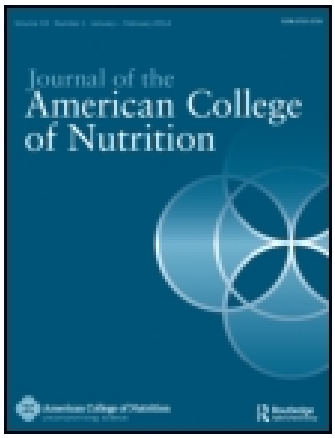

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Randomized Controlled Trial of a Protein Substitute with Prolonged Release on the Protein Status of Children with Phenylketonuria

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Original Research

Randomized Controlled Trial of a Protein Substitute with Prolonged Release on the Protein Status of Children with Phenylketonuria

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Key words: children, phenylketonuria, protein status, protein substitute, transthyretin

Objective: To examine whether a phenylalanine-free protein substitute with prolonged release may be beneficial to the protein status of children with phenylketonuria (PKU) compared to conventional substitutes.

Methods: Sixty children with PKU, 7 to 16 years of age, were randomly allocated to receive either a prolonged-release (test) or the current conventional protein substitute for 30 days. Subjects were additionally sex and age matched with 60 subjects with mild hyperphenylalaninemia and 60 unaffected subjects. The protein status in children with PKU was assessed by albumin, transthyretin, and retinol-binding protein (RBP), and changes throughout the trial period were the primary outcome measures.

Results: Children with PKU did not differ in anthropometry from children with mild hyperphenylalaninemia or unaffected children but they ingested lower amounts of proteins ($p < 0.01$). No differences occurred throughout the trial between or within children with PKU who received the test or conventional substitute for macronutrient intake. Albumin and RBP concentrations were within the age-specific reference range for all children. The rate of protein insufficiency (transthyretin concentration less than 20 mg/dL) did not differ statistically between children receiving test or conventional substitute (recruitment 51.8% vs 53.6%; end of the trial 44.4% vs 50.0%) but mean transthyretin recovered over 20 mg/dL in children who received the test substitute, increasing from 19.1 to 20.7 mg/dL (mean change, 1.6 mg/dL; 95% confidence interval 0.4 to 2.8 mg/dL). In children receiving conventional substitute mean transthyretin changed from 19.0 to 19.2 mg/dL (0.2; −0.2 to 0.6) mg/dL.

Conclusions: Protein substitutes with prolonged release might be beneficial to protein status in children with phenylketonuria.

INTRODUCTION

Phenylketonuria (PKU; Online Mendelian Inheritance in Man, OMIM, phenotype MIM number 361600) is an inherited autosomal recessive disorder of metabolism resulting from a deficiency of phenylalanine hydroxylase (OMIM, gene MIM number 612349; Enzyme nomenclature database, EC (Enzyme Commission) number 1.14.16.1) with an incidence rate of approximately 1 per 10,000 newborns in most of the Caucasian populations of North America and Europe [1,2].

The dietary intervention is the keystone of management of PKU, primarily based on the restriction of phenylalanine (PHE)-containing foods and supplementation with PHE-free protein substitutes [3,4] that should start as soon after birth as possible and continue throughout adulthood to maintain plasma PHE concentration ideally within the range of 2–6 mg/dL (1 mg/dL = 60 μmol/L) [5] and for better physical and neurophysiological health [6,7].

It has been recognized that compliant ingestion of the substitute when regularly distributed during the day can favor stabilization of plasma phenylalanine and tolerance because the retention of proteins and synthesis improve [8,9]. Despite this view, compliance to dietary recommendations may actually decline, mainly in school-age children [10], and result in higher PHE concentration [11] and erratic protein intake. This behavior may be critical at the age when protein synthesis is essential for growth [12] and might negatively affect neurodevelopment [13] and also upset the protein status. One may then wonder whether ingestion of protein substitutes with prolonged release may have a competitive function in modulating plasma PHE and

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in smoothing potential stresses on protein status possibly also related to a heterogeneous observance of the dietary treatment. Although this question is of potential practical and clinical relevance, there are no studies currently in the literature examining the value of protein substitutes with prolonged release in the optimization of PKU management.

