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Keywords

Phenylketonuria · Micronutrients · Essential fatty acids · Bone mineral density · Phenylalanine · Osteopenia · Osteoporosis

Abstract

Introduction: In phenylketonuria (PKU), a natural protein-restricted dietary treatment prevents severe cognitive impairment. Nutrient deficiencies may occur due to strict diet. This study is aimed at evaluating the dietary intake and blood concentrations of micronutrients and essential fatty acids (FA), bone mineral density (BMD) and fracture history in patients on long-term dietary treatment. ***Methods:*** Sixty early diagnosed Dutch patients (aged 1–39 years) were included in a multi-center cross-sectional study. Their dietary intake, blood concentrations of micronutrients, FA, fracture history and BMD were assessed. ***Results:*** Selenium dietary intake and serum concentrations were low in 14 and 46% of patients, respectively. The serum 25-OH vitamin D2 + D3 concentration was low in 14% of patients while 20% of patients had a low vitamin D intake. Zinc serum concentrations were below normal in 14% of patients, despite adequate intake. Folic acid serum concentrations and intake were elevated. Despite safe total protein and fat intake, arginine plasma concentrations and erythrocyte eicosapentaenoic acid were below reference values in 19 and 6% of patients, respectively. Low BMD (Z-score <–2) was slightly more prevalent in patients, but the lifetime fracture prevalence was comparable to the general population. ***Conclusions:*** Dutch patients with PKU on long-term dietary treatment have a near normal nutrient status. Supplementation of micronutrients of which deficiency may be deleterious (e.g., vitamin D and selenium) should be considered. BMD warrants further investigation.

Introduction

Phenylketonuria (PKU; MIM 261600) is an autosomal recessive disorder of phenylalanine (Phe) metabolism caused by a deficiency of the enzyme phenylalanine

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hydroxylase (PAH; EC 1.14.16.1), leading to severe cognitive impairment due to the accumulation of Phe in the brain. With newborn screening and the early institution of dietary treatment, cognitive impairment caused by PKU has nearly been eliminated in developed countries [1]. Based on the level of blood Phe at diagnosis, disease severity is classified as either classic or severe PKU (≥1,200 μmol/L), mild to moderate PKU (600–1,200 μmol/L), or mild hyperphenylalaninemia (360–600 μmol/L). Treatment consists of restriction in dietary Phe intake (an essential amino acid [AA]) through a diet low in natural protein to achieve safe Phe blood concentrations (age below 12 years <360 μmol/L, age ≥12 years <600 μmol/L, during pregnancy <240 μmol/L) [2]. Severely affected patients tolerate <500 mg of Phe, which is <10 g of natural protein per day [3, 4]. To guarantee a sufficient intake of daily protein, patients use designated Phe-free amino acid mixtures (AAM) containing most AA and other micronutrients. Different AAM are available, all with variable compositions. In some, the amount of added nutrients is calculated based on the needed daily calories, while in others advised intakes of protein/kilogram bodyweight is used to assess appropriate nutrient composition [5]. Studies evaluating intake and deficiencies of nutrients in PKU reported variable nutrient intakes and blood concentrations [6–8]. A minority of patients is responsive to a recently available (2009) treatment with tetrahydrobiopterin (BH4), a cofactor of PAH. BH4 increases the dietary Phe tolerance and thus permits diet relaxation [9]. Deficiencies have been reported in responsive patients on a relaxed diet, who might experience difficulties making food choices after years of strict dieting [7].

To optimize treatment, and to prevent deficiencies or potential toxic concentrations of micronutrients, there is a need for more insight into the nutrient intake and blood concentrations in PKU [5].

Furthermore, a decreased bone mineral density (BMD) has been frequently reported in PKU patients on treatment, and our meta-analysis demonstrated a slight decrease in BMD Z-score in PKU patients [10]. One theory is that this might be due to micronutrient deficiencies.

Our main objective was to evaluate the intake of micronutrients and essential fatty acids (FA) from natural protein-containing food and AAM in patients with PKU, and to investigate the association between intake and blood concentrations of these nutrients. The secondary objectives were to investigate BMD and fracture history, and their associations with blood concentrations and intake of micronutrients and essential FA.

