Protein substitutes and BH4 Treatment

Subjects: Nutrition & Dietetics

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The traditional treatment for phenylketonuria (PKU) is a phenylalanine (Phe)-restricted diet, supplemented with a Phe-free/low-Phe protein substitute. Pharmaceutical treatment with synthetic tetrahydrobiopterin (BH4), an enzyme cofactor, allows a patient subgroup to relax their diet. However, dietary protocols guiding the adjustments of protein equivalent intake from protein substitute with BH4 treatment are lacking.

phenylalanine hydroxylase deficiency | hyperphenylalaninemia | PKU | protein substitute | medical formula | amino acid mixture | tetrahydrobiopterin | sapropterin | BH4

1. Introduction

Phenylketonuria (PKU) is an inborn error of phenylalanine (Phe) metabolism caused by deficiency of the Phe hydroxylase enzyme (PAH; EC 1.14.16.1), which catalyzes the conversion of Phe to tyrosine, with the help of the cofactor tetrahydrobiopterin (BH4) [1]. PKU is a rare disorder affecting approximately 1 in 24,000 newborns globally [2], although incidence varies greatly across ethnicities and geographic regions. Infants are usually diagnosed via newborn screening in the first 2 weeks of life and commence treatment if blood Phe levels exceed 360 µmol/L [3]. Untreated, PKU may cause severe neurological impairment with profound intellectual disability [1][3][4].

The traditional treatment for PKU is a Phe-restricted diet, which aims to avoid excessive accumulation of Phe to prevent adverse neurocognitive and psychological outcomes, while also meeting requirements for growth and development [3][5][6]. Phe tolerance, the maximum amount that can be eaten whilst maintaining blood Phe levels in the therapeutic range, varies between patients; it is influenced by the residual PAH activity and therefore the severity of PKU [3], and up to 80% of patients tolerate less than 10 g/day natural protein [7]. Therefore, a low-Phe diet requires supplementation with a Phe-free or low-Phe protein substitute, i.e., a protein replacement formula, based on either free L-amino acids (AA), or casein glycomacropeptide (cGMP) supplemented with free AA. Most protein substitutes contain additional tyrosine, micronutrients, essential fatty acids, and long-chain polyunsaturated fatty acids [6]. Protein substitutes are not only necessary to meet age-appropriate protein requirements for growth and to provide tyrosine [3][6], they also improve Phe tolerance and optimize metabolic control by suppressing blood Phe levels [6][8][9][10]. This is particularly important during illness and trauma, where protein substitutes have a protective role by counter-acting protein catabolism [6].

Although successful, dietary treatment of PKU constitutes a substantial burden for patients and their families. The difficulties to adhere life-long to this restrictive diet, as well as to maintain blood Phe levels within the

recommended range, have called for new therapies to improve patients' quality of life [11]. Over the last 12 years, pharmaceutical adjunct therapies have been licensed including treatment with sapropterin dihydrochloride (a synthetic form of BH4) [12] and enzyme substitution therapy with pegvaliase (pegylated recombinant Phe ammonia lyase, PEG-PAL) [13]. Sapropterin therapy is prescribed to BH4-responsive patients with PKU; pegvaliase is only licensed for adults (≥16 y in Europe) with blood Phe levels ≥600 µmol/L. Both pharmaceutical treatments may be used as monotherapies or in combination with Phe restriction. Kure et al. were among the first to report that oral administration of BH4 to some individuals with mild hyperphenylalaninemia led to a significant reduction in blood Phe levels [14]. Since then, it has been suggested that 20–50% of patients with PKU respond to sapropterin [15][16][17][18][19]. The basis of responsiveness may be associated with different molecular mechanisms. Increased liver BH4 concentrations may stimulate the activity of a partially active mutant PAH enzyme [20], as some mutations can decrease the enzyme affinity for its cofactor [21][22], or may act as a chemical chaperone to stabilize mutant PAH [22][23]. Potential responsiveness to BH4 may be predicted from a patient's *PAH* genotype and/or BH4 loading tests [3][24][25][26]. It varies according to metabolic phenotype—milder forms of PKU are more likely to respond, whereas patients with classic PKU are less likely to do so [2].

