Published in final edited form as:

Mol Genet Metab. 2011 December ; 104(4): 485–491. doi:10.1016/j.ymgme.2011.09.009.

Using change in plasma phenylalanine concentrations and ability to liberalize diet to classify responsiveness to tetrahydrobiopterin therapy in patients with phenylketonuria

Rani H. Singh^{a,b,*} and Meghan E. Quirk^b

^a Department of Human Genetics, Emory University School of Medicine, 2165 North Decatur Road, Decatur, GA 30033 USA

^b Division of Biological and Biomedical Sciences, Nutrition and Health Sciences, Emory University, 2165 North Decatur Road, Decatur, GA 30033 USA

Abstract

Tetrahydrobiopterin (BH₄) responsiveness is currently defined as a decrease in plasma phenylalanine concentrations in patients with phenylketonuria (PKU). This definition does not offer insight beyond the initial assessment of patients, which may lead to treatment ambiguity in patients who only experience an initial decrease in plasma phenylalanine concentrations. We present our experience with a novel classification approach using sequentially-applied criteria. Plasma phenylalanine concentrations were measured at baseline and after one month of BH₄ therapy (20 mg/kg/day) in 58 PKU patients (34M, 24F; age 17.3 ± 11.0 years). Thirty-two patients (55.2%) were classified as –preliminary responders“ at one month, experiencing at least a 15% decrease in plasma phenylalanine concentrations. Preliminary responders’ ability to liberalize their dietary restrictions was then systematically assessed. –Definitive responders“ were defined as preliminary responders who could increase their dietary phenylalanine tolerance by at least 300 mg/day and lower prescribed medical food needs by at least 25% while maintaining metabolic control (plasma phenylalanine <360 μmol/L) and consuming adequate dietary protein. Preliminary responders who could not liberalize their diets according to these criteria were classified as –provisional responders.“ Nineteen patients (32.8% of patients initiating BH₄ therapy) met the definitive responder criteria, increasing dietary phenylalanine tolerance from 704 ± 518 mg/day to 1922 ± 612 mg/day and reducing medical food to 16.7 ± 19.5% of their baseline prescription. Nine patients (15.5% of patients initiating BH₄ therapy) were classified as provisional responders, all remaining on 100% of their baseline medical food prescription. From this classification approach, a subgroup of provisionally responsive patients emerged who experienced an initial decrease in plasma phenylalanine concentrations but who could not substantially increase their dietary

© 2011 Elsevier Inc. All rights reserved.

*Corresponding Author: Rani H. Singh, PhD RD LD Department of Human Genetics 2165 North Decatur Road Decatur, GA 30033 USA Phone: 1-404-778-8519 Fax: 1-404-778-8562 rsingh@emory.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

phenylalanine tolerance or decrease medical food needs. Diet liberalization is an essential component of BH₄-responsiveness classification.

Keywords

phenylketonuria; tetrahydrobiopterin; sapropterin dihydrochloride; Kuvan®

1. Introduction

Patients with phenylketonuria (PKU; OMIM 261600) are instructed to follow a lifelong diet restricted in phenylalanine [1]. Dietary protein adequacy is achieved by adding a phenylalanine-free amino acid medical food, which supplies the majority of protein in the diets of treated patients [2, 3]. The burden of such a limited and often unpalatable diet can lead to treatment non-compliance and prolonged periods of elevated blood phenylalanine concentrations, which can negatively impact a patient's development and health [4-6].

Effective PKU management must strike a balance between diet liberalization and maintenance of blood phenylalanine concentrations in the therapeutic range (preferably 120-360 μ mol/L). Tetrahydrobiopterin (BH₄) is the first drug therapy that may help certain PKU patients strike such a balance. With its potential first clinically identified in four mild hyperphenylalaninemic patients in 1999 [7], BH₄ has since been the subject of numerous investigations and clinical protocols internationally [8-13]. It is believed that pharmacological doses of BH₄ can correct kinetic defects and/or can act as a chemical chaperone [14, 15], thereby increasing and/or prolonging the functionality of mutant phenylalanine hydroxylases harboring some residual activity.

Where conventional diet therapy maintains blood phenylalanine concentrations in the therapeutic range by simply limiting the amount of the offending amino acid ingested, BH₄ therapy enhances the catabolism of phenylalanine and therefore has the potential to improve responsive patients' dietary phenylalanine tolerance [16-18]. The current definition of BH₄-responsiveness typically found in the literature – a clinically significant decrease in blood phenylalanine concentrations, with a threshold usually set at 30% decrease [19] – fails to capture the added benefit of improved intact protein tolerance. Clinical ambiguity can arise when patients experience an initial marked decrease in blood phenylalanine concentrations, but cannot subsequently increase their dietary phenylalanine tolerance.

Assessing dietary phenylalanine tolerance to classify BH₄-responsiveness has been previously suggested [20, 21], but in-depth descriptions of the implementation of such protocols in the clinical setting are still lacking. We present our clinic's approach to and experience with a novel and expanded BH₄-responsiveness classification protocol which uses both change in plasma phenylalanine concentrations and ability to liberalize diet restrictions in patients prescribed the BH₄ analog sapropterin dihydrochloride (Kuvan®; BioMarin Pharmaceutical Inc., Novato, California, USA).