Methods

Study Design

This cross-sectional multicenter study was performed in 3 Dutch metabolic centers (Academic Medical Center Amsterdam [n = 43], Maastricht University Medical Center [n = 7] and Groningen University Medical Center [n = 10]) between May 2013 and May 2014. The inclusion criteria were: PKU diagnosed through newborn screening; age ≥1 year; continuous treatment with a protein-restricted diet using an AAM, a protein-restricted diet with AAM in combination with BH4 treatment or BH4 treatment without dietary protein restriction. While the exclusion criteria were: changes in AAM within a month before inclusion and (planned) pregnancy. The study protocol was approved by the Ethics Committee of the AMC and patients/parents provided informed consent before participation.

Micronutrients and essential FA were assessed in blood after >3 h fasting. An investigator-designed-questionnaire (online suppl. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000465529) was used to evaluate daily dietary intakes, fracture history and amount of physical activity (sports, walking and cycling in the past year). Medication (including BH4) and dietary intake of AAM/natural diet and supplementary vitamins/minerals were assessed. Patient reported intake was compared with the most recent dietary prescription from the treating dietician [11]. Patient records were studied to obtain Phe concentrations from dried blood spots over the last 12 months, and BMD Z-scores from dual-energy X-ray absorptiometry scans (DXA) performed between 2 years before to 6 months after inclusion.

A sample size calculation was performed to determine the minimal number of patients, to detect differences of at least 1 SD between patients and normal values in serum levels of selenium, plasma and erythrocyte levels of docosahexaenoic acid (DHA) and BMD. The minimum number of patients to be included resulted in 7 patients per age-group (to reach significance with type I error rate of 0.01 and type II error rate of 0.20 and based on a desired power of the study of 80%).

Laboratory Measurements

Chemical analyses of micronutrients were performed at one particular clinical chemical laboratory for serum albumin, calcium, phosphate, magnesium, sodium, potassium, transferrin, selenium, zinc, folic acid, 25-OH vitamin D2 + D3, vitamin B12, and whole blood vitamin B1 and B6, and for erythrocyte FA. We compared the patient data with the reference ranges provided by the laboratory. These reference values are based on large groups of control patients and have been validated by our lab. Plasma AA was assessed at the medical center where the patient was under treatment because all laboratories participate in a quality control system for AA measurements.

Detailed information about all laboratory analyses is available in the online supplementary material (online suppl. 2).

Bone Mass Density Measurements

Patient BMD outcomes were compared to BMD reference data from the general Dutch population using Hologic Discovery Imaging equipment. Details on the used reference data for children are presented in an article by van der Sluis et al. [12], who examined 444 subjects aged 4–20 years. According to the International

Table 1. Patient characteristics

Age, years	Frequency, *n* (%)	Age, years, median (IQR)	Male, *n* (%)	BMD, Z-score (SD)
Population				
All	60 (100)	13.0 (6–17)	25 (41.7)	0.45 (–0.18 to 1.24)
1–11	25 (41.7)	6.0 (4.5–9)	10 (40)	0.45 (0.08 to 0.96)
12–17	20 (33.3)	15 (13.3–15.8)	10 (50)	0.42 (–0.49 to 1.06)
18–39	15 (25)	29 (20.8–35.8)	5 (33.3)	0.49 (–0.40 to 2.65)

Age, years	*n*	Total protein intake, median (IQR)	Protein natural sources, median (IQR)	*n*	Protein amino-acid supplement, median (IQR)	BH4 use, *n* (%)
Protein intake, g/day						
All	59	64.8 (43.1–76.6)	14.6 (10.1–26.4)	55	45.00 (25.2–62.2)	14 (24)
1–11	24	39.7 (34.5–58.1)	10.8 (7.9–25.4)	23	25.2 (15.2–39.0)	7 (28)
12–17	20	74.7 (63.9–83.5)	16.3 (12.0–27.3)	19	60.0 (40.0–63.0)	5 (25)
18–39	15	81.3 (72.5–86.1)	19.0 (12.5–28.2)	13	60 (50.5–71.7)	2 (14)

Age, years	*n*	Plasma Phe values, μmol/L, median (IQR)	Percentage of bloodspot Phe values above range per patient, median (range)
Phenylalanine			
1–11	24	302 (193–342)	33 (0–67.5)
12–17	20	611 (401–778)	57 (0–100)
18–39	15	804 (522–978)	60 (0–100)

BMD, bone mass density; IQR, interquartile range; Phe, phenylalanine.

Society for Clinical Densitometry (ISCD), a diagnosis of osteoporosis in children, men and premenopausal women is based on a BMD Z-score <–2 coupled with a significant fracture history [13].

