals provide the major source of micronutrients for the majority of patients, the biochemical micronutrient deficiencies are common.[27] In particular, low plasma levels of carnitine, selenium, and zinc have promoted increased supplementation, as it is widely known that they are involved in the anti-oxidant status.[28–30] Iron deficiency and iron deficiency anemia have been reported and routine evaluation of iron status, including ferritin is recommended.[19,30] High concentrations of blood folate and low in vitamins, especially vitamin B12, have been noticed.[31,32] A high content of folic acid in protein substitutes for the patients results in high plasma folate levels and low plasma homocysteine levels.[32] Functional B12 deficiency can exist despite levels within the reference

range, and it is recommended to evaluate vitamin B12 levels against methylmalonic acid or homocysteine levels. Although mineral and vitamin deficiencies have been reported among individuals with PKU, it is not clear that PKU in and of itself, leads to a specific nutrient deficiency (except Tyr).[15]

Additionally, a typical PKU diet provides low saturated and polyunsaturated fat intake, due to the low intake of Phe-containing animal products. As a consequence, PKU patients have a poor long-chain polyunsaturated fatty acids (LC-PUFAs) status in plasma and erythrocytes, as compared to healthy controls,[33] especially when fat-free AA-mf is consumed. The dietary supply of LC-PUFAs in the first months of life increased erythrocyte status in infants[34] and may have long-term consequences for the development of some cognitive functions in later childhood.[35] Osteopenia and osteoporosis have been reported in children and adults with PKU[36] and may be due to long-standing dietary deficiencies in natural protein, calcium, vitamin D, and/or trace elements, or a primary defect in bone turnover inherent to the disease itself.[15] Other dietary nutrients positively correlated with bone health include docosahexaenoic acid, eicosapentaenoic acid, and total omega-3 fatty acids.[37] Individuals with PKU have significantly diminished plasma levels of these nutrients compared with controls unless the AA-mf is enriched.[38]

D) Additionally, for better dietary control SLPFs are administered to people with PKU to provide energy, variety and satiety in the diet.[39]

Recently, an alternative source of protein substitute, Glycomacropeptide (GMP), with a significant proportion of large neutral amino acids (LNAAs), seems to ameliorate the quality of the PKU diet.[40] Medical food enriched with LNAAs, is especially very important for the dietary control of adolescents and adult PKU patients.[41] Despite a generous nutrient intake for the majority of patients with PKU, achieving the optimal nutrient status is challenging, particularly when a substantial proportion of the nutrient intake is from chemically derived sources with poor bioavailability and little natural protein to aid absorption and utilization. The addition of BH4 (Figure 2), also known as sapropterin dihydrochloride (Kuvan) in those patients who respond by reducing Phe blood levels, is also a cornerstone for the management of the disease.[42] Another promising agent for the lowering blood Phe is Phe ammonia-lyase (PAL), which is still under clinical evaluation.[43] Special attention should always be payed to future mothers with PKU in order to avoid complications during pregnancy and the presence of microcephaly, congenital heart disease etc in their offsprings.[44]

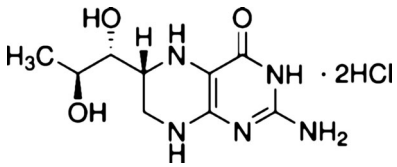


**Figure 2 Chemical structure of BH4**

## *The important role of LNAAs*

The oral ingestion of a LNAA mixture, excluding Phe with the purpose of lowering brain Phe concentrations was first studied in rats in 1976.[45] Targeting the brain requirements, LNAAs supplementation including, tyrosine, tryptophan, threonine, methionine, valine, isoleucine, leucine and histidine aims to restore the disturbed LNAA transport across the BBB, with or without the traditional diet. LNAA enrichment could serve to: (i) decrease brain Phe, (ii) increase brain non-Phe LNAAs, and/or (iii) increase brain monoaminergic neurotransmitter concentrations.[46]

LNAA transporter 1 (LAT 1) is the predominant transport system for all LNAAs across BBB and it is saturated for >95%.[47] Combined with the fact that LAT 1 shows a high affinity to Phe, increased blood Phe concentrations in poorly controlled PKU, strongly increase brain Phe influx, outcompeting the transport of other LNAAs across the intestinal mucosa into the blood and across the BBB into the brain.[48] High brain Phe concentrations have been found to be neurotoxic, consequently, the role of other LNAAs for protein synthesis is diminished. Additionally, impaired cerebral monoaminergic neurotransmitter synthesis may result from outcompeted brain uptake of their AA precursors Tyr and tryptophan (Trp). Specific supplementation with Tyr and Trp improved metabolism of dopamine and serotonin in PKU patients with an unrestricted natural protein intake, however, later studies with large doses of Tyr and Trp had no further effect.[46] LNAA supplementation in the patients was recently found to increase blood and urine melatonin concentrations, resulting in increased brain serotonin synthesis[49] and better cognitive function. This treatment is recommended for adults who are usually on a loose diet.[50] Differences in outcome have been reported depending on the composition, dosing, route of administration and duration of the supplementation period.[46,50,51]

LNAAs typically provide 20–30% of the total protein requirement with 70–80% of protein supplied by natural sources.[46] Total protein has been limited to the DRI of 0.8 g/kg or 1 g/kg.[46] A recent study has tested a new nutritionally complete formula containing LNAAs in patients with PKU, with the purpose of improving the compliance and lowering blood Phe. It was concluded that the older aged children who responded less to the treatment can manipulate their intake and follow the diet incoherently.[41]

Overall, the administration of LNAAs, either alone or in combination with a low-Phe diet, has a potential to improve health outcomes for the older patients who are unable to follow the low-Phe diet. Accordingly to the latest European guidelines, the use of LNAAs cannot be easily adapted for children under the age of 10 years.[7] Further investigation on this field should be done.

