High Dietary Folic Acid and High Plasma Folate in Children and Adults with Phenylketonuria

Linn Helene Stølen • Rina Lilje •
Jens Veilemand Jørgensen • Yngve Thomas Blikrud •
Runar Almaas

Received: 11 April 2013 / Revised: 21 August 2013 / Accepted: 29 August 2013 / Published online: 18 October 2013
© SSIEM and Springer-Verlag Berlin Heidelberg 2013

Abstract Background: PKU patients on a strict low protein diet get most of their folic acid intake from protein substitute. Several protein substitutes contain high amounts of this vitamin. Concern has been raised about the safety of high levels of folic acid, especially in relation to cancer risk.

Methods: This cross-sectional study included 34 children and 22 adults with PKU. A dietary interview was performed and intake of folic acid and vitamin B12 from protein substitute was calculated for patients compliant with their protein substitute. Intakes of folic acid and vitamin B12 were compared with plasma levels of folate, vitamin B12, and homocysteine.

Results: Children aged 2–9 years had the highest intake of folic acid according to RDI (449 %), and children aged 7–10 years had the highest intake of folic acid according to UL (155 %). Median plasma folate level in PKU children was two times the upper reference level and in PKU adults well above. Children between 10 and 13 years had the highest level of plasma folate. Young children had both a high intake and high plasma levels of vitamin B12. Homocysteine levels were low or in the lower part of the normal reference range in most patients.

Conclusion: Children with PKU are at a particular risk of receiving folic acid high above RDI and many children with PKU receive doses above the UL. Many PKU patients have a very high level of plasma folate related to a very high content of folic acid in many of their protein substitutes.

Introduction

Phenylketonuria (PKU) is a rare inborn error of metabolism resulting from deficient activity in the enzyme phenylalanine hydroxylase (PAH) that catalyzes the conversion of phenylalanine to tyrosine. Mainstay treatment of PKU is dietary restriction of phenylalanine and natural protein, to maintain blood phenylalanine in a nontoxic range. The diet is based on low phenylalanine foods, and natural protein is replaced by a phenylalanine-free protein substitute supplemented with tyrosine, vitamins, and other micronutrients. Supplementation of micronutrients thus seems necessary to prevent deficiencies. However, little is known about the optimal levels of micronutrients in the protein substitutes and their bioavailability.

We have observed that PKU patients, both children and adults, often have plasma folate levels above the reference range and also above cutoff levels for measurements. There are several commercial protein substitutes available containing different amounts of folic acid. For patients adhering with the low protein diet, the intake of folate from natural sources is minor compared to the amount obtained from protein substitutes (Wiig et al. 2013). The same is true for vitamin B12, as the diet allows very limited amounts of animal products (Wiig et al. 2013).

Folate is a cofactor for the synthesis of nucleic acids and required for cell division and growth. It is required for

Communicated by: Nenad Blau, PhD

Competing interests: None declared

L.H. Stølen (✉) • R. Lilje • J.V. Jørgensen • R. Almaas
Department of Paediatric Medicine, Women and Children's Division,
Oslo University Hospital, Box 4950 Nydalen, 0424, Oslo, Norway
e-mail: lstolen@ous-hf.no1

Y.T. Blikrud
Department of Medical Biochemistry, Division of Diagnostics and
Intervention, Oslo University Hospital, Oslo, Norway

R. Almaas
Department of Pediatric Research, Women and Children's Division,
Oslo University Hospital, Oslo, Norway

DNA repair processes and increases DNA stability through methylation. Low folate status in pregnant women during the first trimester is associated with increased risk of neural tube defects (Smithells et al. 1976; Pitkin 2007). Concern about the need for folic acid supplementation in pregnancy is probably the reason why some protein substitutes are supplemented with higher amounts of folic acid. While a higher dietary intake of natural folate is linked with reduced cancer risk (Duthie 2011; Figueiredo et al. 2009), there are also reports that supplementation with synthetic folic acid actually increases cancer risk (Cole et al. 2007; Wien et al. 2012; Ebbing et al. 2009; Figueiredo et al. 2009), cancer mortality, and all-cause mortality (Ebbing et al. 2009).