2. Material and Methods

2.1. Patient Eligibility

Patients seen at the Emory University Genetics Clinic were recruited from October 2008 through October 2009 to participate in a yearlong clinical trial evaluating BH₄-responsiveness. Inclusion criteria were: being diagnosed with hyperphenylalaninemia or PKU and being at least 4 years of age. Patients were excluded if they were pregnant or breastfeeding, were previously determined to be BH₄-responsive, or had taken biopterin in the previous 8 weeks. Informed consent, and when necessary assent, was obtained from all study participants and from pediatric patients' legal guardians. This study was approved by the Emory University Institutional Review Board.

2.2. Responsiveness Classification Algorithm

Patients were classified using the algorithm presented in **Figure 1**. The approaches are detailed as follows:

2.2.1 Preliminary Responsiveness Criterion: Change in Plasma Phenylalanine Concentrations—Patients' plasma amino acid concentrations were assessed immediately prior to and after one month of BH₄ therapy (20 mg/kg/day). A patient was considered a –*preliminarily responder*“ if their month one plasma phenylalanine concentration was at least 15% lower than their baseline plasma phenylalanine concentration. Patients meeting this threshold continued using BH₄ and proceeded to diet liberalization. Patients not meeting the 15% threshold were classified as –*nonresponders*“ and discontinued BH₄ therapy.

2.2.2. Definitive Responsiveness Criteria: Ability to Liberalize Diet—Preliminary responders' diets were liberalized using an adapted version of a previously published protocol [21]. The approach taken was dependent on the patient's reported dietary prescription compliance and plasma phenylalanine concentrations at the month one assessment (detailed in Sections 2.2.2.1 and 2.2.2.2).

Regardless of diet liberalization approach, two criteria were ultimately used to classify patients. A –*definitive responder*“ was defined as a preliminary responder who could increase dietary phenylalanine tolerance by at least 300 mg/day (approximately 6 grams of intact protein) and decrease medical food need by at least 25% while maintaining their blood phenylalanine concentrations in the therapeutic range (<360 μmol/L) and meeting their age- and sex-specific Recommended Dietary Allowance (RDA) for protein. Preliminary responders who could not increase their dietary phenylalanine tolerance and decrease their medical food needs while maintaining metabolic control were classified as –*provisional responders*.“

Patients who electively ate diets rich in phenylalanine (meeting RDA protein needs through intact protein) and had plasma phenylalanine concentrations <360 μmol/L after one month of BH₄ therapy had no need to have their diets liberalized and were considered definitive responders. If medical food was being consumed, its necessity was evaluated.

2.2.2.1. Diet Liberalization of Patients with Plasma Phenylalanine Concentrations in the Therapeutic Range: Milk Powder Challenge: Patients who reported restricting intact protein and had plasma phenylalanine concentrations $<360 \mu\text{mol/L}$ after one month of BH_4 therapy were instructed to add 20 grams of non-fat dry milk powder (approximately 350 mg phenylalanine or 6.8 grams protein) to their diet each week. A patient's new dietary tolerance was established as the quantity of dietary phenylalanine consumed prior to blood phenylalanine concentration exceeding $360 \mu\text{mol/L}$. After the new dietary phenylalanine tolerance was established, medical food intake was progressively decreased by 25% of baseline prescription each week. The patient's new medical food prescription was established as the intake associated with the last blood filter paper phenylalanine concentration in the therapeutic range, ensuring dietary protein adequacy. Once dietary phenylalanine and medical food tolerance were established, intact protein sources displaced milk powder in the diet.

Female definitive responders of childbearing potential were encouraged to maintain the taste for medical food by consuming a fraction of their baseline prescriptional food, typically 25%, even if intact protein tolerance could meet the patient's RDA. This routine is intended to ease the transition back to diet therapy alone if a woman chooses to discontinue BH_4 therapy during pregnancy.

2.2.2.2. Diet Liberalization of Patients with Plasma Phenylalanine Concentrations Exceeding the Therapeutic Range: Patients who reported consuming medical food and whose plasma phenylalanine concentration exceeded the therapeutic range after one month of BH_4 therapy were instructed to decrease dietary phenylalanine intake by approximately 350 mg (6.5-7 grams of intact protein) per week. A patient's dietary phenylalanine intake was decreased until blood phenylalanine concentration was in the therapeutic range. Medical food intake was then progressively decreased using the method described in Section 2.2.2.1.

Patients consuming completely liberalized diets without medical food and whose blood phenylalanine concentration exceeded $360 \mu\text{mol/L}$ were instructed to decrease dietary phenylalanine intake until metabolic control was achieved, with medical food progressively added back into the diet 25% at a time as needed to ensure dietary protein adequacy.