Dietary Intake and Growth Parameters

Weight/height were collected, and age/gender appropriate Z-scores were calculated based on Dutch population references [14]. Based on the answers provided by patients to the questionnaire, the daily intake of micronutrients, protein and fat was calculated manually. The questionnaire was orally assessed during a routine clinic visit. Results were compared to the recommended daily dietary allowance [15], and the safe advised range (SAR) of intake as recommended by the European Food Safety Authority [16]. The recommended dietary allowance is based on ±2 SD of the required dietary intake and thus provides sufficient intakes for 97.5% of the general population [17].

Statistical Analysis

For all analyses, the Statistical Package for Social Sciences Windows version 19 was used. Descriptive statistics were used to assess intakes and blood concentrations of micronutrients, AA, FA and BMD Z-scores. Concerning laboratory concentrations and dietary intake, we decided that concentrations/amounts outside of the reference range are of interest to study (remarkable). The reference range provides mean ± 2 SD for a healthy age-matched population; hence, remarkable results represent concentrations below 2.5% or above 97.5% of normal. Results are reported as median values and the range has been specified; data are shown separated in age groups equal to available age groups used to indicate reference ranges. In addition, Mann–Whitney U (not normally distributed continuous variables) or chi-square tests (nominal data) were conducted to test differences between patients based on disease severity, BH4 and supplement use (not AAM). Because most data demonstrated a non-normal distribution, those data distributed normally were treated as if they were not. The applied significance level was $p = 0.01$.

Results

Participants

Sixty out of 102 eligible patients (58.8%) agreed to participate in the study. One enrolled patient was not included in the dietary analyses, because the answers given in the questionnaire were inconsistent with the prescribed intake. Of the included 60 patients, the median age was 13 years (range 1–39 years) and 25 (41.7%) were male. No differences were found between male and female patients for all assessed outcomes. None of the participants had restricted mobility, or used medication that affected the bone status. Medications used (other than BH4) ranged from bronchodilators used for asthma to paracetamol used for reducing pain and fever. Detailed patient characteristics are displayed in Table 1.

Protein Intake, Phe Concentrations and BH4 Use

Detailed information on protein intake and Phe concentrations is shown in Table 1. All patients had a total protein intake above minimal safe recommendations. Twenty-four percent of patients had a Phe tolerance of <500 mg per day (severe phenotype). Fourteen patients used BH4; 10/14 had an additional natural protein restriction and AAM, 4/14 were off diet. Twenty-five different AAM were used, most frequently Milupa PKU-2-prima (n = 11), Milupa PKU-2-mix (n = 10), Vitaflo PKU cooler (n = 9) and Milupa PKU-3-advanta (n = 6).

The median percentage of Phe measurements above the recommended range in the year before inclusion varied from 33 to 60%, increasing with age (Table 1); comparable to findings in other cohorts of PKU patients [18, 19].

Laboratory Results

Laboratory results of 2 patients were incomplete: in one AA were not evaluated and in the other only vitamins and FA were measured. The laboratories assessing AA made different choices on AA measured in routine PKU follow up, and therefore the total number of assessed samples differs. We report levels outside of reference ranges with possible clinical implications; tables demonstrating unremarkable intake and laboratory findings are available in online supplements 3–6.

Micronutrients

Out of range dietary intakes and blood levels are reported in Tables 2 and 3, respectively.

Vitamin D

Vitamin D (vitD) intake was below the advised minimum intake of 5 μg/day [20] in 12/59 (20%) patients. VitD supplements were used in 12/60 (20%) patients. The 25-OH vitamin D2 + D3 serum level was below the reference range of 50 nmol/L in 7/59 (12%) patients (4 using AAM, 3 without dietary restrictions) and below 25 nmol/L in 2 of them (3%). The 2 patients with the lowest serum concentrations (21 nmol/L) reported very low intakes: one adult using AAM not containing VitD (Phlexyvits) had a dietary intake of 0 μg/day; one adult using BH4 had a natural protein intake of 64.7 g/day with a VitD intake of 0.80 μg/day. Of the 11 patients using oral vitD supplements, 7 used over-the-counter supplements, 3 had a prescription from their general practitioner and 1 patient had a prescription from the metabolic specialist. Three more patients used over-the-counter multivitamin supplements containing vitD. No significant differences in serum concentrations were found between supplemented and un-supplemented patients (72 vs. 70 nmol/L).