Both high and low blood concentrations of folate and vitamin B12 have been reported in PKU patients compared to controls (Colome et al. 2003; Huemer et al. 2008; Schulpis et al. 2002). Colomé et al. found lower levels of homocysteine (hcy) concurrent with higher concentrations of folate and vitamin B12 (Colome et al. 2003), while Schulpis et al. found higher hcy levels and lower folate and vitamin B12 concentrations compared to controls (Huemer et al. 2008; Schulpis et al. 2002).

We wanted to compare intake of folic acid and vitamin B12 with plasma levels in children, adolescents, and adults with PKU using different types of protein substitutes to identify whether certain groups of PKU patients are at special risk of receiving either too much or too little folic acid. In particular, we wanted to address whether certain age groups are at special risk as vitamin requirements and intake levels of protein substitute are highly age dependent.

Subjects and Methods

The study protocol was approved by the institutional review board at Oslo University Hospital.

Subjects

Children below 18 years ($n = 34$) and adults ($n = 22$) with PKU coming for annual follow-up at Oslo University Hospital between June 2010 and June 2011 were included in this cross-sectional study.

This population includes 23 % of all patients diagnosed with PKU in Norway whereof seven were late-diagnosed patients. Patients on a phenylalanine-restricted diet compliant with their protein substitutes were included. Patients not on diet, on large neutral amino acid treatment, BH4-treatment, or taking supplements containing folic acid or vitamin B12 were excluded. Patients were classified as having classical PKU (Phe < 1,200 $\mu\text{mol/L}$), mild PKU (Phe 600–1,200 $\mu\text{mol/L}$), or mild hyperphenylalaninemia (MHPA) (Phe < 600 $\mu\text{mol/L}$) primarily based on pretreatment

serum/blood phenylalanine concentrations. In Norway, MHPA patients with phenylalanine > 400 $\mu\text{mol/L}$ are treated.

Calculation of Intake

Patients were interviewed about their habitual diet by a metabolic dietitian. Intake of folic acid, vitamin B12, and protein equivalents from protein substitute was calculated for all patients.

Patients used protein substitutes from SHS (XP Maxamaid, XP Maxamum, Lophlex/Lophlex LQ, PKMax, Anamix Junior), VitaFlo (PKU gel, PKU Express, PKU Cooler), and PreKUlab (Avonil). Protein substitutes were categorized into two groups: high folic acid (HighFA) protein substitutes with folic acid of 9.57–12.44 $\mu\text{g/g}$ protein (XP Maxamaid, XP Maxamum, Lophlex/Lophlex LQ, PKMax) and lower folic acid (LowerFA) protein substitutes with folic acid of 3.1–6.7 $\mu\text{g/g}$ protein (PKU gel, PKU Express, PKU Cooler, Anamix Junior, and Avonil).

Three to four days weighed dietary registrations were reviewed for a subgroup of children and adults. Folate intake from foods and intake of natural protein were calculated with “Mat på Data 5.1,” a Norwegian food analysis program that uses data from the Norwegian Food Composition Database 2006 (The Norwegian Food Safety Authority and The Norwegian Directorate of Health & The University of Oslo 2006).

Blood Measurements

Blood samples were drawn fasting in the morning or at least 4 h fasting to measure plasma folate, vitamin B12, and total hcy at the time of the annual visit. Median serum phenylalanine levels from the last year were calculated.