In responders, the BH4-induced decrease in blood Phe concentrations usually enables an increase in Phe/natural protein tolerance and, thereby, some relaxation of the Phe-restricted diet with lowering or cessation of protein substitute use. However, Phe tolerance is also affected by other factors including severity of PKU, patient's age, dosage of protein substitute, growth rate, and target blood Phe concentrations [3][27]. Additionally, it has been shown that some adolescents and young adults with PKU are able to tolerate more natural protein than prescribed when challenged [28]. This supports a periodic re-evaluation of Phe tolerance in all patients including responders to BH4 therapy.

The ultimate goals of BH4 treatment are to (1) allow dietary Phe relaxation and (2) obtain good metabolic control. If either objective is not achieved and sustained long term, continuation of BH4 treatment should be reconsidered. Protein substitutes are a major supplier of nutrients, not only of protein, but also of vitamins and minerals, leading to concerns about the impact on nutritional status of patients taking BH4 when they are stopped [29][30]. This highlights the importance of a systematic and gradual approach when considering reduction of protein substitute, while maximizing natural protein intake in patients on BH4 treatment, in order to avoid impairment of metabolic control and maintain nutritional status. To date, few dietary protocols are available to guide such adjustments [31].

2. Protein Equivalent Intake from Protein Substitute with BH4 Treatment

This is the first time that changes in protein equivalent intake from protein substitute with BH4 treatment have been assessed systematically, although other systematic reviews or meta-analyses have investigated the effects of BH4 treatment on blood Phe control and dietary Phe tolerance [32][33][34]. We have demonstrated that PKU patients with long-term BH4 responsiveness had a significant increase in dietary Phe and natural protein intake when on BH4 treatment. This enabled the majority of responsive patients to reduce the dose of protein substitute, and 51% (157/306) were able to stop protein substitute. However, almost half (149/306) of long-term responders continued

to require some protein substitute, even though Phe and natural protein tolerance substantially improved. In this group, the protein substitute dose could be reduced in 28% (42/149) but remained unchanged in 14% of patients (21/149). In 58% (86/149) of patients on BH4 with protein substitute, the authors did not report if the dose was adjusted. Overall, the extent of reduction of protein equivalent intake from protein substitute, the time needed for change, as well as approaches to adjusting the PKU diet varied widely between studies. These findings highlight the need for guidance on when and how to decrease or stop protein substitute intake with BH4 treatment.

Pooled analysis of 10 studies showed that protein equivalent intake from protein substitute significantly decreased after a median BH4 treatment of one year (range: 0.5–5 years). Where half or more of the responsive patients were able to reduce or stop the use of protein substitutes, dietary Phe tolerance (as either expressed in mg/kg/day or mg/day) had increased by 2.5- to 4.3-fold [29][35][36][37][38][39][40][41][42]. In contrast, three studies reported a Phe tolerance increase <1.5-fold [43][44][45], and two of them failed to show a meaningful reduction (i.e., ≥25% from baseline) in median [45] or mean [44] protein equivalent intake from protein substitute after 1 year of BH4 treatment. Aldámiz et al. [44] attributed these findings to the inability of the BH4 loading test “cut off” of 30% decrease in blood Phe concentrations to identify true (i.e., long-term) responders correctly. When a 50% decrease in blood Phe as cut-off was used in a new loading test protocol [46], all responders were able to consume normal diets without protein substitute in the long term [44]. Most studies included in this systematic review used ≥30% decrease in blood Phe levels as a criterion to define BH4 responsiveness and showed successful long-term outcomes. However, BH4 therapy was discontinued in some patients (*n* = 27) mainly due to unsatisfactory blood Phe control when additional Phe/natural protein was added longer term [16][17][37][39][40][42][47].