The main objective of this study is to evaluate whether a protein substitute with prolonged release may be beneficial to the protein status of children with PKU compared to conventional substitutes.

MATERIALS AND METHODS

Subjects

In this single-center clinical trial, 60 children with PKU were recruited consecutively and randomized, between January 1, 2010, and February 28, 2013, among those undergoing routine monitoring at the Regional Center for Congenital Metabolic Diseases, San Paolo Hospital, Milan, Italy. At this center, subjects with hyperphenylalaninemia routinely undergo a clinical visit once a year, including assessment of growth and biochemistry. Inclusion criteria for children with PKU were as follows: age 7–16 years and plasma PHE $>$ 6 mg/dL in the newborn period ($<$21 days of age) treated by a PHE-restrictive diet. Exclusion criteria were as follows: being treated with tetrahydrobiopterin (BH4), exhibiting an inflammatory condition, and/or concurrent diseases. Subjects administered prior to recruitment with a prolonged-release protein substitute were additionally excluded.

Children with PKU were sex and age matched ($\pm$ 1 year) for baseline comparison with 60 non-PKU subjects with mild hyperphenylalaninemia (MHP; plasma PHE 2–6 mg/dL in the newborn period) not exhibiting an inflammatory condition or concurrent diseases and 60 unaffected subjects. Children with MHP, who were allowed to follow a normal unrestricted diet, were recruited among those followed at the Regional Center for Congenital Metabolic Diseases, and unaffected children were recruited among those undergoing minor surgery at the Day Surgery Clinic of the San Paolo Hospital.

Parents of potentially eligible children with PKU and MHP were contacted by telephone 7 $\pm$ 1 days before the scheduled annual visit, and an e-mail communication with a diet diary and instructions attached was sent to those willing to participate.

On the day of recruitment (baseline), which was the day of visit for children with PKU or MHP or the day of surgery for unaffected children, subjects were assessed for confirmation of eligibility. The parents of children or the legal guardian received a detailed explanation of the aim of the study and signed a consent form. The Ethics Committee of the hospital approved the study protocol.

Anthropometric Measurements

Measurements of body weight and height were obtained at recruitment using a calibrated electronic scale (Seca 799, Seca GmbH & Co. KG., Hamburg, Germany; precision 0.01 kg) fitted with a measuring rod (Seca 220). Anthropometric evaluations were performed by 2 experienced operators. Body mass index was calculated from the ratio of weight to height squared (kg/m^2). WHO AnthroPlus software [14] was used to calculate weight for age, height for age, and body mass index *z*- scores.

Dietary Assessment

Dietary habits were assessed by a 3-day diet diary (including a weekend day) recorded during the week prior to recruitment for children with PKU and MHP and during the second week after surgery for unaffected children. Additionally, PKU subjects recorded a 3-day diary during the last week of the trial period. Energy and nutrient analysis was performed using an *ad hoc* software developed at San Paolo Hospital based on the Food and Nutrient Database issued by the National Institute of Nutrition [15]. The dietary energy and macronutrient intake was worked out for each day and the 3-day average was calculated.

Biochemical Analysis

Fasting blood samples were taken at 9:00 AM $\pm$ 1 hour on the day of recruitment. In children with PKU, blood samples were additionally collected at the end of the trial. Blood samples were assessed for the following indicators of protein status: albumin, transthyretin (prealbumin), retinol-binding protein (RBP). Albumin and transthyretin were measured using a Modular Analytics system (Roche Diagnostics GmbH, Mannheim, Germany). The BN ProSpec System (Siemens AG, Erlangen, Germany) was used to determine RBP. The plasma concentration of PHE was measured using a Biochrom 20 + Amino Acid Analyser (Biochrom Ltd, Cambridge, UK), and the ratio of nonessential to essential amino acids (AA) was calculated. The age-specific reference range of "normality" for protein status was in accordance with the literature [16,17]. *Protein insufficiency* was defined in accordance with Arnold et al. [18] as plasma transthyretin concentration below 20 mg/dL.