Plasma folate was measured using the Roche Elecsys Folate III immunometric assay with a fully automated Roche Modular Analytics E170 immunoassay analyzer. Measuring range: 1.45–45.4 nmol/L. CV ≤ 10 % at 5 nmol/L, ≤ 7 % at 15.5 and 24 nmol/L. Samples with values above 45.4 nmol/L were diluted as described in the Roche manual 1:2 with Elecsys Diluent Universal. Plasma vitamin B12 was measured using the Roche Elecsys Vitamin B12 immunometric assay (Roche Modular Analytics E170). Measuring range: 22–1,476 pmol/L. CV ≤ 8 % at 146 pmol/L, ≤ 7 % at 428 pmol/L. Plasma total hcy was measured by an enzymatic assay using the Liquid Stable (LS) 2-Part Homocysteine Reagent with a fully automated Roche Modular P analyzer. Measuring range: 1–50 $\mu\text{mol/L}$. CV ≤ 5 % at 13.0 $\mu\text{mol/L}$, ≤ 4.5 % at 45.5 $\mu\text{mol/L}$.

Serum phenylalanine was determined at the National Neonatal Screening Unit using tandem mass spectrometry.

Table 1 Artificial and natural protein intake according to recommendations in different age groups

	2–6 years	7–13 years	14–17 years	Adults
<i>N</i> =	10	15	13	22
Recommended total protein intake (g/kg/day)	2.0	1.5	1.0	1.0
Protein intake from substitute (g/day)	38.9 (33.6–52.5)	54 (31.5–80.6)	69.8 (59.8–90)	60 (41–81.4)
Protein intake from substitute (g/kg/day)	1.9 (1.4–2.4)	1.5 (0.8–2.6)	1.1 (0.7–1.4)	0.88 (0.47–1.6) *
Total protein intake (g/kg/day)**	2.2 (1.8–2.7)	1.8 (1.0–2.9)	1.2 (0.9–1.6)	NA

**N* = 21. Protein intake is per actual weight, not corrected for overweight

Total protein intake is calculated from protein substitute and habitual daily phenylalanine exchanges. Total protein recommendations in PKU centers in Europe (g protein/kg/day) are **1–3 years: 2.5–3 g; **4–10 years:** 1.5–2 g; **11–16 years:** 1–1.5 g; **> 16 years:** 1 g

Table 2 Plasma levels of folate, vitamin B12, and homocysteine according to reference levels

	Children	Adults	Reference level
<i>N</i> =	34	22	
Plasma folate (nmol/L)	63.5 (17–123)	45 (19–73.8)	11–30
Plasma B12 (pmol/L)	677 (376–2,280)	490 (246–838)	160–600
Plasma hcy (μmol/L)	4 (2–8)*	6 (4–10)	5–13 (♀), 6–16 (♂)

Hcy homocysteine

**N* = 32

Statistical Analysis

Data are given as median (range). Mann–Whitney U test was used for comparison between groups and the Spearman test for correlations. Kruskal–Wallis test with Dunn’s Multiple Comparison test was used for comparison between more than two groups. A two-tailed $p < 0.05$ was considered statistically significant. Statistical analyses were performed with the software PASW Statistics 18 and with GraphPad InStat version 03.10 for Windows (GraphPad Software Inc, San Diego, California).

Results

Patient Characteristics

A total of 34 children (18 males, 16 females) with a median (range) age of 9.9 (4.1–17.7) years and 22 adults (9 males, 13 females) with a median (range) age of 32 (18–57) years were included. Forty-six percent of the children had classical PKU, 37 % had mild PKU, and 17 % had MHPA. Among the adults, 59 % had classical PKU, 36 % had mild PKU, and 5 % had MHPA. All patients were treated with a phenylalanine-restricted diet and were compliant with a tyrosine-, vitamin-, and other micronutrient-enriched protein substitutes. Median (range) serum phenylalanine during the

last year was 423 μmol/L (217–1,085) in children and 567 μmol/L (262–1,441) in adults. Table 1 shows protein intake from protein substitute according to recommendations.

