Normal Growth in PKU Patients Under Low-Protein Diet in a Single-Center Cross-Sectional Study

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Abstract Dietary phenylalanine restriction in phenylketonuria (PKU) patients is usually mandatory in order to prevent cognitive impairment. The influence of a low-protein diet on growth has raised concerns in families and caregivers. This paper aims to investigate the growth in PKU patients treated with a low-protein diet including supplementation of amino acids and other nutrients according to standard protocols.

We performed a single-center, cross-sectional study on growth in pediatric PKU patients ($n = 51$) treated with low-protein diet over a 20-month period. Height of healthy siblings ($n = 44$) and target height, calculated based on parents' height, served as controls.

No statistically significant differences were found comparing mean height z -scores between patients and siblings ($p = 0.261$). Patients <12 years showed a reduction in mean height z -scores compared to the target height ($p = 0.020$), whereas postpubertal patients ≥ 12 years did not differ significantly in height z -scores compared to the target height ($p = 0.071$). Healthy siblings' height did not differ from target height in neither age group ($p = 0.100$ / $p = 0.301$).

Our results suggest that PKU patients treated with low-protein diet can achieve normal growth with patients making up the leeway after puberty. While prepubertal patients were shorter than expected based on their target height, older patients were within their expected target

height. This study indicates that current practice of low-protein diet in PKU patients allows normal growth.

Introduction

Phenylketonuria (PKU) is an inborn error of L-phenylalanine (Phe) metabolism caused by an autosomal-recessive defect of the *phenylalanine hydroxylase* (PAH) gene (Blau et al. 2010). The global incidence of PKU is estimated between 1:10,000 and 1:15,000, and 1:8,000 in Europe (Blau et al. 2011; Loeber 2007; Widaman 2009).

Since phenylalanine hydroxylase (PAH) catalyzes the conversion of Phe into tyrosine, PKU leads to increased Phe levels in blood (hyperphenylalaninemia, HPA) and body tissues but low tyrosine levels. Tyrosine is a precursor of L-dopa (3,4-dihydroxy-L-phe), which is metabolized to key neurotransmitters and catecholamines (Widaman 2009; Williams et al. 2008).

PAH activity requires the presence of molecular oxygen and its cofactor tetrahydrobiopterin (BH₄), which is acting as a chaperone to prevent PAH misfolding (Scriver 2007; Thöny and Blau 2006; Williams et al. 2008). Inherited defects of BH₄ metabolism due to mutations in genes coding for BH₄ biosynthesis are less common and account for 2–3% of all HPA (Blau 2016; Thöny and Blau 2006).

Depending on the extent of increase of blood Phe concentrations, the severity of the disease can be classified: benign HPA is defined with Phe levels of 120–600 $\mu\text{mol/L}$, mild PKU with Phe levels of 600–1,200 $\mu\text{mol/L}$, and classical PKU with Phe levels $>1,200$ $\mu\text{mol/L}$ (Blau et al. 2010). There are currently a total of 1,040 PAH mutations described (<http://www.biopku.org/home/home.asp>), of which 60% are missense mutations, followed by splice

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variants and deletions (14% each) (Blau et al. 2014; Williams et al. 2008).

Appearing normal at birth, PKU patients would develop neurological complications such as microcephaly, motor dysfunction, seizures, growth failure, dystonia, and spasticity and may progress to severe mental retardation if left untreated (Blau 2016; Widaman 2009; Williams et al. 2008).

Newborn screening (NBS) programs are now introduced in many countries as an efficient tool for early diagnosis and initiation of therapy within the first days after birth. Current guidelines suggest treating patients with Phe levels exceeding 360 or 600 $\mu\text{mol/L}$ with varying intensity depending on the age of the patient and Phe elevations (Burgard et al. 2017; Diamond et al. 1997; van Spronsen et al. 2017; Vockley et al. 2014; Weglage et al. 2001).

To ensure normal growth and development as well as Phe levels within the therapeutic ranges, PKU patients require a balanced diet with careful monitoring of their nutritional intake (van Spronsen et al. 2017; Vockley et al. 2014). The two main components of PKU management are, first, dietary protein restriction and, second, special nutritional products to avoid essential amino acid or other nutrient deficiencies (Panel 2001). The aim of normal growth is at risk in the case of protein over-restriction without appropriate supplementation, as it was observed in early years of PKU treatment (Mereu 1967). In the past decades, dietary products with superior composition, availability of low-protein foods, and improved monitoring of patients aimed to closely mimicking normal nutrition and thus permitting unimpaired growth and development (Vockley et al. 2014).

Nevertheless, there have been concerns, both among affected families and healthcare professionals, that low-protein diet in PKU may still impair normal growth. To investigate this, we studied the height of PKU patients under a low-protein diet in a single metabolic center in comparison to the height of unaffected siblings and the expected target height based on their parents' height.

