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Minireview

Nutrition in phenylketonuria

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ABSTRACT

The same basic principles are used to deliver dietary treatment in PKU that was developed sixty years ago. Dietary treatment is undoubtedly very successful, but it has gradually evolved and been guided commonly by individual experience and expert opinion only. There is little international consensus about dietary practice with improvements in specialist dietary products concentrating on taste and presentation rather than nutritional composition. Many areas of dietary treatment have not been rigorously examined. In particular, the amino acid and micronutrient profile of Phenylalanine-free (phe-free) amino acids requires further study. In different formulations of phe-free amino acids, there are variations in the amino acid patterns as well the amount of essential and non essential amino acids per 100 g/amino acids. The amount of added tyrosine and branch chain amino varies substantially, and in PKU specifically, there is little data about their relative absorption rates and bioavailability. In phe-free amino acids, there is evidence suggesting that some of the added micronutrients may be excessive and so the source and amount of each micronutrient should be scrutinized, with a need for the development of international nutritional composition standards exclusively for these products. There is a dearth of data about the life-long phenylalanine tolerance of patients or the nutritional state of adult patients treated with diet. There is a growing need to measure body composition routinely in children with PKU and with the rise in childhood obesity, it is important to measure body fatness and identify those who are at greatest risk of 'co-morbidities' of obesity. There is necessity for international collaboration to ensure robust data is collected on many basic aspects of nutritional care to guarantee that diet therapy is delivered to the highest standard.

Contents

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1. Introduction

Phenylketonuria (PKU) is a genetic disorder first treated with a low phenylalanine diet sixty years ago, and more recently with the drug BH_4. Diet therapy and nutrition remain a central focus in the treatment of PKU and diet has seen many improvements both in terms of nutritional quality and palatability of specialist dietary products. However, there are many areas where either limited knowledge about the nutritional needs of the normal 'healthy' population and unreliable or under-developed tools to assess nutritional status have hampered progress in developing diet therapy further. In addition, some areas of diet therapy have not received rigorous examination and much existing practice is based on years of experience rather than robust evidence. In this review, we will assess present knowledge concerning protein, amino acids, vitamin and trace element status, in addition to examining the growth and body composition of patients with PKU.

2. Protein requirements

The primary function of dietary protein is to provide amino acids for growth, renewing tissue protein and synthesizing specific nitrogen-containing products [1]. After ingestion, proteins are denatured in the stomach and pass into the small intestine, where the resultant mixture of free amino acids and small peptides is then transported into the enteral cells and secreted as free amino acids into the bloodstream or further metabolized within the gut or liver itself [2]. Protein intake as free amino acids results in a faster rate of appearance in plasma compared to intact protein [3,4].

In the fasted state, amino acids primarily appear from degradation of body protein as the only storage form; while in the fed state dietary proteins provide extra amino acids resulting in net protein synthesis. When amino acid appearance rate exceeds protein synthesis capacity, excess amino acids are degraded. Body proteins are in a constant state of degradation and (re)synthesis (250 g protein/day in adults), while dietary protein intake is much less. Inadequate dietary protein intake, especially of essential amino acids, results in net protein breakdown. More recently, there have been discussions on the importance of protein quality in addition to quantity especially in people with special needs [5].

2.1. Protein requirements for healthy individuals

Three separate terms have been used to define protein requirements (biological need, estimated average requirement [EAR] and recommended dietary intake [RDI]) [6]. Biological need defines the quantity that is consumed by the various metabolic pathways. It is the protein required for deposition and maintenance of equilibrium of N containing substances, with variations for age, growth, pregnancy, lactation, injury and infection. EAR includes correction for digestibility and amino acid pattern, the so called Protein Digestibility Corrected Amino Acid Score (PDCAAS) [1]. RDI is for a population, taking into account variability for both biological need and EAR.

Protein balance is strongly influenced by energy balance. Energy and/or glucose depletion will result in (especially branched chain) amino acids breakdown (gluconeogenesis) to meet minimal glucose requirement. When considering protein requirement, it is assumed energy requirement is met.

2.2. Protein requirements in PKU

Treatment consists of restriction of the essential amino acid phenylalanine by reducing the natural protein intake with concomitant supplementation of all amino acids except phenylalanine to meet protein requirements [7]. Adequate intake of phenylalanine, protein and energy must be provided to prevent breakdown of body tissues, which can lead to elevated plasma phenylalanine concentrations.