Plasma Levels of Folate, Vitamin B12, and Homocysteine

Median plasma folate level in PKU children was more than two times the upper reference level and in PKU adults well above the upper reference level (Table 2). Ninety-one percent of children and 73 % of adults had plasma folate above the upper reference level. None had low plasma levels. Median plasma B12 level was slightly above the upper reference level in children and within range in adults. Fifty-three percent of children and 23 % of adults had plasma B12 levels above the upper reference level. Median plasma hcy was below the reference range in children and at the lower reference range in adults. Sixty-eight percent of children and 18 % of adults had plasma hcy below the reference range. None of the adults had plasma hcy above the reference range.

Intake of Folic Acid from Protein Substitute, Plasma Folate, and Homocysteine

Intake of folic acid from protein substitute was calculated for patients compliant with their protein substitute. Ninety-four percent of the children and 73 % of the adult patients had

Table 3 Intake of folic acid from protein substitute in relation to RDI, plasma folate, and homocysteine levels in different age groups

	2–5 years	6–9 years	10–13 years	14–17 years	Adults/men	Adults/women
<i>N</i> =	6	12	7	9	9	13
RDI*	80	130	200	300	300	400
Folic acid (µg/d)	359 (104–500)	571 (117–747)	700 (120–1,004)	747 (400–1,063)	750 (350–999)	525 (192–795)
Folic acid (% of RDI)	449 (130–625)	439 (100–575)	350 (60–502)	249 (133–354)	250 (117–333)	131 (48–199)
Plasma folate (nmol/L)	67 (28–73)	58 (38–67)	73 (17–123)	66 (30–78)	55 (29–66)	44 (19–74)
<ref level	0	0	0	0	0	0
>ref level	6	12	6	8	7	9
Plasma hcy (µmol/L)	4 (2–5)	4 (3–5)*	5 (3–6)**	5 (4–8)	7 (4–10)	6 (4–10)

RDI Recommended daily intake of folate in Nordic Nutrition Recommendations 2004, *Hcy* homocysteine

**N* = 11

***N* = 6

Table 4 Intake of folic acid from protein substitute in relation to Tolerable Upper Intake Level (UL) in different age groups

	4–6 years	7–10 years	11–14 years	15–17 years	Adults
<i>N</i> =	10	10	10	4	22
UL	300	400	600	800	1,000
Folic acid (µg/day)	300 (104–533)	618 (120–747)	774 (203–1,004)	484 (400–1,063)	660 (192–999)
Folic acid (% of UL)	100 (35–178)	155 (30–187)	129 (34–167)	60 (50–133)	66 (19–99.9)
% > UL	50	60	80	10	0

UL Tolerable Upper Intake Level of folic acid according to the European Commission's Scientific Committee on Food 2000, % > *UL* percent of patients with intake of folic acid from protein substitute above UL

intake of folic acid above the recommended daily intake (RDI) (Nordic Council of Ministers 2004a) (Table 3). One child (3 %) and three adults (14 %) had intakes of folic acid below the RDI. The three adults were using protein substitute tablets containing 4 µg folic acid/g protein. However, none of them had low plasma folate. Children aged 2–13 years had a median intake of folic acid 3.5–4.5 times higher than the RDI (Table 3). Fifty-nine percent of the children, but none of the adult patients, had intakes above the Tolerable Upper Intake Level (UL) (SCF 2000) (Table 4). Half of the children between 4 and 10 years and 80 % of children between 11 and 14 years had intakes of folic acid above the UL (Table 4).

All patients aged 2–13 years had plasma hcy below or at the lower reference range (Table 3). Adolescents between 14 and 17 years had plasma hcy below or in the lower reference range. Adults had plasma hcy slightly below or in the lower/middle reference range.

Folate Intake from Foods and Natural Protein Intake

Weighed dietary registrations in a subgroup of 7 children and 9 adults with a median (range) age of 14 (7–15) years

and 31 (19–47) years showed a median (range) folate intake of 113 (94–202) µg folate/day among children and 177 (98–349) µg folate/day for adults. Natural protein intake was median (range) 12.8 (7.6–31.2) g/day for children and 34.8 (9.9–40.1) g/day for adults.

