Positive effect of a simplified diet on blood phenylalanine control in different phenylketonuria variants, characterized by newborn BH4 loading test and PAH analysis

M. Zimmermann a,1, P. Jacobs a,d,1, R. Fingerhut c, T. Torresani c, B. Thöny b, N. Blau a,2, M.R. Baumgartner a, M. Rohrbach a,*

a Division of Metabolism, University Children’s Hospital and Children’s Research Center, Zurich, Switzerland
b Swiss Newborn Screening Laboratory, University Children’s Hospital and Pediatric Research Center, Zurich, Switzerland
c Division of Clinical Chemistry and Biochemistry and Children’s Research Center, University Children’s Hospital, Zurich, Switzerland
d University of Applied Sciences Bern, Section Health, Bachelor Programme in Nutrition and Dietetics, Bern, Switzerland

A R T I C L E   I N F O
Article history:
Received 18 April 2012
Accepted 18 April 2012
Available online 25 April 2012
Keywords:
PKU
Simplified diet versus classical diet

A B S T R A C T

Until today, the mainstay of phenylketonuria (PKU) treatment is a phenylalanine (Phe)-restricted diet. Strict dietary treatment decreases flexibility and autonomy and still has a major impact on patients and their families. Compliance is often poor, particularly in adolescence. The aim of this study was to investigate the effect of the intake of fruits and vegetables containing Phe less than 100 mg/100 g (‘simplified diet’), as recommended by WHO for all individuals, instead of classical totally restricted diet on the course and treatment control of the disease in a well-characterized PKU cohort (n = 80). All individual blood Phe measurements of each patient (1992–2009) were statistically analyzed before and after diet switch. Epidemiological data, age at diagnosis, PAH mutations, BH4 responsiveness, as well as Phe control measurements and detailed diet information were tabulated in a local database. 62.5% had BH4 loading test and 40% had PAH analysis; 50/80 switched from classical to simplified diet, including 26 classical PKU, 13 moderate PKU, 7 mild PKU and 4 mild hyperphenylalaninemia (HPA). Median Phe levels on a simplified diet did not differ significantly to the median Phe levels on classical diet in all disease groups. Our results indicate that a simplified diet has no negative effect on blood Phe control in patients with hyperphenylalaninemia, independent of severity of the phenotype or the age at diet switch, over the period of 3 years. Thus, a simpler approach to dietary treatment of PKU available to all HPA patients is more likely to be accepted and adhered by patients and might also increase quality of life.

© 2012 Elsevier Inc. All rights reserved.

1. Introduction

Phenylketonuria (PKU; OMIM ID: 261600), the most common inborn error of amino acid metabolism, is an autosomal recessive disorder caused by phenylalanine hydroxylase (PAH) deficiency, the enzyme that converts phenylalanine (Phe) to tyrosine [1]. Hyperphenylalaninemia (HPA) can be caused both by PAH deficiency or by inherited deficiency of enzymes involved in tetrahydrobipterin (BH4) synthesis or recycling [2]. HPA due to a mutated PAH produces a spectrum of phenotypes including classic PKU, moderate PKU, mild PKU and mild HPA [1].

Chronic, untreated, severe HPA in infants and children results in increased levels of blood Phe, which may lead to intellectual disability and motor deficits due to elevated Phe concentrations in the brain [3].

Newborn screening and early initiation of PKU therapy consisting of a Phe-restricted diet have eliminated the major manifestations of the disease [4,5]. Even though diet is affordable and successful strict dietary treatment including highly restrictive nourishment, possible nutritional deficiencies and regular Phe blood level controls still have major impact on patients and their families. Therefore, compliance is often poor, particularly in adolescence [6].

Currently treatment options for the management of PKU are expanding in particular with the introduction of large neutral amino acids, BH4 supplementation (Kuvan®, Merck Serono SA, Switzerland) and, in the future, phenylalanine-ammonia lyase or gene therapy [7].