2.3. Plasma and Blood Amino Acid Analysis

Plasma amino acids were measured at baseline and after one month of BH_4 therapy. A fasting blood sample was drawn from each patient in a heparinized tube and assessed using a Biochrom 30 Amino Acid Analyzer (Biochrom Ltd, Cambridge, UK). During diet liberalization, patients were instructed to spot a filter paper weekly with finger-stick blood drops after an overnight fast. Filter paper amino acid concentrations were analyzed using liquid chromatography/tandem mass spectrometry (Waters 2795 HPLC system/Micromass Quattro micro; Waters Corporation, Milford, Massachusetts, USA), as previously described [22].

2.4. Dietary Intake and Diet Prescription

Patients were instructed to record dietary intake for the three days prior to both baseline and month one assessments and before each filter paper submission. If no 3-day diet record was received, a metabolic dietitian conducted a 24-hour recall to approximate energy, macronutrient, and medical food intake. Diets were analyzed using the Nutrition Data System for Research (University of Minnesota, Minneapolis, MN, USA) diet analysis program. Baseline diet prescriptions were the last prescription recorded prior to the initiation of the protocol. Subsequent changes to the diet prescriptions were established and adjusted by the research metabolic dietitian.

2.5. Statistical Analysis

Data were analyzed using SAS 9.2 (SAS Institute Inc, Cary, NC, USA). Descriptive statistics are presented as count (%) and mean $\pm$ standard deviation. Differences between the classification groups were assessed using Student's *t*-tests for continuous variables and χ^2 -test for categorical variables. A *p*-value < 0.05 was considered statistically significant.

To determine if changes in dietary intake during the first month of BH₄ therapy were associated with preliminary classification, percent change in plasma phenylalanine concentrations after one month of BH₄ therapy was modeled against percent of change in reported dietary intake (energy, protein, phenylalanine, and medical food). Exclusion of diet records containing less than three days did not affect the associations, so all diet records and recalls have been included in the analysis. Due to the diversity of the patient population, the effects of age, sex, baseline diet phenylalanine prescription (a proxy for disorder severity), and bodyweight were evaluated in the models.

3. Results

3.1. Baseline Characteristics and Preliminary Responsiveness Classification

Of the 83 patients approached for study enrollment, 58 participated at baseline, 57 of which returned at month one for preliminary responsiveness classification. Thirty-two patients (55.2% of patients evaluated at baseline) were classified as preliminary responders. The remaining 25 patients (43.1% of patients evaluated at baseline) were classified as BH₄ non-responders. Characteristics of patients at baseline and after one month BH₄ therapy are displayed in **Table 1**.

Mean plasma phenylalanine to tyrosine ratios were significantly different between the two groups at baseline (preliminary responders: 12.9 ± 9.8 , non-responders: 20.4 ± 14.7 ; *p*=0.033) and after one month of BH₄ therapy (preliminary responders: 6.4 ± 6.7 , non-responders: 24.1 ± 15.9 ; *p*<0.0001). These differences were driven by plasma phenylalanine concentrations since plasma tyrosine concentrations did not differ between the groups at baseline (preliminary responders: 50.5 ± 15.5 μ mol/L, non-responders: 52.1 ± 22.4 ; *p*=0.755) or month one (preliminary responders: 46.1 ± 21.4 , non-responders: 48.4 ± 26.3 ; *p*=0.720).

3.1.1. The Effect of Dietary Intake during Preliminary Responsiveness

Assessment—Table 2 displays a summary of the reported intake of 53 patients with complete diet records at both baseline and the month one evaluations. Percent change in reported baseline intake of energy, total protein, phenylalanine, and medical food protein equivalents did not have a relationship with percent change in plasma phenylalanine concentrations between baseline and month one. Models were not improved with the addition of clinical characteristics (all predictor *p-values* >0.05).

3.2. BH₄ Responsiveness: Diet Liberalization

Thirty-two preliminary responders were eligible for diet liberalization assessment. Prior to diet adjustments, one preliminary responder was electively removed from BH₄ treatment while another preliminary responder discontinued BH₄ therapy due to protocol non-compliance. A third preliminary responder was lost to follow-up prior to the establishment of a new diet prescription. One additional patient, who only experienced a 10.8% decrease in plasma phenylalanine concentration after one month of BH₄ therapy, was further evaluated through the diet liberalization process because of a reported 12.9 gram increase in intact protein intake over the first month of BH₄ therapy. A total of 30 patients were assessed using diet liberalization approach. A flow diagram of patient classification is displayed in Figure 2.

3.2.1. Diet Liberalization of Patients with Plasma Phenylalanine

Concentrations in the Therapeutic Range—After one month of BH₄ therapy, three patients eating completely liberalized diets had plasma phenylalanine concentrations below 360 μmol/L and were classified as definitive responders. All three patients no longer needed medical food to meet their age- and sex-specific RDA for protein or to maintain plasma phenylalanine concentrations in the therapeutic range.

Twenty-three patients initiated the milk powder challenge. Of note are two patients who were assessed despite having month one plasma phenylalanine concentrations exceeding 360 μmol/L (439 and 441 μmol/L). Both patients were eating diets high in phenylalanine. To establish their new dietary tolerance they displaced a portion of their intact protein intake with milk powder and proceeded through the challenge.