Plasma Levels of Folate According to Intake

High correlations between intake of folic acid from protein substitutes and plasma folate were found in both children ($r_s = 0.643$, $p < 0.001$, $n = 34$) and adults ($r_s = 0.77$, $p < 0.001$, $n = 26$).

Intake of Vitamin B12 from Protein Substitutes and Plasma Vitamin B12

Children 2–9 years of age had a median vitamin B12 intake of 358 % of the RDI (Table 5). Median intakes of vitamin B12 in children 10–17 years of age and adults were 283 and 270 % of the RDI (Nordic Council of Ministers 2004b). In the youngest age group, median plasma vitamin B12 was 25 % above the upper reference level, and 72 % had plasma vitamin B12 above the upper reference level. In older

Table 5 Intake of vitamin B12 from protein substitute in relation to RDI and plasma vitamin B12 in different age groups

	2–9 years	10–17 years	18 + years
<i>N</i> =	18	16	22
RDI	0.8–1.3	2	2
Vitamin B12 (µg/day)	4.5 (1.6–6.5)	5.7 (1.3–8.0)	5.4 (3.6–7.2)
Vitamin B12 (% of RDI)	358 (154–813)	283 (65–400)	270 (180–360)
Plasma vitamin B12 (pmol/L)*	823 (457–2,280)	596 (376–1,212)	490 (246–838)
< ref level	0	0	0

RDI Recommended daily intake of vitamin B12 in Nordic Nutrition Recommendations 2004

*Kruskal-Wallis test with Dunn's Multiple Comparison test showed significantly different plasma levels between children aged 2–9 years and adults ($p < 0.001$)

Table 6 Differences in intake of folic acid and vitamin B12 and plasma levels according to type of protein substitute used

	Children			Adults		
	HighFA protein substitute	LowerFA protein substitute	<i>p</i> -value*	HighFA protein substitute	LowerFA protein substitute	<i>p</i> -value*
<i>N</i> =	21	13		14	8	
Folic acid (µg/day)	700 (358–1,063)	203 (104–560)	<0.001	750 (500–999)	375 (192–450)	<0.001
Folic acid (% of RDI)	448 (249–625)	133 (60–303)	<0.001	194 (131–333)	100 (48–150)	<0.001
Plasma folate (nmol/L)	69 (44–123)	41 (17–73)	<0.001	57 (41–74)	29 (19–53)	<0.001
Vitamin B12 (µg/day)	5.4 (3.6–8.0)	2.6 (1.6–6.6)	<0.01	5.4 (3.6–7.2)	5.1 (4.2–6.8)	1.000
Plasma vitamin B12 (pmol/L)	750 (415–2,280)	611 (376–1,469)	0.193	552 (299–838)	419 (246–533)	<0.05
Plasma hcy (µmol/L)	4 (3–6)	5 (2–8)**	0.088	6 (4–10)	6.5 (4–10)	0.482
Serum phe last year (µmol/L)	423 (324–1,068)	433 (217–879)	0.753	572 (262–1,127)	532 (385–1,441)	0.868
Age (years)	9.8 (4.1–15.7)	10 (4.1–17.7)	0.944	31.5 (18–46)	34 (19–57)	0.525

RDI Recommended daily intake, *Hcy* homocysteine, *Phe* phenylalanine

*Mann–Whitney U test,

***N* = 11

children and in adults, median plasma vitamin B12 was in the upper reference range. Thirty-one percent of older children and 23 % of adults had plasma vitamin B12 above the upper reference level. None had low plasma levels of vitamin B12. Plasma vitamin B12 levels were significantly higher among 2–9-year-old children than among adults ($p < 0.001$).

Plasma Levels of Vitamin B12 According to Intake

No correlations were found between intake of vitamin B12 from protein substitute and plasma B12 in children ($rs = -0.063$, $p = 0.723$, $n = 34$) or adults ($rs = 0.104$, $p = 0.647$, $n = 22$).