Material and Methods

Patient Cohort

This study was performed in PKU patients, treated at the University Children's Hospital Zurich, who were all diagnosed through NBS and treated immediately after the diagnosis was made. All patients included were treated by low-protein diet, attended periodic outpatient visits, and performed regular home monitoring of Phe levels. We excluded patients with HPA and no need for a protein restriction and those with poor compliance (defined as Phe concentrations mainly above the upper limit of the

tolerance range during the last year before start of the study).

Data were acquired during their annual routine outpatient appointment between July 2015 and January 2017.

An informed consent, signed by the patients or the legal guardians, was obtained before enrolment. This study was approved by the Ethics Committee of the University of Zurich (KEK-ZH-Nr. 2015-0281).

Control Population

The control population comprised patients' siblings and parents. Latter were included to calculate patients' expected target height. Subjects with chronic diseases with relevance to nutrition or development were excluded.

Anthropometry

Height of patients and controls was measured during outpatient appointments. If controls were absent, parents were asked to measure the actual body height and report it to the study coordinator by phone.

To optimize data quality, only three persons (study coordinator and two study nurses) performed the measurements of height. Infants and children <2 years were measured in supine position, all others standing. Body height was measured with a precision of 1 mm on the same measuring devices (horizontal or upright measuring scale). In total, measurements were performed in the outpatient clinic in all patients (100%), the majority of parents (63%), and in over a third of the siblings (36%).

Target height of patients was based on parents' heights and adapted to sex using the formula proposed by Tanner (1986).

Dietary Intake

At the time of study enrolment, all patients included followed a regularly (every 3–6 months) controlled PKU-adapted diet, which was supervised by specialized dieticians and consisted of natural protein restriction and supplementation of amino acids, trace elements, and vitamins. We did not collect details on protein intake of patients or controls.

Statistical Analysis

Height of patients, siblings, and target height were compared using z-scores based on data from the University Children's Hospital Zurich (Braegger et al. 2011).

Statistical analyses were performed using SPSS for Macintosh version 22 (SPSS Inc., Chicago, USA). Independent T-tests were performed to compare the three

groups. Results were considered significant if $p < 0.016$ after Bonferroni correction of the initial significance level of $p < 0.05$. The Kolmogorov–Smirnov test was used to analyze the distribution for continuous variables, which were presented as mean and standard deviation or as median and compared by independent T-tests. Effect sizes were calculated for all statistical analyses.

Results

Study Cohort and Control Population

During the study period, all PKU patients ($n = 56$) who attended the outpatient clinic at University Children's Hospital Zurich agreed to be enrolled. From these, five patients were excluded because of known noncompliance to the diet ($n = 3$), withdrawal from study after initial consent was given ($n = 1$), and lack of response upon contact via phone for data collection ($n = 1$), resulting in a final sample for analyses of 51 PKU patients.

Four siblings were excluded from the total number of 48 because of chronic diseases, as reported by the parents, which may have an effect on growth or development, resulting in a group of 44 siblings.

Anthropometry Findings

Characteristics of patients and controls including sex, age, height, and percentiles are shown in Table 1. No significant differences were found between patients and siblings regarding gender distribution.

Patients' mean (standard deviation) body height was 127.41 cm (± 31.36), which is significantly lower compared to siblings (144.08 cm ± 26.84) while these groups also differed regarding age (siblings: 11.57 years ± 5.17 ; patients: 8.95 years ± 5.18), as illustrated in Fig. 1.

Statistical Analysis

Patients and siblings revealed a negative height z-score resulting in a mean of $-0.29 (\pm 1.00)$ and $-0.03 (\pm 1.17)$, respectively, while target height mean height z-score was $0.17 (\pm 0.80)$ (Table 1). A comparison between height z-scores of patients, siblings, and the target height in scatterplots and boxplots is shown in Fig. 2.

No significant difference was found between the mean height z-scores of patients and siblings ($t = -1.13$, $p = 0.261$, effect size $r = 0.11$).

In contrast to the mean height z-score of siblings ($t = -0.10$, $p = 0.320$, effect size $r = 0.10$), the mean height z-score of patients showed a significant difference to the mean height z-score of target height ($t = -2.54$, $p = 0.013$, effect size $r = 0.25$). Further analyses revealed differences in age groups; while PKU patients < 12 years ($M = -0.28 \pm 0.93$, $n = 35$) differed significantly comparing mean height z-score from target height z-score ($t = -2.38$, $p = 0.020$, $r = 0.25$), this difference was not found for PKU patients ≥ 12 years ($M = -0.31 \pm 1.18$, $n = 16$) ($t = -1.84$, $p = 0.07$, effect size $r = 0.22$).

Discussion

This study aimed to examine the association of dietary therapy in PKU patients and their growth by comparing mean height z-scores of patients with those of their siblings and target heights as controls. We only evaluated patients with good metabolic control, excluding noncompliant patients. This allowed evaluation of a possible impact of dietary treatment on growth. Including siblings for comparison in this study enabled us to control the aspects of social, cultural, and economic characteristics between the groups (Grimes and Schulz 2005).