Meeting nutritional requirements while maintaining blood Phe concentrations within therapeutic range is a central consideration when prescribing pharmaceutical therapies for PKU. Daily protein and micronutrient requirements increase throughout childhood and in women during pregnancy and lactation. With BH4 treatment, it is important to use a stepwise approach to increasing natural protein whilst in parallel reducing protein equivalent intake from protein substitute by similar amounts. Attention should be paid to the quantity as well as quality of natural protein. It is critical to ensure a good mix of animal and plant protein so that natural foods can supply all the nutrients in the amounts that meet requirements. Ongoing evaluation about the need for protein substitute supplementation as well as education about appropriate food choices is essential. We identified only a few studies [17][37][40] that have described in detail how natural protein is increased with BH4 therapy (see Table S1). Of these, the protocol by Singh et al. (2011) was the most thorough [40]. All responsive patients were instructed to add 20g of non-fat dry milk powder (≈350 mg Phe or 6.8 g protein) to their diet each week until new Phe tolerance was established [40], although this may be considered a rapid increase in natural protein intake by some. In practice, it may take several months to determine the final Phe tolerance and establish the ongoing need for a source of protein equivalent from protein substitute. Paras et al. reported a range of 3 months to 3.5 years until full diet liberalization occurred [48]. Caution is necessary in the case of illness episodes, injury, or trauma, as these may all adversely affect metabolic control, and it is established that BH4 is less effective in illness [35]. Protein substitutes offer a protective role by counteracting protein catabolism. It may be considered that, in young children, a small dose of protein substitute should be maintained as it is difficult to re-establish intake specifically for illness episodes or to meet the increased age-appropriate protein requirements during growth phase [49][50]. For others, it will be necessary to evaluate the

need for protein substitute re-introduction or an increase in dose might be required. Some studies have described patients who could initially stop using protein substitute, but for whom it had to be re-introduced [29][40].

Most protein substitutes provide a major supply of vitamins and minerals, and one of the concerns associated with long-term BH4 treatment is the nutritional adequacy of a relaxed diet when protein substitute is stopped or reduced [29]. We found inconsistent results about the impact on micronutrient status. Overall, the reduction in usage of protein substitutes or change in dietary habits with BH4 led to a decreased intake of several essential micronutrients in some [17][29][51][45] but not all studies [52][37][39][41]. Nutritional inadequacies were generally observed when diet was not fully liberalized, particularly when the dose of protein substitute was reduced by at least half of the baseline prescription [29][51], but it was also reported in a subgroup of patients who could relax their diet and stop protein substitute intake [17]. Another concern has been the establishment of healthy eating habits in BH4-treated patients who were well established in their dietary patterns before initiation of BH4 therapy. One of the two studies that investigated change in eating habits after diet relaxation demonstrated poorer eating habits in patients treated with BH4, despite training and education [29]. Although there was some recovery (e.g., re-increase of fruit intake) after 2 years of treatment, consumption of fish and dairy products remained markedly lower than healthy peers and was replaced by a higher intake of potatoes and pasta [29]. Similar findings were also reported by Hennermann et al. [17] who observed that normal bread, normal pasta, eggs, sausages, and meat were well accepted when dietary treatment was relaxed, while milk and dairy products were poorly accepted, and fish was completely refused by all patients. Growth impairment was found only in 2/9 studies [43][44]. This was evident at baseline and it did not improve with BH4 therapy, possibly due to the limited increase in dietary Phe tolerance coupled with a slight decrease in protein equivalent from protein substitute and thus total protein intake. Overall, our results indicate that long-term BH4 therapy does not seem to have a negative impact on total protein intake, and hence on growth. Nonetheless, there is still a risk of inadequate protein quality and of micronutrient deficiencies, which may be attributable to an embedded high-carbohydrate, low-protein disordered eating pattern that may take many months and years of education and counselling to improve. Further investigations in larger prospective studies including patients from different age groups and with all forms of PKU are needed to confirm the effects of BH4 treatment on dietary adequacy and growth.

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