Differences According to Type of Protein Substitute Used

Both children and adults using HighFA protein substitutes had a significantly higher intake of folic acid (both in absolute levels and in percentage of recommendation) and plasma folate levels than children and adults using LowerFA protein substitutes (Table 6). Children using HighFA protein substitutes had a significantly higher intake of vitamin B12 than children using LowerFA protein substitutes ($p < 0.01$), but plasma levels of vitamin B12 did not differ between the groups. Plasma B12 was significantly higher in adults using HighFA protein substitutes ($p < 0.05$). There was a tendency to slightly higher plasma hcy in children using LowerFA substitutes

($p = 0.088$), but there was no difference among adults. Besides, there was no difference in median serum phenylalanine between the groups during the last year.

Discussion

The main finding of this study was that many patients with PKU have very high levels of plasma folate related to a very high content of folic acid in their protein substitutes. Children with PKU are at a particular risk of receiving too much folic acid and many patients receive doses above the UL. Young children had both high intake and high plasma levels of vitamin B12. Hcy levels were low or in the lower part of the reference range in most patients.

Our findings are in accordance with other studies reporting about twice as high folate levels in PKU children and young adults than in controls (Colome et al. 2003; Huemer et al. 2008). A recent report of 19 Norwegian late-treated adults found high intakes of folic acid and corresponding high blood folate concentrations (Wiig et al. 2013). Only one PKU center in Greece reported of lower folate levels in PKU children with good compliance compared to PKU children with less adherence to diet and to controls (Schulpis et al. 2002). Intake of folate among the patients with good compliance did not meet reference nutrient intakes for folate (Schulpis et al. 2002). Even though we tend to give some more total protein than our actual recommendations as shown in Table 1, the protein intake of our PKU children is within the range of other European PKU centers (Ahrling et al. 2009; van Spronsen et al. 2009).

The folic acid content of different protein substitutes varies greatly. The protein substitutes used by our patients ranged from 3.1 to 9.6 µg folic acid/g protein for PKU children aged 6 months or 1 year up until 8–10 years. Protein substitutes suitable from 3 to 4 years or from 8 years of age ranged from 6.7 to 12.4 µg folic acid/g protein. Children often change to an adult protein substitute from age 8 to 10 years, some even from 3 to 4 years. Protein needs are still higher than in adults at this age, but with a HighFA substitute, it gives rise to very high intakes of folic acid.

A limitation of the study is that intake of folate from natural sources, folic acid from supplemented low protein flour and natural protein intake was available for only a subgroup of the patients. Natural protein intake was 270 % higher for adults than children, while folate was only 57 % higher. We also found high correlations between intake of folic acid from protein substitutes and plasma folate, indicating that protein substitute is the main source of folic acid. Another study of 19 Norwegian late-treated adults found that about 85 % of dietary folate equivalents (DFEs)

originated from folic acid in the protein substitute. The remaining 15 % were from specially manufactured low protein foods, vegetables, fruits, and small amounts of dairy products (Wiig et al. 2013).

According to European recommendations, there is no evidence for risk associated with high intakes of folates from natural sources, but a high intake of folic acid from supplements may mask hematological symptoms caused by deficiency of vitamin B12 (SCF 2000). The risk of this is low in our patients compliant with protein substitute since they have good or high vitamin B12 status. However, little is known about the fate and effects of unmetabolized folic acid from high doses of folic acid supplements (Smith et al. 2008). A trial in which adults were supplemented with 800 µg folic acid daily showed that medium serum folate increased from 8.8 to 62.3 nmol/L. Hazard ratios for cancer incidence, cancer mortality, and all-cause mortality were significantly higher among subjects with serum folate > 62.7 nmol/L than among subjects with serum folate between 8.6 and 23.9 nmol/L (Ebbing et al. 2009). A recent systematic review and meta-analysis found a borderline significant increase in overall cancer frequency and increased risk of prostate cancer with folic acid supplementation (Wien et al. 2012). Folic acid suppresses tumor development and progression if supplementation is given before the establishment of neoplastic lesions. However, folic acid administration stimulates tumor progression and growth of already established preneoplastic lesions (Smith et al. 2008; Ulrich and Potter 2007). Whether high intakes of folic acid in PKU patients are beneficial or have adverse long-term effects is yet unknown, but the levels are well above the recommendations. Late-diagnosed PKU patients commencing dietary treatment at older age could have precancerous lesions with tumor progression affected by high intakes of folic acid.