Of the 23 patients who initiated the milk powder challenge, 15 were classified as definitive BH₄ responders while the other 8 were classified as provisional responders.

3.2.2. Diet Liberalization of Patients with Plasma Phenylalanine

Concentrations Beyond the Therapeutic Range—Four patients following liberalized diets had plasma phenylalanine concentrations exceeding 360 μmol/L after one month of BH₄-therapy (range: 496-1504 μmol/L). Patient 1, who reported consuming his full medical food prescription while eating approximately 500 mg phenylalanine/day, failed to reduce his dietary phenylalanine intake enough to lower his blood phenylalanine concentrations into the therapeutic range (as per the protocol outlined in section 2.2.2.2).

Since his reported phenylalanine intake was less than 300 mg above his prescription, this patient did not meet the responsiveness criteria and was classified as a provisional

responder. Patient 2 was not consuming medical food at baseline but progressively decreased his phenylalanine intake in his second month of BH₄ therapy. After adding 25% of his original medical food prescription to his diet, Patient 2's blood phenylalanine concentrations fell within the therapeutic range. His dietary tolerance was increased by 1,100 mg phenylalanine/day as compared to his baseline prescription and he was classified as a definitive BH₄-responder.

Patients 3 and 4 electively followed completely liberalized diets prior to baseline and neither consumed medical food. Both patients attempted to decrease their dietary phenylalanine intake and incorporate 25% of pre-BH₄ medical food prescription into their diet. Due to noncompliance issues, these patients' blood phenylalanine concentrations never fell in the therapeutic range. Since patient 3 was the participant with a 10.8% decrease in plasma phenylalanine concentration after one month of BH₄ therapy, he was ultimately classified as a non-responder. Patient 4 could not be accurately classified.

3.2.3. Differentiating Provisional Responders from Definitive Responders—The changes in dietary phenylalanine tolerances and medical food prescriptions after diet liberalization are detailed in **Table 3**. All definitive responders who underwent diet liberalization could tolerate at least twice the dietary phenylalanine they could at baseline. In comparison, none of the provisional responders could double their prescription or meet the 300 mg phenylalanine/day criterion. Medical food was discontinued in 10 of the 19 definitive responders. An additional four female definitive responders could meet their dietary protein needs through their phenylalanine tolerance, but remained on a reduced medical food prescription. Thus, only five of the 19 definitive responders had nutritional needs for their medical food prescription. In comparison, all nine provisional responders continued on 100% of their medical food prescription, with one participant needing a slight increase due to growth.

Provisional responders were similar to definitive responders in terms of baseline plasma phenylalanine concentrations, month one plasma phenylalanine concentrations, and reported change in dietary intake between baseline and month one assessment. Provisional responders were comprised entirely of children and adolescents (range of baseline age: 4.6-17.8 years) while definitive responders encompassed a wider age range (6.1-36.8 years), leading to a significant difference in age between the groups ($p=0.038$). All other demographic characteristics were similar between the groups.

3.3. Summary of Responsiveness Classification

Preliminary responders comprised 55.2% (32/58) of participants who were evaluated at baseline. This group was further differentiated into definitive BH₄-responders and provisional responders, 32.8% (19/58) and 15.5% (9/58) of patients who initiated the protocol, respectively. This protocol resulted in 8.6% (5/58) of patients being unclassified due to protocol non-compliance and loss to follow-up.

4. Discussion

While the current definition of BH₄-responsiveness in the literature appears simple, in clinical practice responsiveness determination is less straightforward and many factors must be considered. Our approach to BH₄-responsiveness classification differs from other previously reported protocols. First, the minimum change in plasma phenylalanine concentrations during the responsiveness testing period was lowered to 15% from the typical 30% cutoff. This criterion allowed us to identify one additional definitive responder who only experienced a 25.4% decrease in plasma phenylalanine concentrations, but could subsequently increase his dietary phenylalanine intake by 1,100 mg/day. This lower cutoff appears appropriate for protocols of longer duration. The length of time used to assess preliminary responsiveness in our approach was longer than in other protocols, which generally span from 8 to 48 hours [23]. The extension of the testing period beyond a single BH₄ dose has been suggested to identify “slow responders” [24]. One month of therapy was selected for the current protocol to maximize the number of potential responders identified and to minimize patient burden of repeated clinic visits. It should be noted that the longer the testing period, the more likely changes in blood phenylalanine concentrations are to be affected by other factors such as dietary intake or illness. While our data suggest that percent change in baseline dietary intake of energy, protein, phenylalanine, and medical food did not have an association with percent change in plasma phenylalanine concentrations, these results are preliminary and subject to diet record reporting biases by the study participants. Additionally, medical food consumption was fairly consistent in the majority of patients between baseline and month one in our clinic population, which may lead to the false assumption that changes in medical food consumption have no effect on plasma phenylalanine concentrations. To prevent the effects of potential confounders, it would be of value to determine how our responsiveness classification varies between the shorter and longer protocols to expedite the determination process while maximizing accuracy.