The great majority of patients with PKU follow a natural-protein-restricted diet, which represents a nutritional challenge because it is necessary for the patient to restrict the amount of natural protein consumed in order to reduce Phe intake while avoiding the effects of a deprived diet. Recent studies had demonstrated that an increase
in natural protein of 50% from allowed foods, such as fruits and vegetables containing Phe 51–75 mg/100 g, did not destabilize overall Phe control [8,9].

PKU due to PAH deficiency is generally not associated with BH₄ deficiency, but in a subset of individuals with PKU, oral supplementation with BH₄ can potentially be effective leading to significant reduction of blood Phe concentration [10–13]. Subsequently it has also been shown that certain PAH gene mutations, with substantial residual activity, seem to be associated with a BH₄-sensitive phenotype of PKU [10,14]; however the relation between genotype and phenotype is complex [15].

Based on the findings of MacDonald and co-workers [8] we developed a modified and adapted concept of a simplified dietary program in PKU. The aim of this study was to investigate the effect of the intake of fruits and vegetables containing Phe less than 100 mg/100 g, in quantities recommended by WHO, on the course and treatment control of the disease in a well characterized PKU cohort (n = 80), including data on genotype and BH₄ responsiveness. We hypothesized that the simplified diet could present an effective treatment option for all patients with HPA caused by mutations in PAH independent of genotype, severity of disease or BH₄ responsiveness. Our findings reveal that intake of vegetables and fruits containing Phe less than 100 mg/100 g, in quantities recommended by WHO for all individuals, comparable to a vegan diet excluding cereals and pulses, has no destabilizing effect on the biochemical control of Phe in all PKU patients.

2. Methods

2.1. Database

Patient data were reviewed retrospectively for epidemiological data, age at diagnosis, PAH mutations, BH₄ responsiveness, control Phe measurements as well as detailed diet information. Collected data were entered anonymously in a local database (Microsoft Office Access 2003); the study was reviewed and approved by the local IRB at the University Children’s Hospital Zurich. All patients diagnosed between 1955 and 2005 that were regularly monitored and followed on a clinical basis in the period 1992–2009 were enrolled. Data regarding Phe measurements were collected from 1992 until 2009. Statistical analysis was performed by Pearson’s chi-square test.

2.2. Diagnosis and classification of hyperphenylalaninemia

Patients are identified by nationwide Swiss newborn screening program based on dried blood spots (DBS) obtained from a heel prick taken at the age >72 hours indicating Phe > 120 μmol/l, and subsequently confirmed and quantified by diastase pancreatic amino acid analysis. Diagnosis was primarily based on maximal blood Phe measurement before initializing treatment (diet) and classified as follows: Phe > 1200 μmol/l classical PKU, Phe 800–1200 μmol/l moderate PKU, Phe 600–800 μmol/l mild PKU, and Phe 120–600 μmol/l mild HPA [16–18]. BH₄ deficiency was excluded by measurement of urinary pterins and DHPR activity in DBS.

2.3. BH₄ loading test and BH₄ responsiveness

BH₄ loading test was performed to identify BH₄ responsiveness in PKU [13] All patients with blood Phe concentration of >400 μmol/l were challenged orally with BH₄ (20 mg/kg body weight) at time T₀ and T₂₄ hours [19]. Blood Phe concentration was measured before and 4, 8, 12, 24, and 48 hours after the first load to detect also some additional slow-responders [20]. BH₄ responsiveness was defined as reduction of blood Phe by >30% within 24 hours after BH₄ loading or a reduction of <20% after 8 hours and >20% but <30% after 24 hours [21–23].

2.4. Laboratory methods

Serum amino acids, pterins in urine or from dried blood spots, and DHPR activity from dried blood spots were analyzed as described [24,25]. For regular diet control phenylalanine was determined from DBS with the bacterial inhibition test until 2004, and afterwards by tandem mass-spectrometry.