Intakes of vitamin B12 were also high in our study. Children 2–9 years of age had the highest intake of vitamin B12, about 3.5 times higher than recommended. About 70% of patients in this group had plasma vitamin B12 levels above the upper reference range, while this was the case for only around 25% of older children and adults. There was no correlation between intake of vitamin B12 and plasma levels. A strict low protein diet contains little or no animal products and few sources of vitamin B12. PKU patients, following a more liberal diet, have more natural sources of vitamin B12 in the diet. Two studies both found significantly higher serum/plasma levels of vitamin B12 in PKU children and young adults than in controls (Colome et al. 2003; Huemer et al. 2008). On the other hand, Schulpis et al. found lower intakes and lower vitamin B12 levels in PKU children with good dietary compliance than in PKU patients with less compliance and controls (Schulpis et al. 2002), probably because of lower vitamin

B12 concentrations in the protein substitute. Functional vitamin B12 deficiency can exist despite levels within the reference range, and it is recommended to evaluate vitamin B12 levels against methylmalonic acid or hcy levels (Vugteveen et al. 2011). There is no reported danger related to high intake of vitamin B12, but the composition of the protein substitute should probably be adjusted to more age-appropriate levels.

Conclusion

A high content of folic acid in several protein substitutes for PKU patients results in high plasma folate levels and low plasma hcy levels. Children and adolescents with PKU are at a particular risk of receiving folic acid high above the RDI and many children with PKU receive doses above the UL. None of the patients had low plasma folate levels. The same protein substitutes are recommended for adults and children from 8 to 10 years, sometimes from 3 to 4 years. Folic acid content should be reduced in several protein substitutes available, and the supplemented levels of folic acid and vitamin B12 should have a more age-adjusted profile.

Acknowledgments The authors would like to thank the National Neonatal Screening Unit for the analysis of serum phenylalanine. We would also like to thank Cathrine Åkre Strandskogen for help with revising the manuscript.

Take-Home Message

Several protein substitutes for PKU contain high levels of folic acid giving rise to intakes above European Tolerable Upper Intake Levels and high plasma folate levels. Children are at a particular risk.

Contributions of Individual Authors

Linn Helene Stølen: conception and design of the study, collection of data, analysis of data and interpretation of data, drafting the article

Rina Lilje: conception and design of the study, collection and interpretation of data, revision of the manuscript

Jens Veilemand Jørgensen: contributed in discussion of design, acquisition of data, and revision of the manuscript

Yngve Thomas Blikrud: contributed in discussion of design, responsible for biochemical analyses, participated in writing and revision of the manuscript

Runar Almaas: conception and design of the study, interpretation of data, participated in writing and revision of manuscript

Guarantor: Linn Helene Stølen

Compliance with Ethics Guidelines

Conflict of Interest

Linn Helene Stølen has received travel grants/accommodation/meeting expenses from Nutricia and Vitaflo.

Rina Lilje has received travel grants/accommodation/meeting expenses from Nutricia and Vitaflo.

Jens V. Jørgensen has received travel grants/accommodation/meeting expenses from Nutricia.

Yngve Thomas Blikrud has received travel grants/accommodation/meeting expenses from Nutricia.

Runar Almaas has received travel grants/accommodation/meeting expenses from Nutricia.