### *Glycomacropeptide (GMP): a new hopeful natural medical food*

Glycomacropeptide is a 64-amino acid phosphorylated glycopeptide, released from kappa casein by the action of chymosin during the cheese-making process that has been adapted for use as a protein substitute in PKU.[40] While GMP in pure form contains no Phe, the process of extracting and refining GMP results in inclusion of trace quantities of this AA.[52,53] However, given the low-Phe content of GMP, it has been used in the manufacturing of a variety of palatable GMP-MF that are low in Phe and high in protein content.[53]

Pure GMP presents an absence of the aromatic AAs (Phe, Trp and Tyr) with the content of the LNAAs, threonine and isoleucine to be two to three times the amount of other protein substitutes based on *L*-AA supplements. Commercial preparations of GMP-MF are modified by enhancing levels of Trp, arginine, leucine, histidine and Tyr, which are naturally deficient in pure GMP.[52,53] This addition is necessary in order to meet the daily intake requirement of these essential AAs, which cannot be synthesized *de novo* by the body.[1] Medical food based on GMP is also supplemented with small amounts of indispensable AAs, including methionine and lysine, which are important for endogenous biosynthesis of carnitine, as well as with vitamins and minerals to be ideally suited for the PKU diet as mentioned above.

High concentrations of LNAAs in GMP-mf may reduce intestinal absorption of Phe and transport into the brain. PKU mice fed a low-Phe diet containing GMP showed a significant 20% reduction in brain concentration of Phe compared with PKU mice fed a low-Phe amino acid diet.[54] The expansion of plasma Threonine from GMP may also improve Phe tolerance. GMP-MF, compared to conventional AA-MF, improves protein retention and Phe utilization,[40] potentially improves long-term bone health[55] and has an intestinal anti-inflammatory activity that may be important in the long-term health in PKU.[40] Reduced gastrointestinal side effects noted by patients consuming GMP-mf are supported by evidence that GMP is a prebiotic.[56] It demonstrates several unique biological properties and is currently used as a food ingredient with applications to infant formula, gastrointestinal health, and obesity.[57]

Highly acceptable low-Phe, high protein food and beverages that provide 5–15 g of protein and only 15–25 mg Phe per serving can be made with GMP to provide an alternative to AA formula for those with PKU with a daily allowance of ≥300 mg Phe. These GMP foods include beverages, either milkshake-like or clear, fruit flavored, pudding, puffed cereal, crackers, salad dressings, and a snack bar.[53]

So far, many studies have shown that GMP may be suitable for the management of PKU.[58–62] Zaki and coworkers concluded that GMP may be used to replace 50% of the protein intake to improve the nutritive value and palatability of diet and to provide a more satisfactory diet with no toxicity or side effects reported in patients on that regimen.[60] Oppositely, Daly and coworkers,[62] assumed that GMP-mf was more acceptable than AA-mf, blood Phe control declined with GMP-mf but when titrating the dose, blood Phe control remained within target range. They concluded that the additional intake of Phe may have contributed to the change in blood Phe concentration. As commercial GMP-mf contain residual Phe (1.5–1.8 mg/g protein), it is important to establish via longitudinal, long-term studies the safety both in terms of growth and Phe control in children.[7]

Very recently, it was reported that GMP-mf contains no or tiny amount of carnitine.[63] The US national Institute of Health reports[64] that carnitine biosynthesis occurs primarily in the liver and kidneys from the AAs lysine and methionine, as mentioned above. This endogenous production of carnitine covers only 25% of the daily requirements, whereas the rest (75%) should be administered by food. Carnitine plays a critical role in energy production. It transports long-chain fatty acids into the mitochondria so that they can be oxidized to produce energy. It also transports the toxic compounds generated out of these cellular organelles to prevent their accumulation. Given these key functions, carnitine is concentrated in tissues such as skeletal and cardiac muscles that use fatty acids as a dietary fuel.[64] The human body produces sufficient quantities of carnitine to meet the requirements of most healthy people. For genetic or medical reasons, some individuals, such as preterm infants[65] and PAH deficiency patients[29] do not have enough carnitine in their blood, so for them carnitine is a conditionally essential nutrient. In 1990, our team was the first who reported the necessity of carnitine in PKU diet. We concluded that in those patients, low carnitine blood levels were established.[29] Recent studies also reported reduced plasma carnitine concentrations in children with PKU.[66] Thus carnitine supplementation of AA-MFs does not seem to affect carnitine and acylcarnitine status in patients with PKU.[29,67] More recently, Stroup *et al.*[68], from metabolomics evidence relating supplementation of medical

foods with carnitine, showed no significant differences in plasma carnitine concentrations of adolescent and adult patients receiving AA-MFs versus GMP-MFs, despite higher supplementation of AA-MFs with carnitine compared with GMP-MFs. Additionally, excretion of trimethylamine N-oxide (TMAO), a product of bacterial metabolism of carnitine and choline, was determined high in patients receiving AA-MF versus those supplemented with GMP-MF. They concluded that carnitine supplementation of AA-MFs showed reduced bioavailability due, in part, to bacterial degradation to TMAO, whereas the bioavailability of carnitine is greater with prebiotic GMP-MFs.

### *AA-mf vs. GMP-mf administration*

The target point for PKU control is the restriction of natural protein, so the diet should be supplemented with a Phe-free mf. Consequently, the AA-based low-Phe diet provides approximately 80% of protein needs from synthetic AAs and 20% from the intact protein found in fruits and vegetables.[7] Oppositely, a GMP-based low-Phe diet supplemented with limiting AAs provides approximately 70% of protein needs from intact protein and only 30% of protein from synthetic AAs. This is an example to replace the current PKU diet from synthetic AA to natural, based on intact protein. Van Calsar *et al*. reported that GMP-mf provides 30% more histidine, methionine, leucine and tryptophan and 50% more tyrosine than AA dietary reference values.[40] It is clear that in PKU patients *L*-AAs may be poorly used and are less efficacious than the low Phe-peptide based Glycomacropeptide.[69]

As compared to the casein-rich protein, there is evidence of less efficient transfer of AAs into tissue and plasma proteins with AA supplements.[70] AAs delivered as dietary protein (casein) may support whole-body protein metabolism better than ingestion of crystalline *L*-AAs, casein hydrolysates or soy protein,[71] with nitrogen losses to be lower with intact protein than with free-AAs. The slower digestion and absorption of GMP compared to AA-mf promotes satiety and may modulate control of postprandial blood glucose levels.[72] This is important given that many individuals with PKU have a hungry feeling when consuming a diet with AA-mf. GMP has the advantage of being of lower osmolarity than the regular AA formula.[73]