The dosage of the phenylalanine-free L-amino acids (phe-free amino acids) or protein substitute is determined both by the individual phenylalanine tolerance and the total protein requirement (i.e. natural protein + phe-free amino acids = total protein intake). Recommendations for the optimal amount of phe-free amino acids are based on protein recommendations for healthy individuals and additional factors that may influence protein utilization in PKU. They are founded by: a) protein recommendations for healthy individuals, b) the nutritional and assimilation of phe-free amino acids compared with natural (intact) protein, c) growth and (d) studies investigating metabolic control with differing dosages of phe-free amino acids.

In PKU, although there is no data to support that a higher dose of phe-free amino acids is required when giving amino acids instead of intact protein as the main source, an incremental compensation factor of 1.2 is used in the Dutch guidelines (unpublished data). This is in line with the USA RDA in which an adjustment is proposed of approximately 20% to compensate both for losses due to digestibility and protein quality for mainly vegetarian diets [8].

2.3. The nutritional value of L-amino acids

In PKU, the majority of non-phenylalanine protein is supplied by phe-free amino acids. Studies in healthy adults, investigating the bioavailability of amino acids mixtures compared to natural protein, demonstrate differences in the rate of absorption in the gut [9,10]. Amino acids delivered as dietary protein (casein) may support whole-body protein metabolism better than ingestion of crystalline L-amino acids, casein hydrolysates or soy protein [11]. Nitrogen losses are lower with intact protein compared with free amino acids.

Recently, a new low phenylalanine whey based protein substitute has been developed for PKU, called glycomacroprotein (GMP). It is derived from cheese whey (naturally low in phenylalanine) and it is supplemented with phe-free indispensible amino acids. In PKU, recent short term studies in mice and humans demonstrate a slower absorption, less degradation of L-amino acids and improved protein retention with glycomacroprotein compared with phe-free amino acids [12,13].

The composition of phe-free amino acids has received little study. It is possible they may be deficient in one or more indispensible amino acids, their ratios may be sub-optimal or a specific component of the natural food itself may be essential for differences in L-amino acid utilization. Amino acid patterns of phe-free amino acids designed for infants, children and adults are primarily based on that of human milk.

In different formulations of phe-free amino acids, there are variations in the amino acid patterns as well the amount of essential and non essential amino acids per 100 g/amino acids, which may influence bioavailability. In PKU, there is very little data comparing their relative absorption rates and bioavailability. Also the amount of tyrosine and the branch chain amino acids (BCAA) added to phe-free amino acids vary between individual products. This may be particularly important as the BCAA share the same transporter in the blood brain barrier and the gut as phenylalanine [14]. More studies are needed to define the optimal amino composition of phe-free amino acids.

2.3.1. Metabolic control and protein substitute dosage

Evidence based guidelines on protein requirement for the general population are still incomplete [1], and so for people with PKU much research is required, particularly for adults. In PKU, the few published guidelines (Guidelines of the Medical Research Council 1993; the Ross Metabolic Nutrition Support System) advocate a higher protein intake than for the general population [15,16]. Even so, a Cochrane review concluded that there was little evidence to support this recommendation [17], although increased dosages of phe-free amino acids have been shown to improve natural protein (Phenylalanine) tolerance [18–20].

The metabolic and dietary handbooks advise to divide the daily intake of phe-free amino acids into at least three equal parts, and to combine the intake of natural protein with the amino acid supplement. The studies of Monch and MacDonald are convincing on the positive effect of an even and frequent distribution of phe-free amino acids [19,21,22].

Although higher dosages of phe-free amino acids appear safe, and dosage may require individual titration according to phenylalanine tolerance and metabolic control, this has to be balanced against the demanding regimen of administering phe-free amino acids at least three times daily. They are expensive, particularly if they are being ineffectively utilized.

2.3.1.1. Tyrosine. In PKU, tyrosine is an indispensable amino acid because it is not supplied endogenously via phenylalanine hydroxylation or only to a very limited degree; it is important that it is supplemented in the diet. L-tyrosine is a precursor of several biologically active substances, including catecholamine neurotransmitters (dopamine, norepinephrine, and epinephrine), thyroxin and melanin skin pigments. Quantitatively, the amount of tyrosine needed for catecholamine and thyroid hormone synthesis is small [23]. Tyrosine requirement in children with PKU aged 6 to 9 years was estimated at 16.3 to 19.2 mg/kg/day [24]. The MRC [15] recommended that phe-free amino acids provide 100–120 mg/kg/day of tyrosine, which is 5 times the amount recommended for non-PKU school age children [24]. Many widely used phe-free amino acids in Europe contain 9 to 11% of their protein equivalent as tyrosine. However, tyrosine is the least soluble of all the amino acids and if protein substitute is a powder and reconstituted as a drink, it is common for tyrosine particles to separate, which unintentionally forms waste residue in the container, so the actual dose of tyrosine that some patients receive is unknown.