Mutation analysis for all 13 exons of PAH gene was performed as described, and mutations were confirmed, if available, by carrier analysis of parents (PAH reference accession number ENSG00000171759) [26].

2.5. Dietary control

Phe levels were regularly monitored. Guthrie cards with the dried blood spots taken 1 hour after breakfast or dinner were sent to the newborn screening center. Frequency of measurements to monitor Phe levels and recommended Phe reference concentrations depend on age were as follows: 0–2 years: every 1–2 weeks/100–300 μmol/l; 2–10 years: every 1–4 weeks/100–400 μmol/l; >10 years: at least once a month/100–600 μmol/l. Dietary intake of Phe was assessed in all patients before diet switch.

2.6. Simplified diet

2.6.1. The classical diet

The classical diet in PKU is a diet strictly reducing natural protein intake, supplemented with essential amino acids. It can be defined as a vegan diet; however, no cereals and pulses are allowed. Only protein-free products such as pure sugar, products with high sugar content, butter and oil can be consumed without being weighted and calculated. For all other food products, patients must follow precise prescriptions. To ensure quantity of Phe intake, food is always weighted. In circumstances where variations of the diet are required or desired exchange list of food products are mandatory. However, complete exchange lists, including all needed information, are so complex that application is challenging. In case a product is not listed, or exchange lists are not available at all, the amount of Phe has to be analyzed and calculated.

2.6.2. The Zürich concept of the simplified diet in PKU

2.6.2.1. First months. We advocate breastfeeding in combination with Phe-free amino acid mixture as single nutrition for the first 6 months as most countries do followed by potatoes mixed with vegetables or plain fruits as most countries do with monitoring of Phe levels every 3 days up to a weekly basis.

2.6.2.2. After the weaning period. A simplified diet allows PKU patients to eat fruit, vegetables and special low protein food in normal, unrestricted quantities. To illustrate how much of a specific product can be offered the “rule of thumb” is used (in German called rule of fist). Following the recommendation of the World Health Organization to consume at least 400 g of vegetables daily [27], several countries introduced “5 a day” campaigns to encourage the consumption of at least five portions of fruit and vegetables each day. Germany, Austria, and Switzerland used the palm of the hand to measure a portion [28]. Our center has incorporated this widely used measure into the PKU-diet approach to simplify measurement of portion of fruits and vegetables containing Phe less than 100 mg/100 g. To demonstrate and document the rule, a fist is shown, followed by an open hand. The five fingers represent five portions (five a day). The amount of fruits or vegetables that can be placed on the palm of one hand represents the quantity of a specific fruit or vegetable for 1 day. The rule of thumb has no limiting character by itself, however it is rather used as a practical guideline, to reduce the overestimation effect of parents regarding the daily recommend quantities of fruits and vegetables for specific age groups.
Generally all fruits and vegetables were recommended free if consumed with variation. Similar to the national campaign "5 a day" we advocate the use of all other fruits and vegetables. Limitations include fruits and vegetables with >100 mg phenylalanine such as avocado, broccoli, Brussels sprout, passion fruit and kale. We recommend only one portion of these. Of note, potatoes and sweetcorn are not defined as vegetables, and thus must be calculated.

Peas and sprouts such as bean sprouts can be used, but must be calculated too.

Special low protein food including bread, pasta and breakfast cereals may be eaten ad libitum.

The quantities of potatoes, rice and maize are discussed with the parents and then fixed for a period of time. Parents and later the patients are instructed to estimate the fixed quantities, and are advised to control-scale periodically. Beyond calculating and weighting amino acid-mixtures, no calculations are needed. Blood Phe levels are regulated by adjusting the quantity of amino acid mixture, potatoes, rice and maize. For toddlers a lump sum of 25 mg Phe for fruits, vegetables, and protein-free special foods each was counted. For older children and adults 50 mg is calculated (Table 1).