Intervention

The study protocol scheduled oral administration of the substitute—that is, a test prolonged-release PHE-free protein substitute (Afenil Micro 3H, Piam Farmaceutici S.p.a., Genoa, Italy), with nutritional value of 385/kcal/100 g (protein equivalent 73.6%; carbohydrate 15.9%; fat 2.4%; fiber 2.6%) or the current conventional substitute administered at the time of recruitment (nutritional value range 307–334 kcal/100 g, protein

equivalent range 70%–79%; duration of action shorter than approximately 2 hours)—for 30 days. Preparation of the test protein substitute included the use of sodium alginate as hydrophilic carrier to prolong the release [19]. The manufacturing process consists of a wet granulation phase with an aqueous solution for the creation of microtablets that incorporate the active components of the substitute and then a drying phase in a static oven. This technology improves the matrix effect of sodium alginate. Additionally, to enhance the organoleptic characteristics and permeability, the microtablets were coated after compression with a hydroxypropyl-methyl cellulose and stearic acid film. The final microtablets were cylindrical in shape with a smooth surface (diameter: 3 mm; height: 3.8–4 mm). The release of the substitute was assessed in vitro from microtablets according to the method reported by Rodriguez et al. [20], by a dissolution test using 900 mL of 0.1 M HCl as dissolution medium, which was stirred at 75 rpm at 37 $\pm$ 0.5°C. Samples were withdrawn from 8 dissolution runs at predetermined time intervals throughout a 3-hour period and assessed for concentration of substitute by reversed-phase high-performance liquid chromatography with ultraviolet-visible detection. The average overall release time was approximately 3 hours, with a percentage of about 65%, 85%, and 99% of substitute released within 1, 2, and 3 hours, respectively. The amount of substitute and the number of daily doses recommended (3 to 4) were determined based on age, body weight, and PKU status. Forty-one (68.3%) children were administered with 3 doses/day (test substitute 21/30; conventional substitute 20/30) and 19 (31.7%) with 4 doses/day (test substitute 9/30; conventional substitute 10/30). The number of doses/day recommended to a child during the trial was the same that he or she was receiving in the period before the trial. Administration of the substitute assigned was started within 2 days of recruitment. Although substitutes were packaged in identical opaque coded cans, it was not possible to blind the trial because the test substitute was formulated in film-coated microtablets, differing from the conventional substitute, which was a powder formulation or liquid ready-to-drink. The statistician involved in the trial was unaware of the substitute until codes were broken after the completion of the data analysis. The substitutes were consigned to parents at recruitment, and at the end of the trial cans that were not consumed were returned to the investigators.

Acceptability

Subjects with PKU were asked to complete a questionnaire at the end of the trial about acceptability of the substitute, based on a 5-point Likert scale.

Outcome Measures

The primary outcome measure was the change in the protein status of children with PKU at the end of the trial with respect to baseline. Secondary outcome measures were the change in plasma PHE and acceptability of the substitute.

Sample Size and Randomization

The sample size was calculated to detect a change of 0.1 mg/dL or more in mean albumin in children treated with the test substitute. Recursive calculation was performed throughout the recruitment period. Admitting a 2-tailed type I error level of 5% with a power of 80% and assuming expected reference mean and SD of albumin as estimated in children already recruited, 28 subjects with PKU were needed in each treatment group (resulting from a mean and SD of 4.5 mg/dL and 0.18 mg/dL, respectively). Overall, 60 children with PKU were recruited and assigned to the test ($n = 30$) or conventional ($n = 30$) substitute, based on a computer-generated randomization list stratified according to sex and age (7–9, 10–13, and 14–16 years). A block size of 4 units was used.