Treated patients with PKU have lower fasting tyrosine concentrations, but high post-prandial plasma tyrosine concentrations after consuming a dose of their phe-free amino acids. Although no clinical symptoms have been reported in patients with PKU, with post prandial tyrosine concentrations greater than 200 μmol/l, some have suggested that the amount of tyrosine added to phe-free amino acids is excessive [24,25]. A Cochrane review concluded that no recommendations could be given on tyrosine supplementation in PKU [26].

3. Dietary phenylalanine

In patients with severe PKU, there is a negligible or very minimal capacity to oxidize phenylalanine. Phenylalanine is an indispensable, aromatic amino acid. It is essential for protein synthesis and to maintain phenylalanine homeostasis [27] with the remainder being hydroxylated to tyrosine. In non PKU, in vivo studies indicate that 27–41% of L-phenylalanine is converted into tyrosine within 5–8 h of intake [28,29].

3.1. Requirements for phenylalanine in non-PKU

Defining any indispensable amino acid requirement is challenging as no method of assessment is entirely reliable [1]. The traditional technique used to determine amino acid requirements was nitrogen balance. However, several new methods have been developed, particularly the indicator amino acid oxidation and breakpoint analysis method, using stable isotopes, and this is now considered the best approach [30]. For a combination of the aromatic amino acids phenylalanine and tyrosine, the WHO 2007 [1] advocate a best estimate for adults of 25 mg/kg/day, although estimated mean requirements range from 15 to 39 mg/kg/day [28,30]. For infants and children, the WHO requirements for aromatic amino acids are given in Table 1. There is some evidence to suggest that tyrosine as a phenylalanine sparing effect (up to 78% of dietary phenylalanine need [30]), but the WHO (2007) [1] concluded it was not possible, at present, to define a specific value for this. The optimal dietary ratio of phenylalanine to tyrosine is suggested to be 60:40.

3.2. Phenylalanine requirements in PKU

It is particularly difficult to define phenylalanine needs in PKU. Pencharz et al. [31] has proposed that requirements are lower than those of the normal population by the amount of phenylalanine lost via obligatory oxidation [31]. Hence the phenylalanine requirement for patients with PKU should represent the intake of phenylalanine required for protein synthesis and growth. Obligatory oxidation of phenylalanine is estimated to be approximately 26% of the phenylalanine requirement in adult men [30]. An indicator amino acid oxidation study showed that the mean phenylalanine requirement for prepubertal children aged between 6 and 13 years with classical PKU was 14 mg/kg/day (with an upper 95% confidence interval of the mean of 19.5 mg/kg/day) [32].

In PKU, the individual dietary phenylalanine tolerance is pragmatically determined and is influenced by many factors (Table 2). It is defined as the amount of phenylalanine per kg/body weight that is tolerated to achieve plasma phenylalanine concentrations within a target range. In PKU, generally phenylalanine tolerance/requirements is highest in early infancy (ranging from 55 mg/kg/day at 0–3 months of age to 27 mg/kg/day at 12 months [33]). After the age of 1 year, there is a slow and steady decline in tolerance per kg/body weight, and even from the early days of treating PKU with diet it has been recognized that children with classic PKU, on dietary treatment usually only tolerate between 200 and 500 mg/day (0.2–0.5 g daily) [34–36]. Patients with a milder form of PKU (untreated blood

Table 1
Aromatic acid requirements for children [1].

Age (years)	Aromatic amino acids mg/kg/day
0.5	31
1–2	22
3–10	18
11–14	17
15–18	16
>18	15

Table 2
Factors affecting phenylalanine tolerance.