Another beneficial effect of GMP is its prebiotic function in reducing the enteric bacteria and enhancing the synthesis of beneficial short-chain fatty acids. It is well known that there is no prebiotics in many infant AA-mf as has been previously reported, taking into account that the full nutritional characteristics of these products do not usually represent the nutrient profile typically found in the regular infant formulas.[74] AA patterns of Phe-free AAs prepared for infants, children and adults are primarily based on that of human milk. In different formulations of Phe-free AAs, there are variations in the AA patterns as well as the amount of essential and no essential AAs per 100 g/AAs, which may influence bioavailability. Additionally, the amount of Tyr and the Branch Chain Amino Acids (BCAAs) added to Phe-free AAs, vary between individual products.[39,46] This may be particularly important as the BCAAs share the same Phe transporter system in the BBB and the gut, reducing Phe absorption and consequently brain Phe levels.[1,46]

Among young adults patients with PKU-treated since birth with an AA diet, increasing dietary protein intake was associated with decreasing glomerular filtration rate.[75] In an inpatient metabolic study, the AA diet introduced a greater dietary potential renal acid load and evoked adaptions consistent with metabolic acidosis based on reduced serum bicarbonate levels, compared with the GMP diet.[76,77] Reduced renal function in PKU could decrease renal $1,25(OH)_2D$ synthesis and reduce fractional calcium absorption, leading to a decline in bone mineral density (BMD), contributing to skeletal fragility in PKU. This hypothesis was tested by Stroup *et al*., and they also concluded that, AA-mfs provide a high dietary acid load and increase urinary excretion of renal net acid, calcium and magnesium, compared with GMP-mfs.[78] GMP also attenuated the metabolic stress and bone fragility compared to a low amino acid supplemented diet in PKU mice.[55,79] In contrast, GMP-mfs contain no or tiny amounts of carnitine as mentioned above, whereas AA-mfs products are enriched with this biocatalyst.

It is clear that GMP-mf offer several advantages compared to conventional AA-mf administration. In the future, advances in biotechnology and the development of new protein purification technologies will speed the transition from AAs to intact protein in designing medical foods for inherited metabolic disorders.

### *Special low protein foods (SLPFs)*

SLPFs organized in sub-groups include baby cereals, bread, breakfast cereals, cakes/mix cake/pancake mix, chocolate/energy bars/jelly, cookies, flour, ice cream, milk replacers, pasta, rice and savory foods. People with PKU need these products to provide energy, variety and satiety in the diet. While their ingestion satisfies energy needs, they also help support free AAs anabolism, improve dietary adherence and thereby help maintain blood Phe control within target ranges.[7] It is estimated that in patients with the most severe forms of the disorder, SLPF provide 50% of the total energy intake. Patients with milder phenotypes, with a higher natural protein tolerance are likely to be less dependent on their use.[80]

Pena *et al*., identified the number of SLPF available in Europe and analyzed their nutritional composition. They found that Lipid and CHO composition of SLPF subgroups as compared with those of regular foods was higher in 58 and 92%, respectively. Additionally in 75% of the SLPF sub-groups the energy content was higher than in regular foods.[80] As overweight is a matter of concern in PKU, especially in older females[15] with poor Phe control, it is essential to prevent nutritional status imbalances and examine other aspects of nutrition (e.g. differences in dietary patterns) that may influence cardiometabolic markers,[81] beyond blood Phe control**.** It is important that industry ensures a high quality of SLPF in respect to taste, acceptability, palatability and appearance to help improve patient adherence with the diet, but a careful analysis of nutritional profile of all products is also desirable to improve the quality of information, in order to better match the nutrient needs of each patient.[80]

### *The utilization of sapropterin dihydrochloride (Kuvan)*

Tetrahydrobiopterin (BH4) is an essential cofactor of the PAH enzyme. The aromatic amino acid hydroxylases, PAH, tyrosine hydroxylase and tryptophan hydroxylase, all require (6*R*)-*L*-erythro-5,6,7,8-tetrahydrobiopterin (BH4) for catalytic activity. Sapropterin is a synthetic dihydrochloride salt formulation of the naturally occurring, biologically-active 6*R*-diastereomer of BH4.

The safety and efficacy of BH4 are approved by FDA and it is recommended for PAH responders and deficient patients. The therapeutic efficacy of BH4 depends on a number of factors, such as pharmacokinetics and pharmacodynamics properties.[82] Pharmacological response following oral administration of BH4 appears to be delayed, as demonstrated by a reduction in blood Phe concentrations 8–24 h after each administration. The recommended dose of sapropterin is 5–20 mg/kg/day.[82] Greater reductions in plasma Phe occur with doses of 10 or 20 mg/kg/day compared with 5 mg/kg/day.[83] Responsiveness to BH4 has been arbitrary, defined as a reduction of at least 30% of plasma Phe levels during a loading test with BH4 compared to the baseline Phe value.[9,84,85] It is estimated that 20–60% of PKU patients are BH4-responsive, whereas patients with mild PKU are more likely to respond.[42] Criteria to identify individuals for which a trial of sapropterin therapy may be appropriate vary widely and range from including all patients, prioritizing patients by clinical need or adherence history, or limiting therapy to a known mutation or enzyme activity.[15,42]

In the neonatal period, a BH4 loading test is performed immediately after a positive newborn screening result, using 20 mg/kg BH4/24 h, that allows the differentiation between responders and deficient patients. The drug is administered orally directly after taking a baseline blood Phe sample and blood samples are also taken at 4, 8 and 24 h after the loading, with the patients being on a normal diet during the test. However, this kind of test can be more useful knowing the genotype of the patient.[86,87]

In most parts of the world, BH4 is prescribed to patients from four years of age onwards and in accordance with the latest European guidelines: in any patient without two known null mutations for PKU or two known BH4 responsive mutations, a 48 h BH4 loading test should be done by analyzing the blood Phe concentrations before and after a single daily dose (20 mg/kg /day) on two consecutive days.[87]