2.7. Statistics

All diet control measurements were entered in a datasheet followed by statistical analysis of paired chi-tests to compare differences in Phe concentrations depending on diet. Calculations regarding differences by statistical analysis of paired chi-tests to compare differences in Phe levels had increased in the age group >16 years. In the age group >16 years we noted significantly more Phe levels within the recommended reference range in the simpliﬁed diet when compared to classical PKU (p=0.0096). Patients diagnosed before 1980, as well as some patients with mild HPA, had no BH4 loading test.

Molecular analysis of the PAH gene to identify disease causing mutations was performed in 32 patients (40%). Lack of reimbursement of the costs through their health insurance for molecular analysis was the most likely reason for the limited tests. The most frequent mutation in PAH was c.782G>A (p.R261Q) found in 13/64 alleles.

Table 1

<table>
<thead>
<tr>
<th>Classical calculation</th>
<th>Phe mg</th>
<th>&quot;Calculation&quot; simplified diet</th>
<th>Phe mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low protein flakes (Loprofin® Aple-Honig)</td>
<td>40 g</td>
<td>PKU milk (Loprofin®)</td>
<td>200 ml</td>
</tr>
<tr>
<td>PKU milk (Loprofin®)</td>
<td>200 ml</td>
<td>Hazel nut and cocoa spread (Nutella®)</td>
<td>10 g</td>
</tr>
<tr>
<td>Low protein bread (Heilbronner Weissbrot Loprofin®)</td>
<td>30 g</td>
<td>Water (Nutella®)</td>
<td>300 ml</td>
</tr>
<tr>
<td>Hazel nut and cocoa spread (Nutella®)</td>
<td>10 g</td>
<td>Margarine</td>
<td>10 g</td>
</tr>
<tr>
<td>Water</td>
<td>300 ml</td>
<td>Potatoes</td>
<td>200 g</td>
</tr>
<tr>
<td>Low protein bread (Echtes Bauernkruizer® Loprofin®)</td>
<td>60 g</td>
<td>Olive oil</td>
<td>200 g</td>
</tr>
<tr>
<td>Margarine</td>
<td>10 g</td>
<td>Nutella banana cake</td>
<td>50 g</td>
</tr>
<tr>
<td>Cucumber</td>
<td>20 g</td>
<td>Herbal tea</td>
<td>200 ml</td>
</tr>
<tr>
<td>Apple</td>
<td>20 g</td>
<td>Olive oil</td>
<td>15 g</td>
</tr>
<tr>
<td>Potatoes</td>
<td>120 g</td>
<td>P-AM 2® SHS</td>
<td>35 g</td>
</tr>
<tr>
<td>Mixed vegetables</td>
<td>100 g</td>
<td>Fruits, 3 portions</td>
<td>50</td>
</tr>
<tr>
<td>Olive oil</td>
<td>15 g</td>
<td>Vegetables, 2 portions</td>
<td>50</td>
</tr>
<tr>
<td>Nutella banana cake</td>
<td>50 g</td>
<td>Low protein food</td>
<td>50</td>
</tr>
<tr>
<td>Herbal tea</td>
<td>200 ml</td>
<td>Total</td>
<td>400</td>
</tr>
<tr>
<td>Low protein waffle (Loprofin®)</td>
<td>15 g</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Kiwi</td>
<td>85 g</td>
<td></td>
<td>21.3</td>
</tr>
<tr>
<td>Low protein pasta (Loprofin®)</td>
<td>60 g</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Cucumber</td>
<td>70 g</td>
<td></td>
<td>9.8</td>
</tr>
<tr>
<td>Tomato</td>
<td>70 g</td>
<td></td>
<td>16.8</td>
</tr>
<tr>
<td>Olive oil</td>
<td>15 g</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Orange</td>
<td>90 g</td>
<td></td>
<td>24.3</td>
</tr>
<tr>
<td>P-AM 2® SHS</td>
<td>35 g</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>410</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. Results

3.1. PKU cohort of Zurich

The patient cohort of Zurich, identified by national newborn screening program, contains 300 patients with increased blood Phe levels (>120 μmol/l). Two hundred thirty-three patients were diagnosed with mild HPA (120–600 μmol); 220 (74%) of those were not clinically followed as repeat blood Phe measurements showed constantly Phe <300 μmol/l. Consequently, those patients were not included in the study. A total of 80 patients were included.