- In vivo phenylalanine hydroxylation rate
- Net protein catabolism
- Non-protein energy ratio
- Growth rate
- Age
- Gender
- Compliance
- Dosage of phe-free amino acids
- BH4 treatment
- Target blood phenylalanine concentration
- Pregnancy

NB: van Spronsen demonstrated no significant correlation between in vivo hydroxylation rates and phenylalanine tolerance [41], but this was in a small group of patients, almost all with classical PKU.

phenylalanine concentrations less than 1000 μmol/l), usually tolerate in excess of 500 mg/day dietary phenylalanine [37–39]. By comparison, in non-PKU, the third US National Health and Nutrition Examination Survey (NHANES 111) demonstrated that mean daily dietary phenylalanine intakes for all life stages and gender groups was as high as 3.4 g/day [8].

In PKU, van Spronsen has demonstrated that there is a clear relationship between phenylalanine tolerance at 2 years of age and at 10 years of age [40], although it is unknown how this tolerance relates to older patients with PKU, who may have a differing target blood phenylalanine range. There is clearly a need to evaluate the phenylalanine tolerance of all patients periodically, but particularly at the times of rapid growth, changes in body composition or treatment change. MacLeod demonstrated in a small of group of 8 adults with PKU, that almost all tolerated additional prescribed phenylalanine intake (by 15–173%) without significantly increasing blood phenylalanine concentration [41]. van Rijn also demonstrated in 6 well controlled adults with PKU that they tolerated more phenylalanine than prescribed [42]. However, the results from these small studies may not reflect the majority of adult patients.

Surprisingly, there have been no longitudinal reports documenting life time phenylalanine intakes in patients adhering to long term diet therapy; there have also been few reports documenting the phenylalanine intake of adolescent patients. Also the difference between prescribed and actual intake of phenylalanine in patients is rarely reported. It is possible that some patients are consuming a higher phenylalanine intake than prescribed, but because blood phenylalanine concentrations are within target ranges, dietary phenylalanine intake is not measured precisely. MacDonald et al. [43] demonstrated that an unlimited consumption of low phenylalanine foods (including a range of fruits and vegetables) increased prescribed phenylalanine intake by 31%, without loss of dietary control, when actual consumption of all food intake was calculated.

3.3. Phenylalanine deficiency

It has been many years since there have been reports of symptomatic phenylalanine deficiency in PKU. Hanley et al. [44] reviewed the symptoms associated with deficiency. These include generalized amino aciduria, anorexia, listlessness, alopecia, perineal rash, poor and variable growth in preschool children and even death. It is possible that young children, in particular, are at risk of phenylalanine deficiency if they have excellent blood phenylalanine control but refuse most dietary phenylalanine sources because of food faddiness or feeding difficulties. There is little data examining the 'actual' dietary intakes of toddlers with PKU. There is also a risk of phenylalanine deficiency in maternal PKU, particularly during the third trimester when the fetal liver metabolizes the maternal phenylalanine and fetal growth phenylalanine requirements increase.

3.4. Allocation of dietary phenylalanine

Phenylalanine comprises 4–6% of all dietary protein containing foods. Practical methods for prescribing phenylalanine intake vary throughout the world and it is either allocated by prescribing total daily phenylalanine allowance, where all phenylalanine containing foods are calculated in the diet, or by phenylalanine exchange systems (i.e. pre-calculated, prescribed portions of foods all containing the same amount of phenylalanine) [45]. No one method is shown to be better than another, and a comparison of blood phenylalanine control in 10 mainly European centers, indicated that blood phenylalanine control was similar in younger children despite differing dietary systems [46]. Although many PKU centers advocate that phenylalanine containing foods are weighed, with time carers or patients are more likely to estimate portion size by 'eye' only [47,48]. In addition, controlled studies to develop non-weighed aids to estimate phenylalanine exchanges have been unsuccessful [49].

4. Growth and body composition

4.1. Longitudinal growth

In the early years of treating PKU, the desire to achieve normal phenylalanine concentrations, using very restrictive diet therapy, led to growth impairment [50] and malnutrition, probably with a secondary negative effect on intellectual function [44]. In the 1990's, poor linear and head circumference growth were reported in German children with mean protein intakes ranging from 2.24 g/kg/body weight until 2 years of age and about 1.98 g/kg/body weight until 6 years (energy intakes were not reported) [51]. Further studies from the Netherlands, Germany and France also reported poor physical growth, with negative height z-scores, although data on nutrient intakes were unreported [52–54]. In Dutch children, mean height z-scores deteriorated between birth until 3 years of age [52], and there was no relationship between growth and the severity of blood phenylalanine control [55]. In France, Dhondt et al. demonstrated improved height z-scores after relaxation of diet at 8 years of age [54]. Results from earlier studies in the United States, who had a less strict approach to early management did not report problems with growth impairment [56,57]. Moreover, when nutritional recommendations from the UK Medical Research Council Party on PKU [15] were followed physical growth was considered normal [58,59], with higher protein intakes associated with the best growth [58]. More recently, another French centre reported growth retardation despite energy and total protein intakes above the recommend dietary allowances (RDA's) [60]. However, mean dietary zinc intakes were low and 19 of 20 patients studied had plasma zinc deficiency [60]. There was no direct correlation between zinc intake, status and growth.