Treatment with BH4 should only be described in cases of proven long-term BH4 responsiveness, defined as an increase in the amount of natural protein intake, during a 6 month trial with BH4. The treatment trial starts at 10–20 mg of BH4 per kg bodyweight and natural protein intake is increased (with the advice of a dietician), the BH4 dose is adjusted, and the AA supplements are decreased accordingly, while blood Phe concentrations are still maintained in the target range.[7] Increases in dietary Phe intake from natural protein are reported to range from less than two-fold to more than three- or four-fold over baseline intake.[84,88] The best approach for determining increased Phe tolerance is to add incrementally a natural protein (e.g. powdered milk) that can be easily calculated, measured and adjusted without significantly affecting the patient's usual diet. This can then be converted to dietary protein if the Phe levels remain in the target range. Regular blood Phe monitoring and evaluation of dietary intake records are important throughout the Phe challenge and subsequent dietary modification. Illness, missed sapropterin dosage, or changes in diet, exercise or lifestyle may complicate the evaluation of the test. After determining increased Phe tolerance, dietary modification can be made to include reevaluation of requirements for medical food and SLPFs. Reeducation of the patient and/or family on implementation of the new dietary regimen is essential.[15] Regular monitoring of blood Phe levels and dietary intake is required to assess compliance and nutritional adequacy. A reassessment of Phe tolerance may be needed subsequent to major changes in growth, body mass, or lifestyle. There are few studies describing the long-term effect of sapropterin therapy along with modified dietary management.[84,88] Diet liberalization has the potential to augment improved adherence to treatment with associated flexibility in food choices and quantity in addition to enhancing the nutritional quality of the diet.[89]

Several studies provide a relationship between predictors of BH4 responsiveness and BH4 long-term response, including Phe levels at diagnosis, Phe:Tyr ratio, Phe tolerance before BH4 treatment and genotype.[86,90–92] There are mutations of PAH involved, typically 'mild' such as p.Y414C or p.E390G that strongly predict BH4 responsiveness, while others strongly predict non-responsiveness, e.g. p.R408W.[93] Although there is a general relationship between the particular variants in the PAH gene and patient's BH4 responsiveness, the PAH genotype is not always related to phenotype.[9,93]

BH4 treatment below the age of four years may be promising[94] because starting BH4 treatment at a young age can be beneficial for a healthy dietary pattern, containing more natural protein intake.[38,39] Based on the results of the SPARK (Safety Paediatric EfficAcy PhaRmacokinetics with Kuvan) study, the European Medicines Agency has recently included children below 4 years old, for whom the previous standard care was a Phe-restrictive diet.[95] The results of this study, which was the first clinical trial of sapropterin in patients aged less than 4 years old with BH4-responsive PKU, mild PKU or mild HPA in Europe, showed that daily dosing with 10 or 20 mg/kg/day sapropterin was well tolerated and in combination with a Phe-restricted diet, led to a significant improvement in Phe tolerance compared with only a Phe-restrictive diet.

Clinical data regarding the safety and efficacy of the use of this drug in pregnancy is limited. However, sapropterin dihydrochloride has been used during pregnancy anecdotally, but only if women are known to be BH4 responders before pregnancy.[96] Since drug studies in pregnancy are not feasible, experience is based on a small number of case reports and small cohort studies. Therefore, no prospective data are available regarding the indication, dose and management of BH4 during pregnancy.[7]

### *Phenylalanine ammonia-lyase (PAL)*

Phenylalanine ammonia lyase (PAL) is a prokaryotic enzyme that converts phenylalanine to ammonia and trans-cinnamic acid, which is then quickly converted into hippurate and excreted in urine.[97] Attempts have been focused for the utilization of PAL in the PKU control administrating it orally to decrease Phe content in the gut or injected to reduce blood phenylalanine levels.

Unhappily, issues involving immunogenicity and other side effects appeared. The evaluation of PAL enzymes from multiple species, and subsequent studies in animal models led to the development of a recombinant *Anabaena variabilis* produced in Escherichia coli conjucated with PEG (pegylated PAL) to reduce immunogenicity (rAvPAL-PEG).[43] Recently in another experimental model the rAvPAL-PEG given orally, showed promising results with a mean blood Phe level reduction of around 40% with no serious adverse reactions. It was observed that Phe reduction occurred in a dose- and loading-dependent way.[98] More recently, the administration of pegvaliase, (rAvPAL-PEG) to PKU adults showed promising results in lowering Phe levels, without/or little immunoreaction symptoms.[99,100]

### *Maternal PKU*

Maternal PKU syndrome refers to the teratogenic effects of elevated maternal blood Phe during pregnancy.[44] Untreated maternal PKU or hyperphenylalaninemia may result in nonphenylketonuric offspring with neonatal sequelae, especially intellectual disability, microcephaly and congenital heart disease. The risk of congenital abnormalities increases with increasing maternal blood Phe concentration.[44]

The aim of managing maternal PKU is for women to be on strictly controlled diet preconception and onwards, with regular Phe monitoring showing levels between 120 and 360 μmol/l.[7,101] For women trying to conceive and during pregnancy, untreated Phe blood concentrations above 360 μmol/l should be reduced.[3,7] Contraceptive strategies should only be discontinued after stable Phe concentrations, within the target range, have been achieved for at least 2 weeks, thus preventing sustained Phe concentrations above or below the target range.[7]

In addition to elevated maternal blood Phe concentrations, evidence of harm has also been shown for blood Phe concentrations <120 μmol/l during pregnancy.[102] In this case, Phe intake must immediately be increased by 50–100 mg per day, especially during the second and third trimester of pregnancy, where there is an increased Phe requirement, due to fetal-maternal anabolism. Otherwise, PKU pregnant women who remain constantly in a low natural protein intake with blood Phe concentrations below 120 μmol/l are at higher risk to deliver Intrauterine Growth Restricted (IUGR) fetuses. Other significant factors associated with abnormalities found in the infant include low pre-pregnancy weight, poor weight gain during pregnancy and low intake of protein from medical food.[7] Inadequate energy intake, with weight loss especially during the first trimester is common due to poor appetite associated with nausea and vomiting, dislike of low protein foods and inability to consume the Phe-free *L*-amino acid supplements. Therefore, frequent monitoring of dietary intake during pregnancy is essential to ensure nutrient adequacy, with proper proportion of protein, fat, and carbohydrates, and adequate but not excessive energy intake. AA supplements usually supply sufficient Tyr (intake should be at least 6 g

per day). The recommendations for folic acid is 400 μg per day preconceptually and during the first 12 weeks of pregnancy.[7]

In the presence of these risks, all females with PKU must be monitored for the duration of their lives (levels should be determined with protocols similar with the ones used for neonatal screening),[103] being counseled early in their childhood and having a longstanding trusting relationship with their PKU team.[104] Co-monitor in conjunction with practitioners from an experienced metabolic center and close collaboration with the obstetrician should be considered essential during pregnancy, and in the post-partum period. High blood Phe concentrations above target range imply a high-risk pregnancy, necessitating the need for high-resolution ultrasound and fetal echocardiogram. Laboratory examinations included, vitamins B12, 25-OH $D_3$, folic acid, ferritin, total protein and albumin concentrations in serum should be done at regular intervals. Close dietetic advise in relation to the results of the above-mentioned laboratory tests is indicated.