Statistical Analysis

Children with PKU who discontinued the assigned substitute within the first week of ingestion and/or failed to complete blood samplings were excluded from the data analysis, which was conducted on an intent-to-treat basis. Descriptive data are reported as mean (SD) or number of observations (percentage). The Kolmogorov-Smirnov test was used to assess the normality of distribution of continuous variables. Baseline comparison among groups (PKU, MHP, unaffected) was performed by one-way analysis of variance (ANOVA) or the Kruskal-Wallis test, as appropriate. Significance of *post hoc* multiple comparison was adjusted using Bonferroni correction. Overall difference in change of the protein status between the 2 treatment groups of children with PKU was tested by 2-way ANOVA. Student's t test for independent samples or the Mann-Whitney U test, as appropriate, was also used to compare between groups, and within-group comparisons were tested by the paired Student's t test or the Wilcoxon test. A significance level of 0.05 was assumed and the statistical tests were 2-tailed. SPSS software, version 19.0 (SPSS Inc., Chicago, IL), was used for the statistical analysis.

RESULTS

Subjects

Of the 60 randomized children with PKU, 55 were analyzed. Fig. 1 details the progress of children with PKU throughout the trial. No significant difference occurred among PKU, MHP, and unaffected children for any anthropometric characteristic (Table 1). The block randomization of children with PKU resulted in a similar distribution of sex, age, and anthropometry in the 2 treatment groups (minimum $p = 0.585$). Among the children with PKU analyzed, 40 (test substitute $n = 20$, conventional substitute $n = 20$) exhibited mild PKU (neonatal PHE ranging from 6 to 20 mg/dL) and 15 (test substitute $n = 7$, conventional substitute $n = 8$) classical PKU (neonatal PHE > 20 mg/dL).