The female–male ratio was 1:1. Classical PKU was found in 41 individuals (51.25%), 16 individuals had moderate PKU (20%), 10 individuals (12.5%) had mild PKU, and 13 individuals (16.25%) had mild HPA. All patients were on protein-restricted diet.

The majority of patients (n = 57) were of a central European ancestry (Germany 2, Switzerland 55): 9 patients originated from southeast Europe (Turkey 5, Kosovo 1, Macedonia 2, Serbia 1) or southern Europe (Italy 5, Spain 1, Portugal 1); one patient was of Arabian origin (Lebanon and 2 from Iran). From 6 patients we had no information about their ethnic origin. Consanguinity was confirmed in 8 families (Switzerland 3, Turkey 2, Italy 1, Iran 1, Lebanon 1).

Fifty-one out of eighty patients (63.7%) had a 48-hour BH4 loading test in the neonatal period with 20 mg/kg at 0 and 24 hours. Significantly more BH4 responders were identified among mild HPA when compared to classical PKU (p=0.0096) Patients diagnosed before 1980, as well as some patients with mild HPA, had no BH4 loading test.

Molecular analysis of the PAH gene to identify disease causing mutations was performed in 32 patients (40%). Lack of reimbursement of the costs through their health insurance for molecular analysis was the most likely reason for the limited tests. The most frequent mutation in PAH was c.782G>A (p.R261Q) found in 13/64 alleles.

3.2. Median blood Phe levels in patients on simplified diet

Seventy-three out of eighty patients had classical Phe restricted diet at the beginning of their treatment at diagnosis; the remaining 7 patients were on a simplified diet approach from birth on. Fifty patients chose to switch to the simplified approach whereas 23 patients refused to switch and remained on classical restricted diet.

Individual blood Phe measurements ranged from 5 to 2630 μmol/l in classical PKU, from 5 to 1800 μmol/l in moderate PKU, from 5 to 1250 μmol/l in mild PKU and from 5 to 884 μmol/l in mild HPA, respectively. The median blood Phe level on a simplified diet did not differ significantly to the median Phe level on classical diet and were 355 μmol/l in classical PKU, 313 μmol/l in moderate PKU, and 336 μmol/l in mild PKU (Table 2). Only 4 participants diagnosed with mild HPA did switch diet; they were not included in our statistical analysis.

In addition median Phe levels were compared in different age groups (Table 3), including the groups age 2–9 years, age 10–16 years and age >16 years. In the age groups 2–9 and 10–16 Phe levels did not differ significantly; however in the age group >16 years we noted significantly more Phe levels within the recommended reference range in the group on simplified diet when compared to the classical diet (Table 3).

In particular, the use of fruits and vegetables containing Phe less than 100 mg/100 g in quantities as recommended by the WHO had no negative effect on plasma Phe levels independent of severity of the disease and the age of diet switch. Phe intake had increased in most patients after diet switch (Table 1).

4. Discussion

A simplified and modified diet, allowing intake of fruits and vegetables containing Phe less than 100 mg/100 g in quantities as recommended...
Limitations of our study include small sample size as well as overcorrection to the classical diet and the lack of motivation to change. The simplification of PKU revealed that our approach is safe also in children.

The referred age is related to the actual age at time of diet switch. All Phe measurements in μmol/l; n°: total number of measurements.