The diet liberalization phase of BH₄-responsiveness was a critical element of our protocol. While it has been reported that BH₄-responsive patients can have an increase in dietary phenylalanine tolerance and a decreased need for medical food [8, 12, 16, 17, 25, 26], it is known that this is not the case for all patients who experience the threshold change in blood phenylalanine concentrations. A substantial subset of patients that we describe as “provisional responders” was identified by the diet liberalization criteria.

The provisional responder group highlights critical aspects of BH₄ responsiveness determination that must be considered when implementing clinical protocols. Two unrelated provisional responders each had a biological sibling who did not experience a decrease in plasma phenylalanine concentrations during the month-long trial of BH₄. While it has been documented that responsiveness cannot necessarily be predicted from a patient's genotype [27, 28], discordant responsiveness classification between biological siblings begs for further evaluation. Additionally, two of the nine provisional responders had acute illness at baseline, believed to inflate their blood phenylalanine concentrations and cause misclassification. Thus, illness or other catabolic states at baseline and/or follow-up must be taken into consideration, as they can lead to false-positive or false-negative classification.

The remaining provisional responders, however, had no remarkable changes in reported health status or dietary intake over the course of the first month of BH₄ therapy.

In the end, all nine provisionally responsive patients were prescribed duplicative PKU treatments: a maximum BH₄ prescription along with their entire pre-BH₄ medical food prescription. These patients emphasize the need for establishing guidelines for what constitutes BH₄-responsiveness. It is interesting to note that the provisionally responsive patients were comprised entirely of pediatric patients. While there is a possibility that provisional responsiveness is a function of age, it should be noted that we had pediatric patients as young as 6 years of age who met this dietary tolerance threshold. Significant increases of dietary phenylalanine tolerance—far beyond 300 mg phenylalanine per day—have also been previously observed in pediatric patients [17]. While we used absolute cutoffs for diet liberalization in this protocol, alternative dietary criteria could be considered (such as a doubling of dietary phenylalanine prescription, creating age- or weight-adjusted dietary criteria, etc). The purpose of the diet tolerance criteria is to prevent the over-management of patients, considering the expense of either treatment approach. Continued BH₄ therapy in a patient who cannot substantially increase their dietary tolerance can only be justified if it improves long-term metabolic control or improves a clinically significant secondary outcome (such as quality of life, ADHD symptomatology, etc) as compared to diet therapy alone. These benefits have yet to be demonstrated specifically in the provisionally responsive patients. Until they are, the added benefit of BH₄ therapy as opposed to diet therapy alone must be evaluated on a case-by-case basis.

We are not the first clinic to identify a group of patients who cannot increase their dietary tolerance despite an initial marked decrease in plasma phenylalanine concentrations. In 2005, Lambruschini et al [18] reported three patients who experienced 45.7-74.5% decrease in blood phenylalanine concentrations 21 hours after BH₄ loading, but could not improve their diet prescription and subsequently stopped BH₄ therapy. Additionally, Trefz et al report two “pseudo-responders” with initial responses of 60.8% and 33.7% decreased, respectively, who could not increase their dietary phenylalanine tolerance [29]. While it is possible that our provisionally responsive group is an artifact of our month-long protocol—that is all nine patients are false-positive responders—the emergence of a similar subgroup of patients in alternative and shorter protocols [18, 29] suggests that they represent a legitimate subgroup of patients. A need exists to systematically identify and closely follow these patients to form a uniform guideline for proper management.

Two caveats to our proposed diet titration guidelines are exemplified by Patient 4, who remains unclassified due to protocol noncompliance. First, the patient was not actively managed prior to the initiation of BH₄ therapy. The lack of an established diet prescription hampered our ability to definitively classify him. Secondly, after consuming an unrestricted diet for the majority of his adult life, this patient would not reduce his intake to sufficiently lower his blood phenylalanine concentrations into the therapeutic range even with BH₄ therapy. For diet liberalization criteria to be successfully applied, patients initiating BH₄ must be closely monitored by their metabolic clinic, must have a current diet prescription, and must be willing to comply with the diet liberalization process.

In conclusion, using both changes in plasma phenylalanine concentrations and ability to liberalize dietary restrictions as criteria to determine BH₄-responsiveness in patient with PKU led to the identification of a sub-group of provisional responders. This classification approach aids in the identification of patients who can use BH₄ to both liberalize dietary restrictions while achieving blood phenylalanine concentrations in the therapeutic range.

Acknowledgments

We would like to acknowledge the contributions of our entire research team, especially Mary Jane Kennedy and Sarah Travis for their roles as study coordinator and research metabolic dietitian, respectively. We would also like to acknowledge the scientific advisors, clinicians, and physicians who assisted in the development and implementation of this trial, including Laura Ward and Drs. Marian Evatt, Thomas Ziegler, Paul Fernhoff, Muhammad Pervaiz, and Phyllis Acosta. Finally, we would like to thank the study patients and their families for their participation in this study.

Funding: The data presented are part of an investigator-initiated trial funded by BioMarin Pharmaceutical Inc. This study was also supported in part by PHS Grant UL1 RR025008 from the Clinical and Translational Science Award program, National Institutes of Health, National Center for Research Resources.