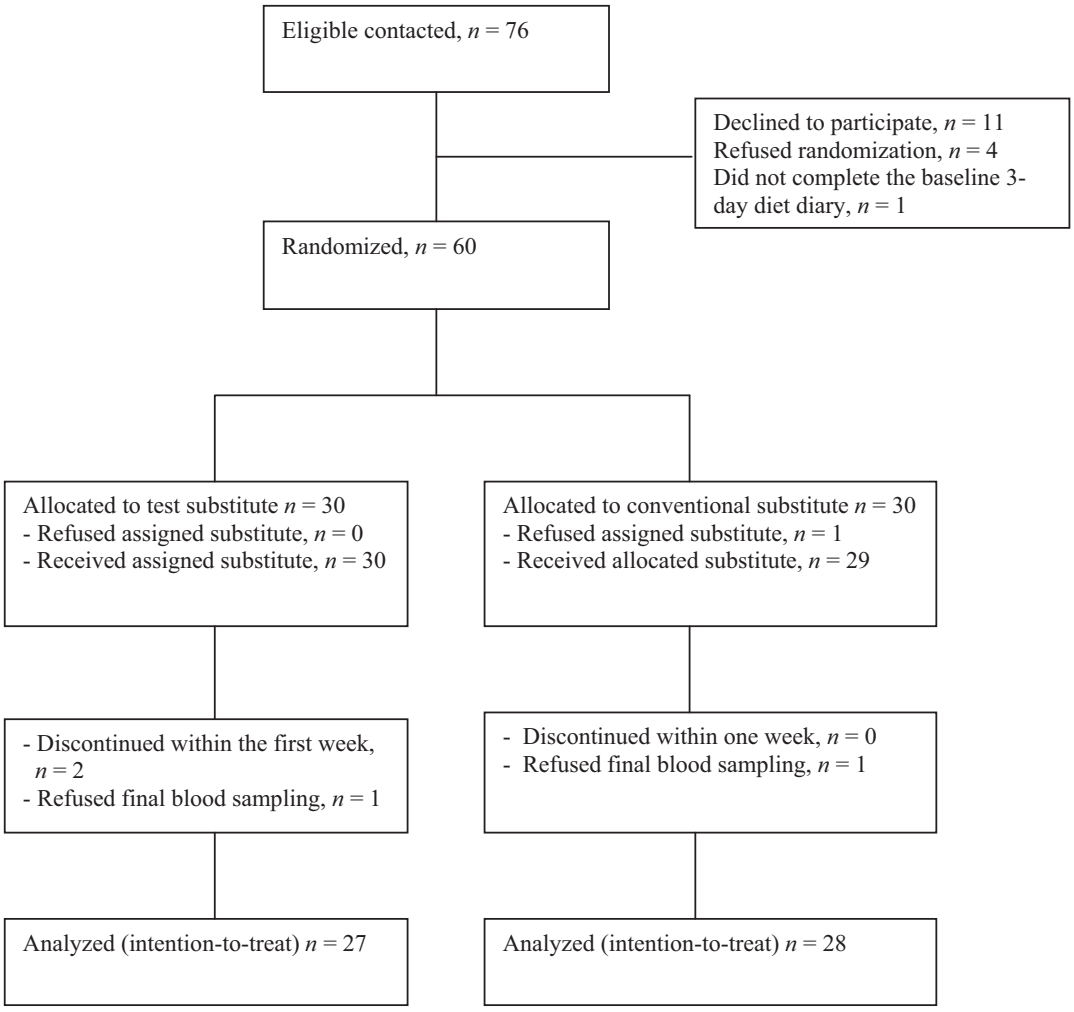

Fig. 1. CONSORT flow diagram of children with phenylketonuria.

Energy and Macronutrient Intake

Children with PKU ingested more calories (kcal/kg/day) than children with MHP but less than unaffected children, and lower amounts of proteins (% of total energy) than both children with MHP and unaffected children (Table 1). In children with PKU no change occurred throughout the trial period in energy or macronutrient intake (minimum $p = 0.673$), and no differences were observed between children who received the test or conventional substitute both at baseline and at the end of the trial (minimum $p = 0.367$). The mean (SD) protein intake at baseline was 12.2% (2.6) vs 12.4% (2.6) of total energy ($p = 0.865$) in children who received the test or conventional substitute, respectively; that is, 1.9 (0.8) vs 2.0 (0.9) g/kg/day. At the end of the trial the corresponding values were 11.9% (2.8) vs 12.3% (2.5) % of total energy ($p = 0.686$) and 1.8 (1.0) vs 2.0 (0.9) g/kg/day. The intake of PHE (mg/day) did not differ between PKU groups at baseline or end of the study and no within-group change was observed (minimum $p = 0.350$). At baseline mean (SD) PHE intake was 403 (213) vs 392 (227) mg/day in children who received the test or conventional substitute, respectively. At the end of the trial the corresponding values were 392 (207) vs 400 (208) mg/day.

Protein Status

Table 2 reports the protein status of children with PKU compared to children with MHP and unaffected subjects. Children with PKU exhibited lower albumin, transthyretin, and RPB and higher AA ratio than children with MHP (maximum $p = 0.01$) and unaffected children (maximum $p < 0.001$). Albumin and RPB concentrations were within the age-specific reference range for all children. All children with MHP and unaffected children showed concentrations of transthyretin within the reference range and 2 PKU subjects had transthyretin levels below the lower limit. Twenty-nine (52.7%) children with PKU had transthyretin concentrations below 20 mg/dL. The AA ratio ranged from 1.7 to 3.5, 1.5 to 3.0, 1.5 to 2.5 in children with PKU and MHP and unaffected children, respectively.

Table 1. Sex, Age, and Baseline Anthropometry and Dietary Energy and Macronutrient (% of total energy) Intake of Children with PKU Compared to Sex- and Age-Matched Children with MHP or Unaffected Children[1]

Variable	PKU ($n = 55$)	MHP ($n = 60$)	Unaffected ($n = 60$)	p Value[§]
Sex (boys)	24 (43.6)	26 (43.3)	26 (43.3)	0.999
Age (years)	9.2 (3.4)	9.3 (3.3)	9.2 (3.2)	0.983
Anthropometry				
Weight-for-age z-score	0.13 (0.82)	0.20 (1.01)	0.25 (0.88)	0.778
Height-for-age z-score	−0.23 (1.03)	−0.11 (0.98)	0.13 (0.95)	0.138
Body mass index z-score	0.41 (1.01)	0.32 (1.00)	0.24 (0.92)	0.648
Dietary intake				
Energy				
Total kcal/day	1762 (399)[a]	1632 (465)[a]	1985 (483)[b]	<0.0001*
kcal/kg/day	60 (4.3)[a]	56 (4.4)[b]	63 (4.0)[c]	<0.0001*
Protein (%)[§§]	12.3 (2.6)[a]	16.5 (2.8)[b]	15.3 (2.8)[b]	<0.0001*
Carbohydrate (%)	57.1 (6.8)	54.2 (6.7)	55.1 (6.7)	0.066
Fat (%)	31.6 (6.3)	29.5 (6.4)	29.6 (5.8)	0.127