Table 1 Sex, age, height, and percentile of patients and controls

	Patients ($N = 51$)	Control 1: siblings ($N = 44$)	Control 2: target height ($N = 51$)	p_1/p_2
Sex (female)	43.1%	34.1%		0.373/0.556
Age (years), mean (SD)	8.96 (5.17)	11.66 (5.07)		0.012/–
Height (cm)				
Height, mean (SD)	127.41 (31.36)	144.08 (26.84)	171.90 (7.96)	0.007/–
Height, median [P25–P75]	135.0 [102.6–152.8]	142.5 [121.5–170.25]	174.0 [165.0–177.5]	
Height z-score University Children's Hospital, mean (SD)	$-0.29 (1.00)$	$-0.03 (1.17)$	$0.17 (0.80)$	0.261/0.013

p_1 p -value of comparison between patients and control 1: siblings, p_2 p -value of comparison between patients and control 2: target height

Fig. 1 (a) Height to age separated for male and female patients and siblings; (b) regression line of patients (full line) and siblings (dashed line), shown ages 2 until 18 years (1;1;1: R2 Linear = 0.941 2;2;2: R2 Linear = 0.912)

As a main result, we found that patients' growth did not differ significantly from their siblings. This is in accordance with other reports (Acosta et al. 2003; Belanger-Quintana and Martínez-Pardo 2011; Hoeksma et al. 2005; Huemer et al. 2007; McBurnie et al. 1991; Rocha et al. 2013). In contrast, there have been studies that showed reduced height in PKU patients (Dobbelaere et al. 2003; Verkerk et al. 1994), but considerable changes in dietary practices in recent years as well as better availability of medical foods were not yet considered in these studies (Ahring et al. 2009; MacDonald et al. 2004).

Further, we observed a significant reduction of mean height z-scores in patients <12 years compared to z-scores of their target height, and this difference is not fully explained. One reason for slower growth development in this age group might be the relatively lower intake of natural or total protein but we cannot comment on this as the detailed protein intake was not part of data collection. During puberty, patients obviously catch up in height and therefore no longer differ if ≥ 12 years of age. Siblings showed no significant difference to target height neither in pre- nor in postpubertal groups. This suggests that PKU patients may have a small delay in reaching puberty and thus in growth development. Already at birth, studies have shown a reduced body length in PKU patients, thus indicating intrauterine growth retardation (Verkerk et al. 1994; Weglage et al. 1994; Zaffanello et al. 2002). After birth, growth of children with PKU was shown to be

impaired even with early start of treatment (Dobbelaere et al. 2003; Schaefer et al. 1994). Patients born before 1980s did not show any catchup growth during puberty (Evans et al. 2016; Theile et al. 1990). In contrast, our study suggests that PKU patients under low-protein diet can realize a catchup growth during puberty.

Interpretation of our results should be made under consideration of a risk of self-reporting bias (Gorber et al. 2007; Niedhammer et al. 2000) since body height of one third of the parents and almost two thirds of siblings were based on parental report. In contrast, patients' growth data were recorded precisely as measuring took place on the same measuring device by only three different persons in a standardized manner.

Since we included 22 patients (43%) with migration background, the results of our study may be influenced by variability of the ethnic backgrounds. Different studies have shown that migration can have an impact on growth (Boas 1940; Kaplan 1954; Lasker 1952; Shapiro and Hulse 1939). This suggests that patients and siblings, who grew up together, finally show similar growth while there is a reduction in growth of prepubertal patients compared to their target height.

Another possible confounding factor is that the calculation of the target height, as based on Tanner in 1986 (Tanner 1986), should possibly be adapted, as the Western population is evolving to a stagnation of growth (Cole 2003). Therefore, the calculated target height may be an

Fig. 2 Height z-scores of patients, siblings, and target height in scatterplots. **(a)** separated by gender and boxplots; **(b)** displaying each two outliers in the sibling and in the target height group with

individual height z-scores being: ID-number (z-score): 109 (−3.34); 75 (−4.20); 154 (2.19); and 124 (−1.99)

overestimation explaining the reduction in mean height z-scores in our prepubertal patients.

In conclusion, our study indicates that normal growth can be achieved in early treated PKU patients receiving a low-protein diet. Velocity of prepubertal growth in patients with low-protein diet deserves attention in future research. Multicenter, prospective studies including more precise recording of the low-protein diet would however still be desirable to strengthen the finding from our study and to provide international reassurance for the safety of PKU diet.

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Take-Home Message

After a prepubertal delay in growth, PKU patients treated with low-protein diet can achieve normal growth similar to their sibling and in accordance with their target height.

Compliance with Ethics Guidelines

Jana Matic, Nina A. Zeltner, and Johannes Häberle declare that they have no conflict of interest.

Jana Matic and Johannes Häberle have planned the study and performed the data collection. Jana Matic, Nina A. Zeltner, and Johannes Häberle have performed data analysis. Jana Matic has prepared the first draft of the manuscript and figures, which were revised by all authors. All authors have seen and approved the final version of the manuscript.

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