Abbreviations

PKU	phenylketonuria
BH₄	tetrahydrobiopterin
RDA	Recommended Dietary Allowance

References

1. National Institutes of Health. Consensus Development Conference Statement: phenylketonuria: screening and management, October 16-18, 2000. *Pediatrics*. 2001; 108:972–982. [PubMed: 11581453]
2. Acosta PB, Yannicelli S, Marriage B, Mantia C, Gaffield B, Porterfield M, Hunt M, McMaster N, Bernstein L, Parton P, Kuehn M, Lewis V. Nutrient intake and growth of infants with phenylketonuria undergoing therapy. *J Pediatr Gastroenterol Nutr*. 1998; 27:287–291. [PubMed: 9740198]
3. Acosta PB, Yannicelli S, Singh R, Mofidi S, Steiner R, DeVincentis E, Jurecki E, Bernstein L, Gleason S, Chetty M, Rouse B. Nutrient intakes and physical growth of children with phenylketonuria undergoing nutrition therapy. *J Am Diet Assoc*. 2003; 103:1167–1173. [PubMed: 12963945]
4. Paine RS. The variability in manifestations of untreated patients with phenylketonuria (phenylpyruvic aciduria). *Pediatrics*. 1957; 20:290–302. [PubMed: 13452670]
5. Holm VA, Knox WE. Physical growth in phenylketonuria: I. A retrospective study. *Pediatrics*. 1979; 63:694–699. [PubMed: 312489]
6. Brumm VL, Azen C, Moats RA, Stern AM, Broomand C, Nelson MD, Koch R. Neuropsychological outcome of subjects participating in the PKU adult collaborative study: a preliminary review. *J Inher Metab Dis*. 2004; 27:549–566. [PubMed: 15669671]
7. Kure S, Hou DC, Ohura T, Iwamoto H, Suzuki S, Sugiyama N, Sakamoto O, Fujii K, Matsubara Y, Narisawa K. Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency. *J Pediatr*. 1999; 135:375–378. [PubMed: 10484807]
8. Belanger-Quintana A, Garcia MJ, Castro M, Desviat LR, Perez B, Mejia B, Ugarte M, Martinez-Pardo M. Spanish BH₄-responsive phenylalanine hydroxylase-deficient patients: evolution of seven patients on long-term treatment with tetrahydrobiopterin. *Mol Genet Metab*. 2005; 86(Suppl 1):S61–66. [PubMed: 16165389]