PKU = phenylketonuria, MHP = mild hyperphenylalaninemia.

[1]Values are means (SD) or number of observations (percentage).

[§]Significance of differences among groups (one-way analysis of variance or Kruskal-Wallis test).

[§§]Protein intake is reported as a combination of natural protein (i.e., food sources) and synthetic amino acids (i.e., phenylalanine-free protein substitutes) among subjects with PKU. Mean (SD) protein intake from food sources and from substitute was 0.5 (0.3) g/kg/day and 1.5 (0.47) g/kg/day, respectively; that is, 3.0% and 9.3% of daily energy intake.

*Statistically significant. Different superscripts indicate significant difference between 2 groups after Bonferroni correction (a vs b, $p < 0.05$; a vs c, $p < 0.05$; b vs c, $p < 0.05$).

At the end of the study, children with PKU exhibited albumin, transthyretin, and RPB within the reference range, except a 12-year-old boy receiving the conventional substitute (transthyretin concentration of 18.3 mg/dL). No overall significant difference between PKU groups was found for protein status (Table 3). The rate of protein insufficiency did not differ between children receiving the test or conventional substitute both at baseline (51.8% vs 53.6%; $p = 0.889$) and at the end of the trial (44.4% vs 50.0%; $p = 0.875$). A within-group analysis revealed an increase of transthyretin in children who received the test substitute (mean individual increase, 1.6 mg/dL; 95% confidence interval, 0.4 to 2.8 mg/dL), with an overall median changing from 19.3 to 21.0 mg/dL ($p < 0.05$) and a median individual increase of 1.5 mg/dL ($p < 0.05$). The change in albumin concentration was close to statistical significance in the test group (Table 3).

Table 2. Baseline Protein Status of Children with PKU Compared to Sex- and Age-Matched Children with MHP or Unaffected Children[1]

Variable	PKU ($n = 55$)	MHP ($n = 60$)	Unaffected ($n = 60$)	p Value[§]
Albumin (g/dL)	4.5 (0.2)[a]	4.6 (0.2)[b]	4.7 (0.2)[c]	<0.0001*
Transthyretin (mg/dL)	19.0 (6.4)[a]	22.7 (3.8)[b]	22.8 (5.2)[b]	<0.0001*
RBP (mg/L)	25.6 (9.4)[a]	31.8 (6.3)[b]	34.7 (8.7)[b]	<0.0001*
AA ratio	2.7 (0.8)[a]	2.1 (0.4)[b]	2.0 (0.3)[b]	<0.0001*

PKU = phenylketonuria, MHP = mild hyperphenylalaninemia, RBP = retinol-binding protein, AA = nonessential to essential amino acids ratio.

[1]Values are means (SD).

[§]Significance of differences among groups (one-way analysis of variance or Kruskal-Wallis test).

*Statistically significant. Different superscripts indicate significant difference between 2 groups after Bonferroni correction (a vs b, $p < 0.05$; a vs c, $p < 0.05$; b vs c, $p < 0.05$).