In the most recent growth study, Huemer et al. demonstrated normal growth patterns with total protein intakes 20 to 40% above the RDA's [61]. In addition, body composition did not differ significantly from controls [61]. More interestingly, fat free mass correlated with natural protein intake [61] which was in good accordance with a Dutch study. This underlined the importance of the protein quality in order to promote an optimal head circumference growth in the first 3 years of life [62].

Considering the lack of recent data on growth on current dietary management and the need to clarify the really impact of a semi-synthetic diet, the definition of a reliable biochemical parameter to measure protein status to classify patients at risk would be helpful. Low prealbumin concentrations seem to be related with growth restriction in classical PKU [63] while patients with milder forms of PKU may also be at risk of protein insufficiency, especially if not taking adequate amounts of protein substitute [64]. Guidance on assessment of protein status is given in Table 3.

Table 3
Assessment tools for assessing protein status.

Assessment tools for assessing protein status	
Dietary records to assess protein intake	Calculating dietary protein and energy intake from food records is cheap and easy but unreliable because of differences in compliance, over/underestimating reported intake, differences in food tables and food composition labels on food packaging
Biochemical markers	• Hepatic Transport Proteins (total protein/albumin, prealbumin, retinol binding protein) • Plasma amino acids (prandial and post prandial) • IGF-1
Anthropometry	Growth, height for age (target height) and head circumference <2 years of age
Assessment of lean body mass	Densitometry, DXA [68], Bioelectrical impedance analysis [86–89]

4.2. Anthropometry and body composition measurements

Comparing growth measurements to a "good reference" is important to determine if patients are receiving adequate nutrition, although choosing the most appropriate growth charts is problematic. When national data is unavailable, care should be taken when comparing the growth of PKU patients with reference growth charts as they only represent how a cohort of children grew in a particular place and time. However, even the growth data from a specific population may vary widely when different criteria are used to collect the dataset population and varying cut-offs are applied to define optimal growth [65]. The WHO (2007) [66] growth charts should be considered as a standard rather than a reference, since they describe growth expectations of breast fed and healthy children (aged 0–59 months) under optimal environmental and health conditions [67].

There is a growing need to measure body composition in children with PKU. Measurement of body composition may improve the capacity to tailor nutritional management directly to lean mass as weight may be a poor proxy for this [68]. Furthermore, with the rise in childhood obesity in PKU and non-PKU children, it has become increasingly important to measure body fatness in children and identify those who are at greatest risk of 'co-morbidities' of obesity. However, obesity in pediatrics is not easy to define, given that different criteria exist [International Obesity Task Force (IOTF); World Health Organization (WHO); Centers for Disease Control and Prevention (CDC) and National Data] [69].

The measurement of body composition may include direct or indirect measurements of body fat, lean body mass and bone mass. A large body of consistent evidence suggests that body mass index is a practical and valid [70] method of measuring obesity in children over 2 years of age. However, when accurately measured [71], waist circumference appears to be a good predictor of intra-abdominal fat accumulation in adults [72,73], but also in children and adolescents [74–76]. Abdominal fat should be viewed as a risk factor for later metabolic and heart diseases [77,78], increasing the risk for complications in adulthood [79]. Waist circumference percentiles are available [75,80–84], but there is no agreement on the cut-offs to diagnose abdominal obesity [85].

4.3. Overweight and obesity trends in patients with PKU

The first reports of overweight and obesity in PKU appeared in the late 1970's and 80's [56,90]. In later studies, others have reported the same tendency of overweight in PKU [57,91]. An Italian study group, in a sample of 97 patients diagnosed by neonatal screening, concluded that early BMI rebound was associated with overweight [92]. In the same study, the prevalence of overweight at 2 years of age was 19.6%, increasing to 24.7% by the age of 8 years. Some studies have not shown any significant differences in body composition between patients with PKU and controls [60,61], while a recent study, using Plethysmography, indicated increased body fat percentage especially in females above 11 years of age, despite no differences between BMI [93].