Table 2. Dietary intake

Age, years	Total, n	Range	Median	Above SAR, n
Zinc, mg/day				
1	3	4–21	7	2
4–8	14	6–14	10	8
9–13	13	9–34	24	7
≥14 (male)	12	2–25	17	8
≥14 (female)	17	5–32	17	6
Selenium, μg/day				
1	3	11–38	25	–
4–8	14	8–56	24	–
9–13	13	26–91	51	–
≥14 (male)	12	5–125	58	–
≥14 (female)	17	30–125	78	–
Magnesium, mg/day				
1	3	92–276	132	–
4–6	12	107–375	251	–
7–10	9	151–411	277	–
11–18 (male)	12	139–701	386	–
11–18 (female)	11	299–647	487	–
≥19 (male)	4	432–667	495	–
≥19 (female)	8	363–644	503	–
Folic acid, μg/day				
1	3	70–313	127	1
4–6	12	116–380	240	3
7–10	9	137–484	296	2
11–14	9	285–577	457	–
≥15	26	131–1,256	477	2
Vitamin D, μg/day				
All	59	0–40	11	–
Vitamin B6, mg/day				
1	3	0.7–2.3	0.9	–
4–6	12	0.8–3.8	1.8	–
7–10	9	0.8–3.4	1.8	–
11–18	23	0.8–5.7	2.4	–
≥19 (male)	4	2.3–3.8	3.1	–
≥19 (female)	8	2.2–4.0	3.1	–
Vitamin B12, μg/day				
1	3	1–2.4	1.1	–
4–6	12	1–4.3	1.7	–
7–10	9	2.5–3.8	3.3	–
11–18	22	2–7	3.9	–
≥19	12	3.2–7.6	4.8	–
Fat, % caloric intake				
1	3	25–37	31	–
4–17	42	8–46	20	–
≥19	14	6–38	14	–

SAR, safe advised range.

Table 3. Blood concentrations of 25-OH vitamin D2 + D3, selenium, zinc, folic acid, vitamin B6, vitamin B12, and magnesium

	Total, *n*	Range	Median	Compared to reference range, *n*		
				above	within	below
Vitamin 25-OH D2 + D3, nmol/L						
All patients	59	21–195	70	–	52	7
Extra supplementation	12	21–148	66	–	8	4
No extra supplementation	47	21–195	70	–	44	3
Selenium, μmol/L						
All patients	59	0.5–1.4	0.8	–	30	29
Extra supplementation	53	0.5–1.4	0.8	–	28	25
No extra supplementation	6	0.6–1.3	0.9	–	2	4
Zinc, μmol/L						
All patients	58	6.5–19.6	12.4	1	49	8
Magnesium, mmol/L						
All patients	59	0.8–1.0	0.87	24	35	–
Folic acid, mmol/L						
All patients	56	10.7–45.5	38.7	29	27	–
Vitamin B12, mmol/L						
All patients	58	176–862	518.5	11	47	–
Vitamin B6, nmol/L						
All patients	60	82.8–241.9	171.2	55	5	–

Selenium

The daily selenium intakes varied from 5 to 125 μg/day, and 27 patients (45%) had low serum concentrations (7/27 using BH4 with AAM). Of these, 13/27 patients (48%) had a selenium intake below and 6/27 (22%) had an intake above the advised range. None of the patients had an intake above the SAR. The 2 patients with the highest intake (125 μg/day) were females (aged 14 and 30 years) with a mild phenotype. The majority of selenium was ingested through the use of AAM.

No significant differences in serum concentrations were found between supplemented (6/60, 10%) and unsupplemented patients (0.87 vs. 0.80 μmol/L).

Zinc

Despite the dietary zinc intake being well above the norm in 48/59 patients (81%), low serum concentrations were found in 8/59 patients (14%, none using BH4). Only one of these patients had a dietary intake below the advised range and 5 had an intake above SAR. Severe patients showed significantly higher median zinc concentrations than mild patients: 12.5 vs. 10.6 μmol/L.

Folic Acid, Magnesium, Vitamin B6 and Vitamin B12

Patient's intakes of folic acid, magnesium, vitamin B6 and B12 were above the advised range. Folic acid intake was above SAR in 5/26 patients (9%) and serum concentrations were above reference range in 26/56 (46%). Patients used the following supplements: magnesium (n = 1), multivitamin tablets (n = 4), vitamin B complex (n = 1). High blood concentrations were also found for magnesium (9/59, 15%), vitamin B6 (52/60, 89%) and vitamin B12 (11/58, 19%). Patients off diet treated with BH4 did not show elevated magnesium and folic acid concentrations (n = 5). Severe patients (who inherently use more AAM than mild patients) showed significantly higher concentrations of vitamin B12 (median 600 [n = 13] vs. 482 pmol/L [n = 44]), indicating that vitamin B12 is highly available in used AAM. No significant differences were found between supplemented and un-supplemented patients for folic acid, magnesium, vitamin B12 and B6.