BH4 treatment in maternal PKU should be considered with caution, only if women are known to be BH4 responders and strict dietary management does not adequately reduce blood Phe levels.[3,7,105] Breastfeeding may be pursued if the infant does not have PAH deficiency, but the provision of breastfeeding to an infant with PKU whose mother also has PKU requires careful monitoring with regard to the Phe concentrations both in the mother and the infant.[7]

### *Conclusions*

The most serious neurological manifestations presented in PKU are now preventable with early diagnosis of the disease by neonatal screening, as well as the establishment and adherence to the special PKU therapeutic diet. In contrast, a variety of subtle physical, neuropsychological and behavioral impairments have been recognized, even if the PKU patients adhere strictly to the special dietary recommendation. These outcomes stem from a complex array of factors, including the severity of PKU and a variety of other genetic and environmental effects. Full access to medical foods and foods modified to be low in protein, the coordination of medical follow up, dietetic adherence to the special diet, psychological support not only of the patients but also of their parents and genetic counseling, as well as biochemical laboratory tests at regular intervals facilitate good care of individuals with PKU. An interdisciplinary approach is also required to ensure successful transition from pediatric to adult care. New medical foods such as GMP-MF, LNAAs and SPLFs make easier the control of this metabolic disorder as compared previously. BH4 is mostly administrated to those of patients in whom certain mutations are identified. The utilization of the drug results in lowering Phe blood levels even though patients Phe intake is increased. Other drugs, such as PAL, are under investigation, representing a long-awaited potential therapy of this metabolic disorder. Gene therapy is expected to be the future for the management of such a disease.

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## References

1 Scriver CR, Kaufman S. Hyperphenylalaninaemia: phenylalanine hydroxylase deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D, Childs B, Kinzler K, Vogelstein B, (eds). The metabolic and molecular bases of inherited disease, 8th ed. New York: McGrawHill; 2001. p. 1667–724.
2 Mitchell JJ, Trakadis YJ, Scriver CR. Phenylalanine hydroxylase deficiency. Genet Med 2011;13:697–707.
3 Vockley J, Andersson HC, Antshel KM, Braverman NE, Burton BK, Frazier DM, *et al*. Phenylalanine hydroxylase deficiency: diagnosis and management guideline. Genet Med 2014;16:188–200.
4 Singh RH, Rohr F, Frazier D, Cunningham A, Mofidi S, Ogata B, *et al*. Recommendations for the nutrition management of phenylalanine hydroxylase deficiency. Genet Med 2014;16:121–31.
5 Ahring K, Belanger-Quintana A, Dokoupil K, Gokmen-Ozel H, Lammardo AM, MacDonald A, *et al*. Blood phenylalanine control in phenylketonuria: a survey of 10 European centres. Eur J Clin Nutr 2011;65:275–78.
6 Blau N, Bélanger-Quintana A, Demirkol M, Feillet F, Giovannini M, MacDonald A, *et al*. Management of phenylketonuria in Europe: survey results from 19 countries. Mol Genet Metab 2010;99:109–15.
7 van Wegberg AM, MacDonald A, Ahring K, Quintana AB, Blau N, Bosch AM, *et al*. The complete European guidelines on phenylketonuria: diagnosis and treatment. Orphanet J Rare Dis 2017;12:162.
8 Guldberg P, Rey F, Zschocke J, Romano V, Francois B, Michiels L, *et al*. A European multicentre study of phenylalanine hydroxylase deficiency:classification of 105 mutations and a general system for genotype-based prediction of metabolic phenotype. Am J Hum Genet 1998;63:71–79.
9 Blau N, Hennermann JB, Langenbeck U, Lichter-Konecki U. Diagnosis, classification, and genetics of phenylketonuria and tetrahydrobiopterin (BH4) deficiencies. Mol Genet Metab 2011;104:S2–S9.
10 Ahring K, Bélanger-Quintana A, Docoupil K, Gokmen Ozel H, Lammardo AM, MacDonald A, *et al*. Dietary management practices in phenylketonuria across European centres. Clin Nutr 2009;28:231–36.
11 Rohde C, Mütze U, Weigel JF, Ceglarek U, Thiery J, Kiess W, *et al*. Unrestricted consumption of fruit and vegetables in phenylketonuria: no major impact on metabolic control. Eur J Clin Nutr 2012;66:633–38.
12 Webster D, Wildgoose J. Tyrosine supplementation for phenylketonuria. Cochrane Database Syst Rev 2010;8:CD001507.
13 Sharman R, Sullivan K, Young R, McGill J. A preliminary investigation of the role of the phenylalanine:tyrosine ratio in children with early and continuously treated phenylketonuria: toward identification of "safe" levels. Dev Neuropsychol 2010;35:57–65.
14 van Spronsen FJ, Van Dijk T, Smit GP, van Rijn M, Reijngoud DJ, Berger R, *et al*. Large daily fluctuations in plasma tyrosine in treated patients with phenylketonuria. Am J Clin Nutr 1996;64:916–21.
15 Camp KM, Parisi MA, Acosta PB, Berry GT, Bilder DA, Blau N, *et al*. Phenylketonuria scientific Review Conference: State of