4.4. The long term impact of early nutrition

Patients with PKU are exposed from an early age to an abnormal dietary intake. It is unknown if a higher phe-free amino acids intake in infancy will have any effect on the development of future obesity, similar to the non-PKU population [94–96]. Any supplementation of energy intake in early infancy may increase the risk for other metabolic disturbances in the later life [97].

5. Micronutrient status in PKU

In a low phenylalanine diet, animal proteins act as the vectors for many micronutrients, and to avoid their deficiency, vitamins, minerals and trace minerals must be supplemented in the diet (mainly through the phe-free amino acids). In PKU, the micronutrient status is influenced by many factors: type of diet, amount of natural protein [animal or vegetable origin], BH4 treatment, metabolic control, micronutrient composition of the phe-free amino acids, and frequency of their dosage. Patients are at risk of developing micronutrient deficiency if 1) they fail to adhere to their phe-free amino acids, 2) have mild hyperphenylalaninemia, treated with a low protein vegan diet only (without phe-free amino acids) 3) have either relaxed or stopped their dietary treatment but fail to eat animal protein foods and 4) are treated by BH4 in association with a protein restricted diet without supplementation with phe-free amino acids.

5.1. The vitamins

Vitamin D status is better in diet adherent than non adherent patients with PKU [98]. This is related to the vitamin D content of the phe-free amino acids (50–250 IU per 10 g of amino acids equivalent), which, thereby, provides a consistent and regular source of this nutrient. Interestingly, in a single study in PKU, the bone mineral density was low in patients on a strict low phenylalanine diet (with a high and adequate intake of calcium and vitamin D) compared to a relaxed diet (with lower calcium and vitamin D intakes) [98]. This study suggests that the observed impairment in peak bone mass is related to abnormalities in bone metabolism [99] rather than calcium or vitamin D deficiency in PKU [98]. Some studies highlight the role of osteoclastogenesis in the pathogenesis of bone disease in PKU [99,100].

5.2. The group B vitamins

Vitamin B_{12} deficiency is common in older patients with PKU [101–103] and this could potentially cause neurological impairment. As the main vitamin B_{12} sources are meat and seafood, patients on dietary treatment but without adequate vitamin B_{12} and cobalamin from either phe-free amino acids or a separate vitamin/mineral supplement are likely to have a low intake [104–106]. In contrast, the cobalamin content of phe-free amino acids is high (from 0.65 to 9.4 μg/10 g of amino acids equivalents), so the risk of vitamin B_{12} deficiency is minimal if adherence with phe-free amino acids is adequate. Consequently, patients at particular risk of cobalamin deficiency are older patients, who are well known to be poorly compliant with dietary treatment or patients who eat a 'normal' diet that contains minimal vitamin B_{12} sources [101]. As vitamin B_{12} deficiency causes neurological impairment, it is essential it is detected before the appearance of clinical signs. Therefore, it is important to assess the annual vitamin B_{12} status of all patients, regardless of severity of dietary restriction. However vitamin B_{12} concentration within the