9. Burton BK, Grange DK, Milanowski A, Vockley G, Feillet F, Crombez EA, Abadie V, Harding CO, Cederbaum S, Dobbelaere D, Smith A, Dorenbaum A. The response of patients with phenylketonuria and elevated serum phenylalanine to treatment with oral sapropterin dihydrochloride (6R-tetrahydrobiopterin): a phase II, multicentre, open-label, screening study. *J Inher Metab Dis*. 2007; 30:700–707. [PubMed: 17846916]
10. Mitchell JJ, Wilcken B, Alexander I, Ellaway C, O'Grady H, Wiley V, Earl J, Christodoulou J. Tetrahydrobiopterin-responsive phenylketonuria: the New South Wales experience. *Mol Genet Metab*. 2005; 86(Suppl 1):S81–85. [PubMed: 16091307]
11. Shintaku H, Kure S, Ohura T, Okano Y, Ohwada M, Sugiyama N, Sakura N, Yoshida I, Yoshino M, Matsubara Y, Suzuki K, Aoki K, Kitagawa T. Long-term treatment and diagnosis of tetrahydrobiopterin-responsive hyperphenylalaninemia with a mutant phenylalanine hydroxylase gene. *Pediatr Res*. 2004; 55:425–430. [PubMed: 14681498]
12. Steinfeld R, Kohlschutter A, Ullrich K, Lukacs Z. Efficiency of long-term tetrahydrobiopterin monotherapy in phenylketonuria. *J Inher Metab Dis*. 2004; 27:449–453. [PubMed: 15303001]
13. Vernon HJ, Koerner CB, Johnson MR, Bergner A, Hamosh A. Introduction of sapropterin dihydrochloride as standard of care in patients with phenylketonuria. *Mol Genet Metab*. 2010; 100:229–233. [PubMed: 20418136]
14. Erlandsen H, Pey AL, Gamez A, Perez B, Desviat LR, Aguado C, Koch R, Surendran S, Tying S, Matalon R, Scriver CR, Ugarte M, Martinez A, Stevens RC. Correction of kinetic and stability defects by tetrahydrobiopterin in phenylketonuria patients with certain phenylalanine hydroxylase mutations. *Proc Natl Acad Sci U S A*. 2004; 101:16903–16908. [PubMed: 15557004]
15. Pey AL, Perez B, Desviat LR, Martinez MA, Aguado C, Erlandsen H, Gamez A, Stevens RC, Thorolfsson M, Ugarte M, Martinez A. Mechanisms underlying responsiveness to tetrahydrobiopterin in mild phenylketonuria mutations. *Hum Mutat*. 2004; 24:388–399. [PubMed: 15459954]
16. Trefz FK, Burton BK, Longo N, Casanova MM, Gruskin DJ, Dorenbaum A, Kakkis ED, Crombez EA, Grange DK, Harmatz P, Lipson MH, Milanowski A, Randolph LM, Vockley J, Whitley CB, Wolff JA, Bebachuk J, Christ-Schmidt H, Hennermann JB. Efficacy of sapropterin dihydrochloride in increasing phenylalanine tolerance in children with phenylketonuria: a phase III, randomized, double-blind, placebo-controlled study. *J Pediatr*. 2009; 154:700–707. [PubMed: 19261295]
17. Burlina A, Blau N. Effect of BH(4) supplementation on phenylalanine tolerance. *J Inher Metab Dis*. 2009; 32:40–45. [PubMed: 19067227]
18. Lambruschini N, Perez-Duenas B, Vilaseca MA, Mas A, Artuch R, Gassio R, Gomez L, Gutierrez A, Campistol J. Clinical and nutritional evaluation of phenylketonuric patients on tetrahydrobiopterin monotherapy. *Mol Genet Metab*. 2005; 86(Suppl 1):S54–60. [PubMed: 16040265]
19. Blau N.; Muntau, A. EMG Workshop Results. Milupa, Friedrichsdorf; Germany: 2002. Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency..
20. Blau N. Defining tetrahydrobiopterin (BH4)-responsiveness in PKU. *J Inher Metab Dis*. 2008; 31:2–3. [PubMed: 18327672]
21. Singh RH, Jurecki E, Rohr F. Recommendations for personalized dietary adjustments based on patient response to tetrahydrobiopterin (BH4) in phenylketonuria. *Top Clin Nutr*. 2008; 23:149–157.
22. Gregory CO, Yu C, Singh RH. Blood phenylalanine monitoring for dietary compliance among patients with phenylketonuria: comparison of methods. *Genet Med*. 2007; 9:761–765. [PubMed: 18007145]
23. Blau N, Belanger-Quintana A, Demirkol M, Feillet F, Giovannini M, MacDonald A, Trefz FK, van Spronsen F. Management of phenylketonuria in Europe: survey results from 19 countries. *Mol Genet Metab*. 2010; 99:109–115. [PubMed: 19800826]
24. Fiege B, Bonafe L, Ballhausen D, Baumgartner M, Thony B, Meili D, Fiori L, Giovannini M, Blau N. Extended tetrahydrobiopterin loading test in the diagnosis of cofactor-responsive phenylketonuria: a pilot study. *Mol Genet Metab*. 2005; 86(Suppl 1):S91–95. [PubMed: 16290003]

25. Hennermann JB, Buhner C, Blau N, Vetter B, Monch E. Long-term treatment with tetrahydrobiopterin increases phenylalanine tolerance in children with severe phenotype of phenylketonuria. *Mol Genet Metab.* 2005; 86(Suppl 1):S86–90. [PubMed: 16051511]
26. Trefz FK, Scheible D, Frauendienst-Egger G, Korall H, Blau N. Long-term treatment of patients with mild and classical phenylketonuria by tetrahydrobiopterin. *Mol Genet Metab.* 2005; 86:S75–80. [PubMed: 16242984]
27. Trefz FK, Scheible D, Gotz H, Frauendienst-Egger G. Significance of genotype in tetrahydrobiopterin-responsive phenylketonuria. *J Inherit Metab Dis.* 2009; 32:22–26. [PubMed: 18956252]
28. Bercovich D, Elimelech A, Zlotogora J, Korem S, Yardeni T, Gal N, Goldstein N, Vilensky B, Segev R, Avraham S, Loewenthal R, Schwartz G, Anikster Y. Genotype-phenotype correlations analysis of mutations in the phenylalanine hydroxylase (PAH) gene. *J Hum Genet.* 2008; 53:407–418. [PubMed: 18299955]
29. Trefz FK, Scheible D, Frauendienst-Egger G. Long-term follow-up of patients with phenylketonuria receiving tetrahydrobiopterin treatment. *J Inherit Metab Dis.* 2010

Highlights

- We evaluated BH4 responsiveness in 58 patients with phenylketonuria.
- Responsiveness was based on change in plasma phenylalanine and dietary tolerance.
- A subgroup of patients couldn't liberalize their diets despite an initial response.
- Dietary phenylalanine tolerance is vital for BH4 responsiveness classification.

Figure 1. Practical algorithm used to classify BH₄ responsiveness in patients with PKU. Criteria include both change in plasma phenylalanine concentrations and ability to liberalize diet restrictions.

Figure 2.
Flow diagram of BH₄ responsiveness classification of 58 PKU participants.

Table 1

Baseline and month one characteristics of 58 PKU patients who initiated BH₄ therapy. Data are presented collectively and separated into preliminary responsiveness groups.