the science and future research needs. Mol Genet Metab 2014;112:87–122.
16 European Commission. Foods for Special Medical Purposes. Available at:http://ec.europa.eu/food/food/labellingnutrition/medical/index_en.htm (Last accessed 21st July 2018).
17 MacDonald A, Rylance G, Davies P, Asplin D, Hall SK, Booth IW. Administration of protein substitute and quality of control in phenylketonuria: a randomized study. J Inherit Metab Dis 2003;26:319–26.
18 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:S40–43.
19 Robert M, Rocha JC, van Rijn M, Ahring K, Bélanger-Quintana A, MacDonald A, *et al*. Micronutrient status in phenylketonuria. Mol Genet Metab 2013;110:S6–16.
20 Pardridge WM. Blood-brain barrier carrier-mediated transport and brain. Metabolism of amino acids. Neurochem Res 1998;23:635–44.
21 Blau N, MacDonald A, van Spronsen F. There is no doubt that the early identification of PKU and prompt and continuous intervention prevents mental retardation in most patients. Mol Genet Metab 2011;104:S1.
22 Gropper SS, Gropper DM, Acosta PB. Plasma amino acid response to ingestion of L-amino acids and whole protein. J Pediatr Gastroenterol Nutr 1993;16:143–50.
23 Fouillet H, Mariotti F, Gaudichon C, Bos C, Tom D. Peripheral and splanchnic metabolism of dietary nitrogen are differently affected by the protein source in humans as assessed by compartmental modeling. J Nutr 2002;132:125–33.
24 Metges CC, El-Khoury AE, Selvaraj AB, Tsay RH, Atkinson A, Regan MM, *et al*. Kinetics of L-[1-(13)] leucine when ingested with free amino acids, unlabeled or intrinsically labeled casein. Am J Physiol Endocrinol Metab 2000;278:E1000–9.
25 Schoeffer A, Herrmann M-E, Brosicke HG, Monch E. Influence of single dose amino acid mixtures on the nitrogen retention in patients with phenylketonuria. J Nutr Med 1994;4:415–18.
26 Pena MJ, Almeida MF, Dam E, Ahring K, Bélanger-Quintana A, Dokoupil K, *et al*. Protein substitutes for phenylketonuria in Europe: access and nutritional composition. Eur J Clin Nutr 2016;70:785–89.
27 Evans S, Daly A, MacDonald J, Preece MA, Santra S, Vijay S, *et al*. The micronutrient status of patients with phenylketonuria on dietary treatment: an ongoing challenge. Ann Nutr Metab 2014;65:42–48.
28 Schulpis KH, Karakonstantakis T, Bartzeliotou A, Karikas GA, Papassotiriou I. The association of serum lipids, lipoproteins and apolipoproteins with selected trace elements and minerals in phenylketonuria patients on diet. Clin Nutr 2004;23:401–7.
29 Schulpis KH, Nounopoulos C, Scarpalezou A, Bouloukos A, Missiou-Tsagarakis S. Serum carnitine level in phenylketonuric children under dietary control in Greece. Acta Paediatr Scand 1990;79:930–34.
30 Schulpis KH, Papastamataki M, Stamou H, Papassotiriou I, Margeli A. The effect of diet on total antioxidant status, ceruloplasmin, transferrin and ferritin serum levels in phenylketonuric children. Acta Paediatr 2010;99:1565–70.
31 Schulpis KH, Karikas GA, Papakonstantinou E. Homocysteine and other vascular risk factors in patients with phenylketonuria on a diet. Acta Pediatr 2002;91:905–9.
32 Stolen LH, Lilje R, Jorgensen JV, Bliksrud YT, Almaas R. High dietary folic acid and high plasma folate in children and adults with phenylketonuria. JIMD Rep 2014;13:83–90.
33 Moseley K, Koch R, Moser AB. Lipid status and long-chain polyunsaturated fatty acid concentrations in adults and adolescents with phenylketonuria on phenylalanine-restricted diet. J Inherit Metab Dis 2002;25:56–64.
34 Agostoni C, Harvie A, McCulloch DL, Demellweek C, Cockburn F, Giovannini M, *et al*. A randomized trial of long-chain polyunsaturated fatty acid supplementation in infants with phenylketonuria. Dev Med Child Neurol 2006;48:207–12.
35 Willatts P, Forsyth S, Agostoni C, Casaer P, Riva E, Boehm G. Effects of long-chain PUFA supplementation in infant formula on cognitive function in later childhood. Am J Clin Nutr 2013;98:536S–42S.
36 Demirdas S, Coakley KE, Bisschop PH, Hollak CE, Bosch AM, Singh RH. Bone health in phenylketonuria: a systematic review and meta-analysis. Orphanet J Rare Dis 2015;10:17.
37 Rousseau JH, Kleppinger A, Kenny AM. Self-reported dietary intake of omega-3 fatty acids and association with bone and lower extremity function. J Am Geriatr Soc 2009;57:1781–88.
38 Lohner S, Fekete K, Decsi T. Lower n-3 long-chain polyunsaturated fatty acid values in patients with phenylketonuria: a systematic review and meta-analysis. Nutr Res 2013;33:513–20.
39 MacDonald A, Rocha JC, van Rijn M, Feillet F. Nutrition in phenylketonuria. Mol Genet Metab 2011;104:S10–18.
40 Van Calsar SC, Ney DM. Food products made with Glycomacropeptide, a low-phenylalanine whey protein, provide a new alternative to amino Acid-based medical foods for nutrition management of phenylketonuria. J Acad Nutr Diet 2012;112:1201–10.
41 Concolino D, Mascaro I, Moricca MT, Bonapace G, Matalon K, Trapasso J, *et al*. Long-term treatment of phenylketonuria with a new medical food containing large neutral amino acids. Eur J Clin Nutr 2017;71:51–55.
42 Blau N. Sapropterin dihydrochloride for the treatment of hyperphenylalaninemias. Expert Opin Drug Metab Toxicol 2013;9:1207–18.
43 Bell SM, Wendt DJ, Zhang Y, Taylor TW, Long S, Tsuruda L, *et al*. Formulation and PEGylation optimization of the therapeutic pegylated phenylalanine ammonia lyase for the treatment of phenylketonuria. PLoS One 2017;12:e0173269.
44 Rouse B, Azen C, Koch R, Matalon R, Hanley W, de la Cruz F, *et al*. Maternal Phenylketonuria Collaborative Study (MPKUCS) offspring: facial anomalies, malformations, and early neurological sequelae. Am J Med Genet 1997;69:89–95.
45 Andersen AE, Avins L. Lowering brain phenylalanine levels by giving other large neutral amino acids. A new experimental therapeutic approach to phenylketonuria. Arch Neurol 1976;33:684–86.
46 van Spronsen FJ, de Croot 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;33:671–76.
47 Smith QR. Transport of glutamate and other amino acids at the blood-brain barrier. J Nutr 2000;130:1016S–22S.
48 de Groot MJ, Hoeksma M, Reijngoud DJ, de Valk HW, Paans AM, Sauer PJ, *et al*. Phenylketonuria: reduced tyrosine brain influx relates to reduced cerebral protein synthesis. Orphanet J Rare Dis 2013;8:133.
49 Yano S, Moseley K, Azen C. Large neutral amino acid supplementation increases melatonin synthesis in phenylketonuria: a new biomarker. J Pediatr 2013;162:999–1003.
50 Schindeler S, Ghosh-Jerath S, Thompson S, Rocca A, Joy P, Kemp A, *et al*. The effects of large neutral amino acid supplements in PKU: An MRS and neuropsychological study. Mol Genet Metab 2007;91:48–54.
51 Matalon R, Michals-Matalon K, Bhatia G, Burlina AB, Burlina AP, Braga C, *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;30:153–58.
52 Laclair CE, Ney DM, MacLeod EL, Etzel MR (2009) Purification and use of Glycomacropeptide for nutritional management of phenylketonuria. J Food Sci 2009;74:E199–206.
53 Lim K, van Calsar 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;92:176–78.
54 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;138:316–22.
55 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:e45165.
56 Sawin EA, De Wolfe TJ, Aktas B, Stroup BM, Murali SG, Steele JL, *et al*. 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:G590–601.
57 Patel S. Emerging trends in nutraceutical applications of whey protein and its derivatives. J Food Sci Technol 2015;52:6847–58.
58 Ney DM, Stroup BM, Clayton MK, Murali SG, Rice GM, Rohr F, *et al*. Glycomacropeptide for nutritional management of phenylketonuria: a randomized, controlled, crossover trial. Am J Clin Nutr 2016;104:334–45.