Amino Acids

Plasma concentrations were below reference ranges for asparagine (22/59, 37%), 2-aminobutyric acid (10/50, 20%), tyrosine (13/59, 22%) and arginine (33/58, 57%). Plasma hydroxyproline concentrations were elevated (11/40, 28%), as were ornithine concentrations (16/59, 27%; Table 4). Phe concentrations were high in all ages. Low asparagine was significantly more often found in patients using BH4 (2/15 vs. 20/44 not using BH4, or 13 vs. 45%).

Erythrocyte FA

Patient's daily total fat intake was below the minimal recommended 20% of caloric intake in 34/59 (58%) patients. Thirteen of these 34 patients (38%) used AAM

Table 4. Amino acids in plasma, μmol/L

Age, years	Total, *n*	Range	Median	Compared to reference range, *n*		
				above	within	below
2-Aminobutyric acid						
1	3	10–16	13	–	3	–
2–9	16	7–18	10.5	–	7	9
10–17	19	5–27	12	–	18	1
≥18	12	10–19	11	–	12	–
Tyrosine						
1	3	22–129	74	1	2	–
2–9	17	23–118	53	1	11	5
10–17	24	18–103	43.5	2	12	10
≥18	15	31–111	65	3	11	1
Arginine						
1	3	14–60	25	–	2	1
2–9	17	21–66	45	–	12	5
10–17	23	24–62	41	–	7	16
≥18	15	8–64	33	–	4	11
Ornithine						
1	3	50–97	87	–	3	–
2–9	17	30–96	65	10	7	–
10–17	24	35–96	67.35	3	21	–
≥18	15	43–123	73	5	10	–
Asparagine						
1	3	17–40	32	–	2	1
2–9	17	18–48	33	–	15	2
10–17	24	20–48	32.5	–	15	9
≥18	15	23–41	31	–	5	10
Hydroxyproline						
1	3	11–19	14	–	3	–
2–9	15	8–22	12	5	10	–
10–17	24	10–208	18.5	6	18	–
≥18	8	10–17	10	–	8	–

without added FA, 19 (56%) used AAM with added FA, and 2 patients were off diet (6%; Table 2).

All patients had total erythrocyte FA concentrations within the reference range. Concentrations of essential FA linoleic acid (LA; C18:2ω6) and α-linolenic acid (ALA; C18:3ω3) were unremarkable. Elevated concentrations (in up to 38% of patients) were found for homo-γ-linolenic acid (C20:3ω6), docosatetraenoic acid (C22:4ω6), docosapentaenoic acid (C22:5ω6) and DHA; c22:6ω3. For eicosapentaenoic acid (EPA; C20:5ω3), 10/60 patients (17%) showed low concentrations (Table 5).

Median DHA and EPA concentrations were higher both in patients using FA-supplemented AAM and in patients on a free diet, when compared to patients using AAM without FA supplementation (DHA 23 and 22 vs. 16.5 pmol/10E6 cells; EPA 2.7 and 2.4 vs. 2.1 pmol/10E6 cells).

Bone Mass Density and Fracture History

Mean Z-scores for lumbar, femoral and hip BMD were overall normal with Z-scores <–2 in 4.9% (*n* = 2/41), 7.4% (*n* = 2/27) and 5.9% (*n* = 2/34) of patients, respectively (Fig. 1). Median Z-scores by age are shown in Table 6. No differences in BMD were found based on BH4 use or severity of disease. The median physical exercise for adults was 205 min/week, for children 12–17 years 325 min/week and those 1–11 years 180 min/week.

A total of 25 patients (41.7%) suffered one or more fractures. All fractures were caused by compatible trauma and healed without complications. No vertebral fractures were noted. One patient had a positive fracture history as defined by the ISCD; however, the BMD was within normal range.