### Table 2
Comparison of median blood Phe levels of patients on a classical diet and after the switch to a simplified diet in all subgroups of PKU.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Diet switch (n)</th>
<th>Median age (range; SD)</th>
<th>Median Phe on classic diet (n°)</th>
<th>Median Phe on simplified diet (n°)</th>
<th>Chi square test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classic PKU</td>
<td>26</td>
<td>19 (2–51; 11.9)</td>
<td>275 (n = 3688)</td>
<td>355 (n = 677)</td>
<td>p = 0.96</td>
</tr>
<tr>
<td>Moderate PKU</td>
<td>13</td>
<td>10 (2–27.10)</td>
<td>200 (n = 2779)</td>
<td>313 (n = 270)</td>
<td>p = 0.06</td>
</tr>
<tr>
<td>Mild PKU</td>
<td>7</td>
<td>16.5 (2–29.78)</td>
<td>300 (n = 598)</td>
<td>336 (n = 162)</td>
<td>p = 0.42</td>
</tr>
</tbody>
</table>

Comparison of mean blood Phe levels (μmol/l) of patients on a classical diet and after the switch to a simplified diet in all subgroups of PKU. All Phe measurements in μmol/l; n°: total number of measurements.

by the WHO, was used to treat all subgroups of PKU patients and was analyzed with regard to its effect on blood Phe levels. Based on the work of MacDonald and co-workers [9] who had first shown that it appeared to be safe to incorporate into the diet without restriction fruits and vegetables containing Phe 51–75 mg/100 g, we demonstrated that not weight intake of all fruits and vegetables containing Phe less than 100 mg/100 g had no destabilizing effect on the control of blood Phe values in all PKU subgroups also including classical PKU patients. Our PKU cohort is relatively well characterized as it includes also data on BH4 responsiveness and molecular testing of PAH gene mutations.

Our findings in 50 patients who switched from classical restricted diet treatment to a simplified diet reveal that blood Phe levels were not negatively affected by this liberalization. In particular median Phe levels did not change significantly in all PKU subgroups, respectively, classical PKU, moderate PKU, and mild PKU (Table 2). These results confirm the findings previously reported [9]; however to the best of our knowledge we demonstrate for the first time that intake is not limited to fruits and vegetables containing Phe of maximum 51–75 mg/100 g and in particular that this simplified diet model has not adversely affected long term blood phenylalanine control. The results of the analysis of the effect of a simplified diet in different age groups of PKU revealed that our approach is safe also in children under the age of 10 years. Interestingly we noted a positive effect of the simplified diet in the cohort of patients older than 16 years. In addition this approach introduces a pragmatic way of ensuring that patients eat enough but with no excess phenylalanine intake from fruit and vegetables. Both severity of PKU and age at diet switch did not influence ability to allow fruits and vegetables containing Phe less than 100 mg/100 g without measurement.

Thirty patients did not choose to switch diet, mainly because they were used to the classical diet and the lack of motivation to change. Limitations of our study include small sample size as well as overlapping phenotypes using a classification that is based on highest Phe level before treatment initiation (which could be responsible for some misclassification). In particular, the small sample size after diet switch could bias the Phe measurement. Additional limitations include the lack of evidence that gastrointestinal resorption of Phe from fruits and vegetable is similar to other protein sources; thus it is difficult to theoretically predict the effect of increased Phe intake through fruits and vegetables as well as the lack of exact individual calculations of Phe intake after diet switch. However as the goal of the simplified diet is to allow patients intake without measuring, calculation of Phe intake is only possible theoretically based on our recommendation (Table 1).

Compared to the classical Phe restricted diet, our data show that the concept of allowing intake of fruits and vegetable containing Phe less than 100 mg/100 g, in quantities as recommended by WHO, has several important impacts to consider.