59 Daly A, MacDonald A, Evans S, Chahal S. Glycomacropeptide: can we safely advocate its use in children with PKU? J Inherit Metab Dis 2015;38:S35–39.
60 Zaki OK, El-Wakeel L, Ebeid Y, Ez Elarab HS, Moustafa A, Abdulazim N, *et al*. The Use of Glycomacropeptide in Dietary Management of Phenylketonuria. J Nutr Metab 2016;2016:2453027.
61 Pinto A, Almeida MF, Ramos PC, Rocha S, Guimas A, Ribeiro R, *et al*. Nutritional status in patients with phenylketonuria using glycomacropeptide as their major protein source. Eur J Clin Nutr 2017;71:1230–34.
62 Daly A, Evans S, Chahal S, Santra S, MacDonald A. Glycomacropeptide in children with phenylketonuria: does its phenylalanine content affect blood phenylalanine control? J Hum Nutr Diet 2017;30:515–23.
63 Manta-Vogli PD, Schulpis KH. Phenylketonuria dietary management and an emerging development. J Acad Nutr Diet 2018;118:1361–63.
64 El-Hattab AW. Systemic primary carnitine deficiency. In: Adam MP, Ardinger HH, Pagon RA, editors. GeneReviews. Seattle: University of Washington; 2016.
65 Schulpis KH, Papakonstantinou ED, Vlachos GD, Vlachos DG, Antsaklis A, Papassotiriou I, *et al*. The effect of the mode of delivery on the maternal-neonatal carnitine blood levels and antioxidant status. Clin Chem Lab Med 2008;46:680–86.
66 Mutze U, Beblo S, Kortz L, Matthies C, Koletzko B, Bruegel M, *et al*. Metabolomics of dietary fatty acid restriction in patients with phenylketonuria. PLoS One 2012;7:e43021.
67 Weigel C, Kiener C, Meier N, Schmid P, Rauh M, Rascher W, *et al*. Carnitine status in early-treated children, adolescents and young adults with phenylketonuria on low phenylalanine diets. Ann Nutr Metab 2008;53:91–95.
68 Stroup BM, Nair N, Murali SG, Broniowska K, Rohr F, Levy HL, *et al*. Metabolomic Markers of Essential Fatty Acids, Carnitine, and Cholesterol Metabolism in Adults and Adolescents with Phenylketonuria. J Nutr 2018;148:194–201.
69 Ney DM, Murali SG, Stroup BM, Nair N, Sawin EA, Rohr F, Levy HL. Metabolomic changes demonstrate reduced bioavailability of tyrosine and altered metabolism of tryptophan via the kynurenine pathway with ingestion of medical foods in phenylketonuria. Mol Genet Metab 2017;121:96–103.
70 Daenzer M, Petzke KJ, Bequette BJ, Metges CC. Whole-body nitrogen and splanchnic amino acid metabolism doffer in rats fed mixed diets containing casein or its corresponding amino acid mixture. J Nutr 2001;131:1965–72.
71 Officer DI, Batterham ES, Farrell DJ. Comparison of growth performance and nutrient retention of weaner pigs given diets based on casein, free amino acids or conventional proteins. Br J Nutr 1997;77:731–44.
72 Homer K, Dreummond E, Brennan L. Bioavailability of milk protein-derived bioactive peptides: a glycaemic management perspective. Nutr Res Rev 2016;29:91–101.
73 Krissansen GW. Emerging health properties of whey proteins and their clinical implications. J Am Coll Nutr 2007;26:713S–23S.
74 MacDonald A, Cochrane B, Wopereis H, Loveridge N. Specific prebiotics in a formula for infants with phenylketonuria. Mol Genet Metab 2011;104:S55–59.
75 Hennermann JB, Roloff S, Gellermann J, Vollmer I, Windt E, Vetter B, *et al*. Chronic kidney disease in adolescent and adult patients with phenylketonuria. J Inherit Metab Dis 2013;36:747–56.
76 van Calcar SC, MacLeod EL, Gleason ST, Etzel MR, Clayton MK, Wolff JA, *et al*. Improved nutritional management of phenylketonuria by using a diet containing glycomacropeptide compared with amino acids. Am J Clin Nutr 2009;89:1068–77.
77 Ney DM. Does the PKU diet contribute to impaired renal function? J Inherit Metab Dis 2013;36:903–4.
78 Stroup BM, Sawin EA, Murali SG, Binkley N, Hansen KE, Ney DM. Amino Acid Medical Foods Provide a High Dietary Acid Load and Increase Urinary Excretion of Renal Net Acid, Calcium, and Magnesium Compared with Glycomacropeptide Medical Foods in Phenylketonuria. J Nutr Metab 2017:1909101.
79 Solverson P, Murali SG, Brinkman AS, Nelson DW, Clayton MK, Yen CL, *et al*. Glycomacropeptide, a low-phenylalanine protein isolated from cheese whey, supports growth and attenuates metabolic stress in the murine model of phenylketonuria. Am J Physiol Endocrinol Metab 2012;302:E885–95.
80 Pena MJ, Almeida MF, van Dam E, Ahring K, Belanger-Quintana A, Dokoupil K, *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.
81 Schulpis KH, Papassotiriou I, Tsakiris S, Vounatsou M, Chrousos GP. Increased plasma adiponectin concentrations in poorly controlled patients with phenylketonuria normalize with strict diet: evidence for catecholamine-mediated adiponectin regulation and a complex effect of phenylketonuria diet on atherogenesis risk factors. Metabolism 2005;54:1350–55.
82 Feillet F, Clarke L, Medi C, Lipson M, Morris AA, Harmatz P, *et al*. Pharmacokinetics of Sapropterin in Patients with Phenylketonuria. Clin Pharmacokinetics 2008;47:817–25.
83 Lee P, Treacy EP, Crombez E, Wasserstein M, Waber L, Wolff J, *et al*. Safety and efficacy of 22 weeks of treatment with sapropterin dihydrochloride in patients with phenylketonuria. Am J Med Genet A 2008;146A:2851–59.
84 Trefz FK, Burton BK, Longo N, Casanova MM, Gruskin DJ, Dorenbaum A, *et al*. Efficacy of sapropterin dihydrochloride in increasing phenylalanine tolerance in children with phenylketonuriia : a phase III randomized, double-blind, placebo-controlled study. J Pediatr 2009;154:700–7.
85 Steinfeld R, Kohlschutter A, Zschocke J, Lindner M, Ullrich K, Lukacs Z. Tetrahydrobiopterin monotherapy for phenylketonuria patients with common mild mutations. Eur J Pediatr 2002;161:403–5.
86 Anjema K, Hofstede FC, Bosch AM, Rubio Gozalbo ME, de Vries MC, Boelen CCA, *et al*. The neonatal tetrahydrobiopterin loading test in phenylketonuria: what is the predictive value? Orphanet J Rare Dis 2016;11:10.
87 Anjema K, van Rijn M, Hofstede FC, Bosch AM, Hollak CE, Rubio-Gozalbo E, *et al*. Tetrahydrobiopterin responsiveness in phenylketonuria: prediction with the 48-hour loading test and genotype. Orphanet J Rare Dis 2013;8:103.
88 Singh RH, Jurecki E, Rohr F. Recommendations for personalized dietary adjustments based on patient response to tetrahydrobiopterin (BH4) in phenylketonuria. Top Clin Nutr 2008;23:149–57.
89 Singh RH, Quirk ME, Douglas TD, Brauchla MC. BH4 therapy impacts the nutrition status and intake in children with phenylketonuria: 2-year follow-up. J Inherit Metab Dis 2010;33:689–95.
90 Humphrey M, Nation J, Francis I, Boneh A. Effect of tetrahydropterin on Phe/Tyr ratios and variation in Phe levels in tetrahydropterin responsive PKU patients. Mol Genet Metab 2011;104:89–92.
91 Keil S, Anjema K, van Spronsen FJ, Lambruschini N, Burlina A, Bélanger-Quintana A, *et al*. Long-term follow-up and outcome of phenylketonuria patients on sapropterin: a retrospective study. Pediatrics 2013;131:e1881–88.
92 Utz JR, Lorentz CP, Markowitz D, Rudser KD, Diethelm-Okita B, Erickson D, *et al*. START, a double blind, placebo-controlled pharmacogenetic test of responsiveness to sapropterin dihydrochloride in phenylketonuria patients. Mol Genet Metab 2012;105:193–97.
93 Heintz C, Cotton RG, Blau N. Tetrahydrobiopterin, its mode of action on phenylalanine hydroxylase and importance of genotypes for pharmacological therapy of phenylketonuria. Hum Mutat 2013;34:927–36.
94 Burton BK, Adams DJ, Grange DK, Malone JI, Jurecki E, Bausell H, *et al*. (2011) Tetrahydrobiopterin therapy for phenylketonuria in infants and young children. J Pediatr 2011;158:410–15.
95 Muntau AC, Burlina A, Eyskens F, Freisinger P, De Laet C, Leuzzi V, *et al*. Efficacy, safety and population pharmacokinetics of sapropterin in PKU patients <4 years: results from the SPARK open-label, multicenter, randomized phase IIIb trial. Orphanet J Rare Dis 2017;12:47.
96 Grange DK, Hillman RE, Burton BK, Yano S, Vockley J, Fong CT, *et al*. Sapropterin dihydrochloride use in pregnant women with phenylketonuria: an interim report of the PKU MOMS sub-registry. Mol Genet Metab 2014;112:9–16.
97 Hoskins JA, Hollidays SB, Greenway AM. The metabolism of cinnamic acid by healthy and phenylketonuric adults; a kinetic study. Biomed Mass Spectrom 1984;11:296–300.
98 Sarkissian CN, Kang TS, Gamez A, Scriver CR, Stevens RC. Evaluation of orally administered PEGylated phenylalanine ammonia lyase in mice for the treatment of phenylketonuria. Mol Genet Metab 2011;104:249–54.