Table 5. Erythrocyte essential fatty acids: ALA and metabolites, pmol/10E6 cells

	Total, *n*	Range	Median	Compared to reference range, *n*		
				above	within	below
C18:3ω3 LA						
All patients	60	0.0–1.7	0.9	7	46	7
FA in AAM	35	0.0–1.7	0.9	5	28	2
No FA in AAM	20	0.0–1.6	0.8	2	14	4
Off diet	5	0.3–1.0	0.8	–	4	1
C20:5ω3 EPA						
All patients	60	0.5–4.9	2.4	–	47	13
FA in AAM	35	0.5–4.9	2.7	–	31	4
No FA in AAM	20	0.7–3.5	2.1	–	12	8
Off diet	5	1.6–3.6	2.4	–	4	1
C22:5ω3 docosapentaenoic acid						
All patients	60	7.2–23.6	11.6	15	45	–
FA in AAM	35	7.2–19.0	11.6	8	27	–
No FA in AAM	20	8.7–23.6	11.6	7	13	–
Off diet	5	9.8–13.4	11.2	–	5	–
C22:6ω3 DHA						
All patients	60	9.2–42.4	19.7	10	50	6
FA in AAM	35	9.3–42.4	22.6	10	24	1
No FA in AAM	20	9.2–23.9	16.3	–	14	5
Off diet	5	16.2–25.0	22.2	–	5	–

ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linolenic acid; FA, fatty acids; AAM, amino-acid mixture.

Discussion

We evaluated the dietary intake and deficiencies of micronutrients and essential FA, BMD, physical activity and fracture history in a large group of patients with PKU. It is very reassuring that in spite of the complex diet, most blood concentrations of micronutrients are in the normal range. Some exceptions need to be addressed specifically.

Micronutrients

Vitamin D

To investigate the bone status in our patients, we evaluated the intake and blood levels of calcium and vitD, measured BMD and assessed the amount of physical exercise. Calcium intakes and levels in blood were within the required range, and BMD outcomes are discussed below. VitD status was evaluated by measuring the serum vitamin 25-OH D2 + D3 as advised [21]. Serum 25-OH vitamin D2 + D3 levels were below the reference range in 14% of patients, fully comparable to individuals in the general population [20–22] and patients with PKU who are off diet in whom concentrations below reference range are also frequently observed [23]. Of the 2 patients showing concentrations in the range associated with clinical symptoms (<25 nmol/L [19, 20]), 1 patient had a normal intake of natural protein. There was no difference found between patients with and without extra supplementation of vitD. This may be due to the fact that serum vitamin 25-OH D2 + D3 is affected not only by vitD in-

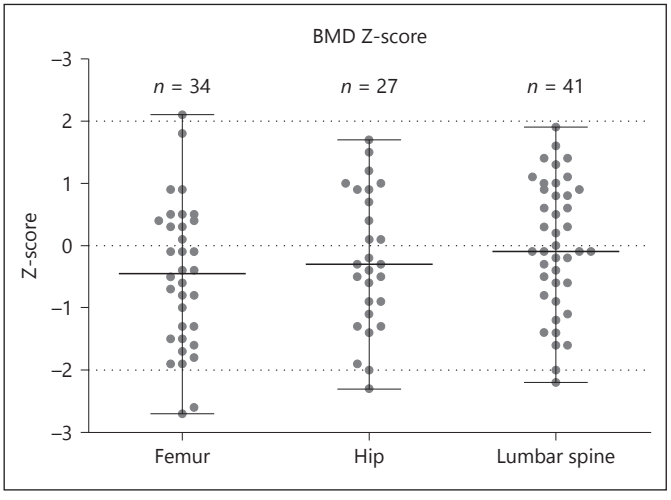

Fig. 1. BMD Z-score. *n*, patients; —, median, minimum, and maximum.

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Table 6. BMD Z-scores by age

Age, years	Median BMD Z-score		
	femur (*n*) (IQR)	hip (*n*) (IQR)	lumbar spine (*n*) (IQR)
4–11	–0.50 (11) (–1.70/0.40)	–0.25 (8) (–1.20/0.70)	–0.10 (18) (–0.95/0.83)
12–17	–0.65 (18) (–1.53/0.43)	–0.40 (17) (–1.10/0.85)	0.25 (18) (–0.95/1.00)
18–39	0.10 (5) (–0.80/0.40)	–0.10 (2) (–)	0.00 (5) (–0.30/1.05)
Total	–0.45 (34) (–1.50/0.40)	–0.30 (27) (–1.10/0.90)	–0.10 (41) (–0.70/0.90)

BMD, bone mineral density; IQR, interquartile range.

take, but also by genetic factors, sunlight exposure and supplement intake adherence [24].

The normal concentrations found in most patients are due to proper monitoring and, if necessary, supplementation. Therefore, we advise to yearly evaluate intake and determine blood concentrations, and to supplement patients when levels are <50 nmol/L (or as according to current guidelines) [20–22, 25].