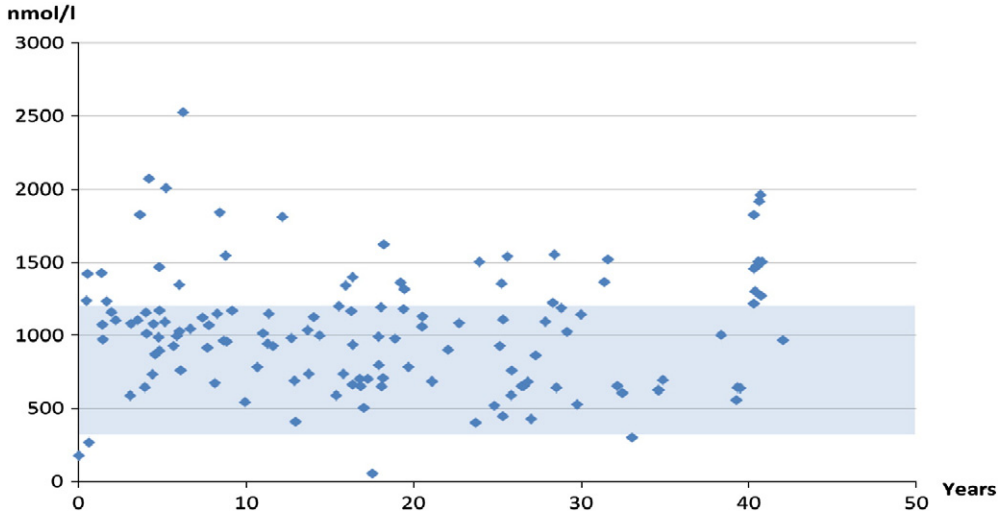

Fig. 1. Cross sectional erythrocytes folates (n = 136) in a cohort of 74 French PKU patients aged from 3 months to 45 years: demonstrating 25% (n = 24) of concentrations were above laboratory reference range (330–1200 nmol/l).

reference range does not automatically imply an adequate vitamin B_{12} status and therefore measuring serum MMA, or alternatively plasma homocysteine, is advised to diagnose functional vitamin B_{12} deficiency [107].

In contrast, folate deficiency has not been described in PKU. Phe-free amino acids are supplemented with substantial amounts of folic acid (24 to 130 μg per 10 g of amino acid equivalent), and so the recommended daily intake/reference nutrient intakes can be exceeded well in excess of requirements. This has been demonstrated by high plasma and erythrocyte folate in a large French cohort from one clinic of in one PKU patients (F Feillet personal data: Fig. 1). As there is now concern about the role of excess folate as a potential cancer factor [108], it is unknown if the apparent excessive folate supplementation of phe-free amino acids is safe. Therefore, it is essential the folate content of phe-free amino acids is re-evaluated; and it considers the contribution from a low phenylalanine diet.

There are very few studies examining pyridoxine (vitamin B_6) status in PKU [102,103,109,110] although it is found in a wide variety of high protein foods. Compared with control subjects, Prince et al. found that PKU patients had a significantly reduced mean protein intake but an increased mean vitamin B6 intake. The mean dietary vitamin B6: protein intake of PKU patients was over two fold the amount of the control group. Mean plasma pyridoxal 5′-phosphate (PLP) and PLP/total B6 in patients were significantly increased compared with control subjects suggesting a reduced turnover of pyridoxine in PKU patients [107].

5.3. The minerals

The trace mineral status has been widely studied in PKU, but particularly zinc and selenium, as they are found in animal protein foods, and they are involved in the anti-oxidant system [111].

Although some studies demonstrate a normal zinc status [112], others indicate deficiency in PKU [113,114]. The zinc content of phe-free amino acids varies from 2 mg to 5 mg per 10 g of amino acid equivalent. If a patient only consumed 20 g/daily protein equivalent from phe-free amino acids, at least 4 to 10 mg/daily of zinc is obtained, which should provide an adequate intake. However, despite relatively high dietary intakes, zinc deficiency is still described [114]. In a cohort from France, 70 of 146 (48%) measurements of plasma