Table 3. Protein Status of Children with PKU Receiving Prolonged-Release or Conventional Substitute, at Baseline and at the End of the Trial

	Children with PKU		
Variable	Prolonged-Release Substitute ($n = 27$)	Conventional Substitute ($n = 28$)	p Value[§]
Albumin (g/dL)			
Baseline	4.5 (0.2)	4.5 (0.2)	0.885
End of study	4.6 (0.2)	4.5 (0.2)	0.334
p Value[§§]	0.068	0.537	
Transthyretin (mg/dL)			
Baseline	19.1 (6.4)	19.0 (6.3)	0.986
End of study	20.7 (6.8)	19.2 (6.0)	0.385
p Value[§§]	0.017*	0.350	
RBP (mg/L)			
Baseline	25.6 (10.0)	25.6 (9.5)	0.997
End of study	26.1 (10.1)	25.5 (8.7)	0.856
p Value[§§]	0.167	0.926	
AA ratio			
Baseline	2.6 (0.9)	2.6 (0.8)	0.897
End of study	2.7 (0.8)	2.6 (0.8)	0.588
p Value[§§]	0.536	0.945	

PKU = phenylketonuria, RBP = retinol-binding protein, AA = nonessential to essential amino acids ratio.

Values are means (SD).

[§]Significance of between-group difference (independent samples t test or Mann-Whitney test).

[§§]Significance of within-group difference (paired samples t test or Wilcoxon test).

*Statistically significant. Significance of difference between PKU groups with 2-way analysis of variance was $p > 0.674$ for any variable.

Plasma Phenylalanine Concentration

Mean plasma concentration of PHE (mg/dL) did not differ between children with PKU who received the test or conventional substitute either at baseline (mean [SD], median, 6.22 [5.40], 5.43 vs 5.96 [5.02], 5.52; $p = 0.887$) or at the end of the trial (4.47 [4.08], 3.70 vs 5.56 [4.92], 4.93; $p = 0.183$), but the decline in PHE concentration was significant in children who received the test substitute, with a mean (95% confidence interval) individual change of −1.75 (−3.34 to −0.16) mg/dL compared to −0.40 (−2.11 to 1.31) mg/dL in children who received the conventional substitute. The median of the individual change was −1.6 mg/dL ($p < 0.01$) and −0.63 mg/dL ($p = 0.732$) in children who received the test or conventional substitute, respectively.

Acceptability

Compliance estimated on the basis of diet diary and substitute not consumed was 81.5% and 82.1% in the prolonged-release and conventional substitute groups, respectively. The questionnaire regarding acceptability was returned completed by 48 subjects (25/27 from the test group, 23/28 from the conventional group). More than 90% of subjects in the conventional group judged the substitute acceptable for palatability, ease of preparation, and ease of ingestion. Among the respondents receiving the test substitute, 84.0% judged it as having better (72.0%) or equivalent (12.0%) palatability compared to the conventional substitute they were receiving before the trial, easier (60.0%) or equivalently easy (24.0%) to prepare, but more difficult to ingest (64%) due to difficulty in swallowing.

DISCUSSION

This is the first randomized trial that evaluated whether ingestion of a protein substitute with prolonged release may benefit the protein status of children with PKU compared to conventional substitutes. The study had adequate statistical power and additionally included for baseline comparison children with mild hyperphenylalaninemia and unaffected subjects. Overall compliance to treatment was comparable between prolonged-release and conventional substitutes. In truth, although it may be plausible that children have maintained standard attitudes during the trial, intraday compliance to the substitute was not assessed and this may be a methodological limitation. Due to this shortcoming, caution should be exercised in drawing definitive conclusions. Acceptability was reported as good enough (>90%) overall, and the dropout rate based on acceptation problems was low, despite the fact that patients with PKU may be reluctant to change their treatment. Indeed, this finding may not be fully surprising. Although the test substitute was difficult to swallow, its film-coated preparation could have made its taste more neutral and tolerable than the current substitute (72% of children receiving the test substitute judged it as having better palatability than the conventional substitute). Moreover, the study included only subjects willing to participate. Lastly, in our center a team of 2 experienced operators (1 pediatrician and 1 dietician) is currently dedicated to providing continuous assistance and encouragement to children with PKU and to their parents, to support a favorable attitude toward acceptance of substitutes and diet and possible treatment changes.

Several studies have compared the anthropometric profile of children with phenylketonuria with the healthy population, and results are controversial and not definitive [21]. In this trial, children with PKU showed a profile not statistically different from unaffected healthy controls, and this agrees with a recent longitudinal study conducted in Austria that examined children of analogous mean age [22].