99 Zori R, Thomas JA, Shur N, Rizzo WB, Decker C, Rosen O, *et al*. Induction, titration, and maintenance dosing regimen in a phase 2 study of pegvaliase for control of blood phenylalanine in adults with phenylketonuria. Mol Genet Metab 2018 in press.
100 Thomas J, Levy H, Amato S, Vockley J, Zori R, Dimmock D, *et al*. PRISM investigators. Pegvaliase for the treatment of phenylketonuria: Results of a long-term phase 3 clinical trial program (PRISM). Mol Genet Metab 2018;124:27–38.
101 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;95:374–82.
102 Teissier R, Nowak E, Assoun M, Mention K, Cano A, Fouilhoux A, *et al*. Maternal phenylketonuria: low phenylalaninemia might increase the risk of intra uterine growth retardation. J Inherit Metab Dis 2012;35:993–99.
103 Loukas YL, Soumelas GS, Dotsikas Y, Georgiou V, Molou E, Thodi G, *et al*. Expanded newborn screening in Greece: 30 months of experience. J Inherit Metab Dis 2010;33:S341–48.
104 Koch R, Trefz F, Waisbren S. Psychosocial issues and outcomes in maternal PKU. Mol Genet Metab 2010;99:S68–74.
105 Feillet F, Muntau AC, Debray FG, Lotz-Havla AS, Puchwein-Schwepcke A, Fofou-Caillierez MB, *et al*. Use of sapropterin dihydrochloride in maternal phenylketonuria. A European experience of eight cases. J Inherit Metab Dis 2014;37:753–62.