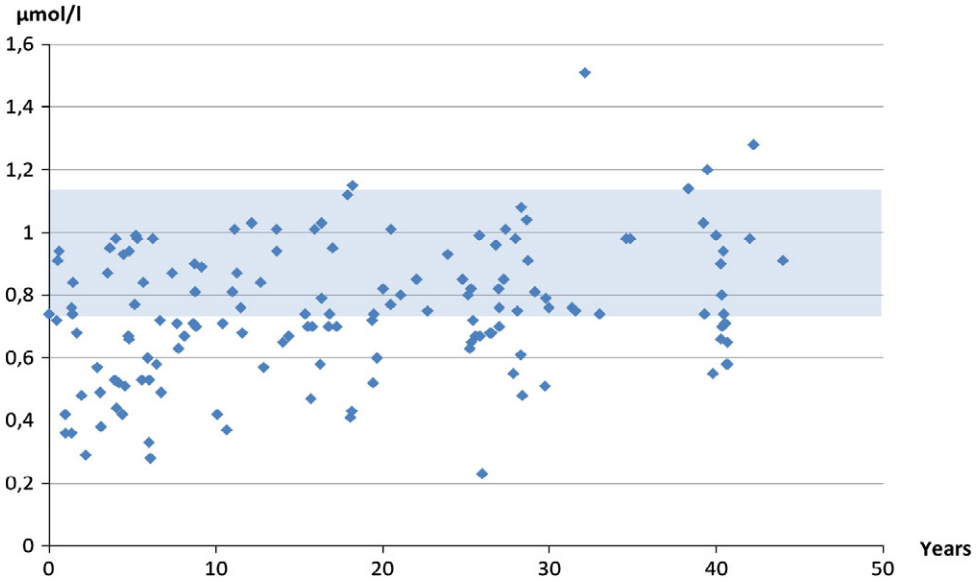

Fig. 2. Cross sectional plasma selenium concentrations (n = 153) in a cohort of 74 French PKU patients aged 0.3 to 45 years. 51% (n = 78) of concentrations were below the lower laboratory reference range (0.75–1.15 μmol/l).

Table 4
The status of vitamin and mineral rarely reported in PKU.

Vitamins/minerals	Supporting evidence to define status
Fat soluble vitamins	
Retinol and tocopherol	No evidence of deficiency
Water soluble vitamins	
Thiamin	No evidence of deficiency [110]
Riboflavin	Inadequately studied
Minerals	
Iron	Studies either demonstrate no deficiency [123] or poor status [124–126]. In iron deficient patients, no correlation has been demonstrated between iron intake and any index of iron status [126].

zinc were below the laboratory reference range of <10.7 μmol/l [F Feillet, personal observation]. These results suggest that the bioavailability of zinc is sub-optimal particularly when the major dietary source is added to phe-free amino acids and not bound by natural protein. In addition, it is well established that zinc can inhibit gut absorption of copper, particularly because it is used specifically for this purpose in Wilson disease [115]. Further studies are required comparing the bioavailability and efficacy of different sources of zinc supplementation added to phe-free amino acids and their effect on zinc and the status of other trace metals.

Selenium deficiency is, perhaps one of the most commonly reported nutritional problems in PKU [116–120] although in some of the older studies no selenium was added to phe-free amino acids. In PKU, the amount of selenium in phe-free amino acids varies from 6 to 16 μg per 10 g of protein equivalent from the protein substitute. As the recommended daily requirements/reference nutrient intake [121] varies from 1 μg/kg/day in childhood to 50 μg/day in adulthood, the selenium content of the L-amino acid supplements may be inadequate. In the French cohort, 51% of plasma selenium concentrations were below the laboratory reference range using phe-free amino acids with added selenium. [F Feillet, personal observation, Fig. 2]. Moreover, gut absorption of selenium is optimized when its main dietary source is animal protein. It is known that carnitine and selenium supplementation improve the anti-oxidant status in PKU patients [116,117] and that BH4 therapy which allows a greater intake of natural protein improves the selenium status in PKU patients [122].

The status of other micronutrients is summarized in Table 4.

6. Recommendations

- Alternative 'intact' protein substitute sources should be explored and developed, as they are likely to be associated with improved protein utilization and dietary adherence.
- Further controlled studies are required examining the ideal dosage of phe-free amino acids particularly the relationship between dosage and improved tolerance of dietary phenylalanine.
- The amino acid profile and micronutrient composition of the phe-free amino acids should be re-examined to develop products that better meet the nutritional requirements of patients with PKU. The introduction of international nutritional standards to regulate the composition of phe-free amino acids is necessary.
- Current supplementation with tyrosine may be excessive and the dosage requires further study.
- Longitudinal data is required about the change in phenylalanine tolerance throughout life, together with data about the 'real' rather than prescribed phenylalanine tolerance.
- There is little data about the nutritional state of adult patients with PKU on diet. More data is required about their protein requirements and micronutrient nutritional status in relationship to diet therapy.
- There is a growing need to measure body composition routinely in children with PKU. This may improve the capacity to tailor nutritional management directly to lean mass.
- With the rise in childhood obesity, it is important to routinely measure body fatness in children and identify those who are at greatest risk of 'co-morbidities' of obesity.
- There is a need for international consensus on the dietary and nutritional treatment of PKU.

7. Conclusions

Nutrition and dietary management in PKU must continue to develop with heightened momentum and in conjunction with the new non-diet treatments. It is important to collect robust evidence about the protein, amino acid, micronutrient requirements together with full knowledge about the growth and body composition of patients with PKU. This will provide essential data to act as a benchmark to compare the outcome of alternative treatments. International standards should be introduced to regulate the composition of phe-free amino acids and many current and accepted dietary approaches need reassessment to ensure that nutritional care is delivered to the highest level.

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