A classical diet is the mainstay of PKU treatment; however in recent years, the nutritional management of PKU has become more complex in order to optimize patients’ growth, development and diet compliance [29]. The classical diet approach ensures the quantity of Phe intake, by constant weighting all food products. Due to the fact that intake of fruits and vegetables are limited on one side, but free quantity of sugar, fat, butter and oil can be used, the diet does not meet criteria for healthy food as advocated by national and international health organizations. It is obvious that durable motivation is needed to weigh, work with exchange lists and calculate for each meal. Some patients are tempted to avoid this struggle by choosing the same foods-products daily. Diet becomes monotone and quality of life might declines.

Especially in young children it is important to have the choice to eat unlimited quantities of food if desired, to ensure optimal development of childhood autonomy. The daily intake is not stable, but depends on several factors such as actual growth velocity, illness and mood. It is a well-know phenomena that children eat less for several reasons and then eat increased portions to compensate. In PKU, compliance is complex being subject to diverse definitions and factors influencing compliance include the nature and nurture of the patient, as well as the inconvenience of the diet, cost and availability of dietary treatment [30]. Not rarely thus, it is common for blood Phe concentrations to be outside optimal target ranges, particularly in teenagers and adults, indicating inadequate compliance.

A simpler approach to dietary treatment of PKU available to all HPA patients is more likely to be accepted and adhered by the patient than a more complex one. Our simplified diet allows patients to eat fruits, vegetables containing Phe less than 100 mg/100 g, and special low protein food in normal and unrestricted quantities, allowing more flexibility. With potatoes, rice, maize and amino acid mixture Phe levels are regulated by adding limited portions of those aliments to meals. Patients learn to estimate the approximate weight of the limited portions, and control-scaling is only performed periodically. For parents with a child with PKU and teenagers with PKU this approach makes life less complicated as this diet has little differences to a non PKU feeding vegetarian situation [8,9].

Specifically for situations where patients are traveling or in a working environment or have no possibilities to prepare their own food it will be simple to order vegetable dishes, salads, rice and potatoes. This will directly decrease the discriminative effect, as patients do not need to explain themselves. In addition, a simplified approach may have significant implications for countries where only limited access to low protein products or dieticians exists, as this approach may open easier treatment options.

It is important however to realize that our approach is a simplification rather than a liberalization of the diet. Continuous monitoring of all patients in close control is mandatory, including regular measurement of blood Phe levels. Intake of small quantities of dairy products or meat is not recommended.

In conclusion, our results indicate that intake of vegetable and fruits containing Phe less than 100 mg/100 g, in quantities as recommended by WHO not only increases the autonomy of the patients.

### Table 3
Comparison of median blood Phe levels on a classical diet and after the switch to a simplified diet in different age groups.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Patients (n)</th>
<th>Median Phe on classic diet (n°)</th>
<th>Median Phe on simplified diet (n°)</th>
<th>Phe reference concentration range</th>
<th>Chi square test</th>
</tr>
</thead>
<tbody>
<tr>
<td>2–9</td>
<td>12</td>
<td>200 (n = 2142)</td>
<td>245 (n = 750)</td>
<td>100–400</td>
<td>p = 0.183</td>
</tr>
<tr>
<td>10–16</td>
<td>13</td>
<td>275 (n = 1240)</td>
<td>406 (n = 122)</td>
<td>100–600</td>
<td>p = 0.4993</td>
</tr>
<tr>
<td>&gt;16</td>
<td>25</td>
<td>400 (n = 1450)</td>
<td>362 (n = 420)</td>
<td>100–600</td>
<td>p = 0.0001</td>
</tr>
</tbody>
</table>

The referred age is related to the actual age at time of diet switch. All Phe measurements in μmol/l; n°: total number of measurements.

Chi square test: total number of measurements.
regarding nutrition, but might also increase the quality of life, and there is evidence that the approach is safe regarding the long-term control of Phe levels measured routinely in all patients. This promising dietary approach may decrease the burden of a restricted diet and may lead to an increased quality of life and compliance. Further studies in larger population will have to validate our findings before broad recommendation on simplified diet can be implemented.

Acknowledgments

We thank Britschgi Corinne for careful control of molecular data.

References