Zinc

Zinc serum concentrations were below the reference range in 14% of patients, despite an intake above SAR in 52% of patients. This finding is comparable to outcomes in other studies [5, 7, 8]. However, the clinical relevance of the observed low zinc concentrations is unclear. It seems probable that the severe restriction of natural protein in the PKU diet decreases zinc absorption, as a low intake of animal protein in a vegetarian diet may decrease the absorption of zinc [26]. Zinc deficiency is associated with growth retardation and sexual maturation abnormalities, decreased wound healing, hair loss, diminished visual dark adaptation and anorexia [27]. Hair loss has been described in other diseases than PKU at serum levels below 11 μmol/L (72 μg/dL) [28] and skin lesions below 9.2 μmol/L (60 μg/dL) [29]. We have not assessed these problems in our population, but if certain symptoms are encountered in patients with PKU combined with decreased zinc blood levels, supplementation could be considered. Future studies need to explore how to effectively increase the zinc uptake in PKU patients of whom many already have intakes exceeding SAR.

Selenium

Low plasma selenium serum concentrations, as seen in 46% of our patients, have previously been reported in PKU [7, 8]. Plasma selenium is an adequate biomarker for assessing the selenium status in patients on a special diet [30]. Selenium is best absorbed as an organic form, while inorganic forms, such as that used in AAM, are less well absorbed. This may also explain why there was no difference found between patients who used extra supplementation and those who did not. Selenium deficiency may lead to cardiomyopathy, depressive symptoms or osteoarthropathy [30–33]. Elevated Phe values already put PKU patients at risk for mood disturbances [34, 35]. Because intake is low in many patients (41%), it seems advisable to annually evaluate the selenium status and consider supplementation if concentrations are below the advised reference ranges (supplementing up to 400 μg/day is considered to be safe in adults [36]). However, the clinical relevance of our findings is unknown and it is yet unclear what amount of supplementation will be needed to achieve normal concentrations of selenium in the blood. Future studies are warranted.

Folic Acid

Folic acid intake and blood concentrations were remarkably high in patients using AAM. Five patients reported an intake above SAR (Table 2). Because all patients off diet showed serum concentrations within normal range, elevated serum concentrations appear to be due to fortified AAM [37]. Our findings are confirmed by the study by Stolen et al. [38] reporting similar results. As there is discussion on the safety of high concentrations [38, 39], it deserves due consideration to lower folic acid amounts in AAM. For example, high levels of folic acid have been associated with interference in DNA and histone methylation, decreased natural killer cell toxicity, an increased risk of cognitive impairment in the elderly and facilitating progression of preneoplastic cells and subclinical cancers. Because this micronutrient is highly available in several AAM, decreasing the concentration of folic acid in these AAM may be advisable [38, 39].

Dietary intake and blood concentrations of magnesium, vitamin B6 and B12 are elevated. However, as intakes are within SAR and these micronutrients are not known to be toxic, adaptation of intake may not be warranted [15]. The fact that there is no difference in serum levels between patients using extra supplementation and those who do not is not surprising because intakes of the named nutrients are overall high.

Amino Acids

In some patients, low values of arginine, tyrosine, asparagine and/or 2-aminobutyric acid were demonstrated. However, there was no indication of clinical implications. The low plasma arginine values did not result from hemolysis as ornithine concentrations were normal. AAM use has been associated with plasma tyrosine fluctuations and effects of extra tyrosine supplementation on outcome are yet unclear [40]. Furthermore, we do not have a clear explanation as to why some of the non-essential amino-acids were low in plasma. They are fully supplemented in the amino-acid mixtures, and we do not think that the minimum 3 h fasting of our patients is attributable to the outcomes either. Future studies are warranted to investigate the need for increased supplementation of specific AA in PKU.

Hydroxyproline (a bone resorption marker) was elevated in adolescents, representing increased protein turnover in bone during growth [41].

Erythrocyte FA

Essential FA are precursors of thromboxanes, leukotrienes and prostaglandins [42]. DHA and EPA are known to have cardio-protective effects [43] and deficiencies may lead to CNS disease or affect the immune system [42]. Normal as well as reduced plasma and erythrocyte FA levels have been reported in patients with PKU [44–49]. It is, however, not clear what these deficiencies mean and if they have a clinical impact on the patients. In patients with PKU, alterations in plasma/erythrocyte levels of FA have been associated with lowered BMD and neurological outcomes [6].