The project has been initiated and performed by the authors. Nutricia and Vitaflo have not been involved in the project neither with respect to initiation, funding, organization of the study, interpretation of data, nor writing of the paper. Nutricia and Vitaflo have not read the submitted paper.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was not required from the institutional review board at the time of the study, since the study included only routine data.

References

- Ahring K, Belanger-Quintana A, Dokoupil K et al (2009) Dietary management practices in phenylketonuria across European centres. *Clin Nutr* 28:231–236
- Cole BF, Baron JA, Sandler RS et al (2007) Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. *JAMA* 297:2351–2359
- Colome C, Artuch R, Sierra C et al (2003) Plasma thiols and their determinants in phenylketonuria. *Eur J Clin Nutr* 57:964–968
- Duthie SJ (2011) Folate and cancer: how DNA damage, repair and methylation impact on colon carcinogenesis. *J Inherit Metab Dis* 34:101–109
- Ebbing M, Bonaa KH, Nygard O et al (2009) Cancer incidence and mortality after treatment with folic acid and vitamin B12. *JAMA* 302:2119–2126
- Figueiredo JC, Grau MV, Haile RW et al (2009) Folic acid and risk of prostate cancer: results from a randomized clinical trial. *J Natl Cancer Inst* 101:432–435
- Huemer M, Fodinger M, Bodamer OA et al (2008) Total homocysteine, B-vitamins and genetic polymorphisms in patients with classical phenylketonuria. *Mol Genet Metab* 94:46–51
- Nordic Council of Ministers (2004a) Folate. In *Nordic Nutrition Recommendations 2004 – Integrating nutrition and physical activity*. Norden, Copenhagen, pp 287–293

- Nordic Council of Ministers (2004b) Vitamin B12. In Nordic Nutrition Recommendations 2004 – Integrating nutrition and physical activity. Norden, Copenhagen, pp 297–300
- Pitkin RM (2007) Folate and neural tube defects. *Am J Clin Nutr* 85:285S–288S
- SCF (2000) Opinion of the Scientific Committee on Food on the tolerable upper intake level of folate. pp 1–13. 19-10-2000. European Commission. Ref Type: Report
- Schulpis KH, Karikas GA, Papakonstantinou E (2002) Homocysteine and other vascular risk factors in patients with phenylketonuria on a diet. *Acta Paediatr* 91:905–909
- Smith AD, Kim YI, Refsum H (2008) Is folic acid good for everyone? *Am J Clin Nutr* 87:517–533
- Smithells RW, Sheppard S, Schorah CJ (1976) Vitamin deficiencies and neural tube defects. *Arch Dis Child* 51:944–950
- The Norwegian Food Safety Authority and The Norwegian Directorate of Health & The University of Oslo (2006) Norwegian Food Composition Database 2006. <http://www.matportalen.no/verktoy/Matvaretabellen/article9866.ece/BINARY/Matvaretabellen+%282006%29+-+hele+tabellen> . Ref Type: Electronic Citation
- Ulrich CM, Potter JD (2007) Folate and cancer—timing is everything. *JAMA* 297:2408–2409
- van Spronsen FJ, Ahring KK, Gizewska M (2009) PKU—what is daily practice in various centres in Europe? Data from a questionnaire by the scientific advisory committee of the European Society of Phenylketonuria and Allied Disorders. *J Inher Metab Dis* 32:58–64
- Vugteveen I, Hoeksma M, Monsen AL et al (2011) Serum vitamin B12 concentrations within reference values do not exclude functional vitamin B12 deficiency in PKU patients of various ages. *Mol Genet Metab* 102:13–17
- Wien TN, Pike E, Wisloff T, Staff A, Smeland S, Klemp M (2012) Cancer risk with folic acid supplements: a systematic review and meta-analysis. *BMJ Open* 2:e000653
- Wiig I, Motzfeldt K, Bjørge Løken E, Kase BE (2013) Nutritional consequences of adhering to a low phenylalanine diet for late-treated adults with PKU. *JIMD Reports* 7:109–116