Characteristic	All Patients (N=58)	Preliminary BH ₄ Responders ^a (n=32)	BH ₄ Non-Responders ^b (n=25)	Difference Between Groups (<i>p</i> -value)
Age (years)	17.3 ± 11.0	15.2 ± 10.3	19.7 ± 11.7	0.128
Gender (male)	34 (58.6%)	21 (65.6%)	13 (52.0%)	0.298
Height (cm)	149.0 ± 23.0	145.0 ± 24.8	153.4 ± 20.1	0.175
Weight (kg)	56.3 ± 30.7	48.4 ± 27.2	63.3 ± 29.7	0.053
Baseline plasma phenylalanine concentration (μmol/L)	693 ± 412	564 ± 307	843 ± 479	0.016
Month 1 plasma phenylalanine concentration (μmol/L)	555 ± 478 ^c	250 ± 213	947 ± 437	<0.0001
Change in plasma phenylalanine concentrations (% change from baseline)	-17.4 ± 58.0 ^c	-55.3 ± 19.8	+31.2 ± 54.6	<0.0001

Values are presented as mean ± standard deviation or n (%); *p*-values are calculated using Student's *t*-test or χ^2 test, as appropriate

^a Preliminary BH₄-responder is a patient experiencing 15% decrease in plasma phenylalanine concentrations after one month of BH₄ therapy

^b BH₄ non-responder is a patient experiencing <15% decrease in plasma phenylalanine concentrations after one month of BH₄ therapy

^c n=57; one patient did not return for month one assessment

Table 2
Reported dietary intake of 53 patients with diet records at baseline and after one month of BH₄ therapy

	Preliminary BH ₄ Responders (n=31) ^a		BH ₄ Non-Responders (n=22) ^b		Association with % Change in Plasma Phenylalanine Concentration ^c	P-value
	Baseline	Month 1	Baseline	Month 1		
Energy Intake (kcal/day)	1803 ± 579	1793 ± 460	1687 ± 481	1846 ± 828		0.516
Total Protein Intake (g/day)	60.9 ± 22.1	57.1 ± 18.6	61.8 ± 14.1	59.5 ± 20.7		0.619
Total Protein Intake (g/kg/day)	1.5 ± 0.6	1.4 ± 0.7	1.2 ± 0.6	1.1 ± 0.5		0.583
Dietary Phenylalanine Intake (mg/day)	1,034 ± 968	876 ± 634	822 ± 802	849 ± 910		0.479
Dietary Phenylalanine Intake (mg/kg/day)	20.3 ± 12.8	17.7 ± 8.2	14.4 ± 12.1	14.1 ± 12.3		0.481
Protein Equivalents from Medical Food Consumption (g/day)	37.6 ± 21.0	36.6 ± 20.7	42.9 ± 17.5	39.2 ± 19.2		0.145
Medical Food Consumption (% of prescription)	87.6 ± 28.9 ^d	85.6 ± 29.6 ^d	82.1 ± 30.2	73.1 ± 35.0		---

^aOne patient excluded for incomplete diet record at baseline

^bThree patients excluded for incomplete diet records at baseline and/or month one

^cLinear regression of % change plasma phenylalanine concentration (between baseline and month one) modeled against % change in reported dietary intake (between baseline and month one) for all participants with a complete diet record at both time points

^dn=29; two patients did not have an established medical food prescription at baseline; excluded from analysis

Table 3

Change in diet phenylalanine and medical food prescription in 19 definitive BH₄-responders and 9 provisional responders.

	Definitive Responders		Provisional Responders
	<i>All Definitive Responders (n=19)^a</i>	<i>Subset of patients who underwent diet liberalization (n=16)^b</i>	<i>All Provisional Responders (n=9)</i>
Baseline dietary phenylalanine prescription (mg/day)	704 ± 518 ^c	512 ± 177 ^d	313 ± 119 ^{c,d}
Liberalized dietary phenylalanine prescription (mg/day)	1922 ± 612 ^e	1958 ± 632 ^f	468 ± 203 ^{e,f}
Phenylalanine tolerance (% of baseline)	356.0 ± 157.4 ^e	403.9 ± 119.0 ^f	149.3 ± 28.3 ^{e,f}
Baseline medical food prescription (grams protein equivalents/day)	43.3 ± 20.3	50.1 ± 13.6	48.8 ± 17.1
Liberalized medical food prescription (grams protein equivalents/day)	7.8 ± 10.5 ^e	9.3 ± 10.9 ^f	49.5 ± 16.6 ^{e,f}
Medical food prescription (% of baseline)	16.7 ± 19.5 ^{e,g}	18.8 ± 19.7 ^f	101.8 ± 5.1 ^{e,f}

^a Includes three patients who did not undergo diet liberalization process due to month one intact protein intake at their RDA

^b Excludes the three patients who did not undergo diet liberalization

^c $p = 0.005$; comparison of all definitive responders and all provisional responders

^d $p = 0.007$; comparison of definitive responders who underwent diet liberalization and all provisional responders

^e $p < 0.0001$; comparison of all definitive responders and all provisional responders

^f $p < 0.0001$; comparison of definitive responders who underwent diet liberalization and all provisional responders

^g $n = 18$; one patient did not have medical food prescribed at baseline