Even though EPA and DHA are frequently supplemented in AAM, EPA is below reference concentrations in 6% of our patients. For this reason, it may be considered to increase EPA supplements in AAM. Significantly lower concentrations of DHA and EPA are found in patients using AAM without FA versus those using FA-supplemented AAM. For this reason, it may be advisable to prescribe FA containing AAM or to supplement. Further research is needed to determine the optimal supplementation dosage and to establish beneficial functional outcomes [6].

Bone Health

We are one of the first to asses fracture risk and the amount of physical exercise in early treated patients with PKU in a cohort of this size. As mentioned, we retrospectively collected BMD data obtained during standard clinical care of patients with PKU. BMD was routinely and regularly assessed to detect low BMD as early as possible with the aim of preventing fractures. We found a lumbar and femoral BMD Z-score <–2 in 4.9 and 7.4% of our patients, respectively. None of the patients had osteoporosis as defined by the ISCD, and the lifetime fracture prevalence of patients seems comparable to the general population (41, 7 vs. 38.2%) [43]. These findings are in line with data reported in our recently published meta-analysis [10] and support our recommendation to only perform a single assessment of BMD with DXA scan in adolescent patients with PKU. Only those patients with a BMD Z-score <–2 and/or a significant fracture history may need follow-up. In this way, unnecessary radiation exposure may also be avoided.

Physical exercise in adults met World Health Organization recommendations (150 min/week). Of children aged 12–17 years, 80% did not meet the recommended 60 min/day of exercise, which is comparable to the general population [50]. We have no reason to believe that insufficient physical activity in this patient group has a negative effect on bone health other than it would in the general population.

Study Limitations

Our study results are overall normal, and in treated patients the diet seems less shortcoming as has been hypothesized in previous literature.

However, this study was an uncontrolled study, covering a wide age range of patients on varying treatment options. Some micronutrient deficiencies were found, but based on our results we can unfortunately not conclude whether these deficiencies are based on shortcomings of the diet (AAM composition), on a lack of adherence of our patients, or on physiological consequences of the disease itself. Even though we present data of one of the largest cohorts published, our study is limited by the low patient numbers and the diversity of the PKU severity/spectrum of disease. This has led to a heterogeneous group when it comes to dietary treatment and therefore dietary intake. For example, AAM used by our patients were diverse and a comparison of different types of AAM was therefore not possible.

Multivariable Linear Regression

Unfortunately, we were not able to properly investigate associations between blood and intake levels of assessed nutrients, nor between outcomes of researched nutrients and BMD. However, because these relations are of great interest to achieve further knowledge on the etiology of nutrient deficiencies and bone health, larger cohort or case-control studies are indicated.

Conclusions

We evaluated dietary intake and deficiencies of micronutrients and essential FA, BMD, physical activity and fracture history in a large group of patients with PKU. It is very reassuring that in spite of the complex diet, most blood concentrations of micronutrients are in the normal range. We did, nonetheless, detect lower blood concentrations of some micronutrients in our population. However, specific complications that may be related to these alterations, other than bone health, were not assessed in this study. Those micronutrients that have been studied in large cohorts as potentially leading to risk (e.g., vitamin D, selenium and EPA) could be considered to be supplemented based on available recommendations. At this time, there is no convincing evidence for supplementation of other nutrients.

Furthermore, we were also able to investigate BMD and fracture history, and we found that although fracture prevalence is normal, a slightly more prevalent low BMD is evident. These findings support earlier conclusions from our recently published meta-analysis about BMD in early treated patients with PKU [10]. The clinical implications may be limited as none of the patients have osteoporosis as defined by the ISCD. However, the meaning of these outcomes is yet unclear and follow up into older age (especially in post-menopausal women) is warranted and advised.

Unfortunately, we were not able to investigate the association between intake and blood concentrations of these nutrients, nor the association of BMD/fracture history with blood concentrations and intake of micronutrients due to the included number of patients and the non-normally distributed data.

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Disclosure Statement

The authors have no conflict of interest to declare.

Author Contributions

S.D., C.E.M.H., and A.M.B. have made substantial contributions to conception and design, analysis and interpretation of data. All authors have participated in the acquisition of data, interpreting data, statistical analysis, drafting the manuscript or revising it critically for important intellectual content. All authors have given their final agreement to the submission after inspection.

Take Home Message

Dutch patients (1–39 years old) with PKU on long-term dietary treatment have a near normal nutrient status; however, supplementation of micronutrients of which deficiency may be deleterious (e.g., vitamin D and selenium) should be considered. BMD Z-scores are within the normal range, but lowered in comparison to the general population, and consequently warrant further investigation.

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