Concerning the dietary intake, lower intake of protein was observed in children with PKU compared to unaffected children, as also reported in other studies [22–24]. One should note, however, that in this trial unaffected children had protein intakes about 50% higher than the one suggested by the Italian Recommended Dietary Allowance [25], and the mean protein intake in children with PKU was around 120% of recommended; that is, within the range of values (113%–129%) for which some authors have found a normal linear growth of subjects with PKU [24].

The analysis of pooled data revealed that the blood protein status was less favorable in children with PKU than in children with MPH or unaffected children, especially regarding concentration of transthyretin. Indeed, though mean albumin concentration in children with PKU was above the midpoint of the reference range and clinically just slightly lower than in children with MHP and unaffected children, transthyretin at baseline was close to the lower limit of the reference range [17], with a rate of protein insufficiency of 53%; that is, similar to values estimated in the literature, ranging from 42% [18] to 55% [26]. The relatively high rate of protein insufficiency may relate, at least in part, with the age of the children (7–16 years). Rocha et al. studied 69 treated subjects with PKU aged 1–27 years and found that all insufficient patients were younger than 15 years, when assuming as a criterion of protein insufficiency z-score value for transthyretin below the fifth percentile [26]. Other studies have investigated transthyretin in children with PKU [26–28]. In particular, Shenton et al. [27] reported lower than normal plasma transthyretin concentrations in children with PKU and suggested that low values may be indicative of secondary malnutrition. The prominent role of transthyretin (half-life of 2 days) as a sensitive and reliable indicator of protein status and its ability to warn about protein disequilibrium long before plasma albumin (half-life of about 20 days) has been recognized [26,28,29]. Interestingly, in this study, although no statistical difference was observed at the end of the trial between children who received the prolonged-release or conventional substitute in the protein status

as a whole, mean concentration of transthyretin recovered over 20 mg/dL in children administered the prolonged-release substitute, with an increase from baseline of 1.6 mg/dL. As pointed out by Arnold et al. [18] and Rocha et al. [26], it would be advisable to maintain transthyretin concentrations above 20 mg/dL in children with PKU. This result was found though neither differences between children who received the prolonged-release or conventional substitute nor any within-group change in dietary caloric or protein or PHE intake was observed throughout the trial period. Based on the above results, the beneficial effect on transthyretin of the substitute with prolonged release should not be excluded. As observed in this study, this benefit might be attributable, at least in part, to the prolonged-release formulation. In particular, ingestion of a substitute with prolonged release could contribute to regulating the protein status better than a conventional substitute and to favor a more harmonized protein synthesis, possibly due to longer retention of proteins. Lastly, although the effect size of the substitute on plasma PHE concentration was not statistically different between children with PKU receiving the test or conventional substitute, the finding that plasma PHE declined throughout the trial only in children who received the prolonged-release substitute supports that protein substitutes with prolonged release may play a positive role in the management of children with PKU.

CONCLUSION

Within the limitations of this study, one can conclude that ingestion of protein substitutes with prolonged release may be beneficial to the protein status and plasma PHE of children with PKU over 6 years of age. Longer and adequately powered longitudinal trials are needed to assess the effective value of protein substitutes with prolonged release in the advancement of management of subjects with phenylketonuria.

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Authors' Contributions

Prof. Marcello Giovannini had primary responsibility for the design of the study and contributed to writing the article. Prof. Enrica Riva participated in the development of the protocol and analytical framework for the study and contributed to writing the article. Dr. Elisabetta Salvatici had responsibility for recruitment of subjects, contributed in clinical assessments of subjects and data collection, and contributed to writing the article. Dr. Graziella Cefalo had responsibility for clinical assessments of subjects, contributed in recruitment of subjects and data collection, and contributed to writing the article. Dr. Giovanni Radaelli supervised the planning and execution of the study, performed the data analyses, contributed to writing the article, and supervised the final version. All coauthors read and approved the article as submitted here and they accept responsibility for the content of the article.

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