ORIGINAL ARTICLE

JIMD ⟨SSIEM⟩ WILEY

Bone mineral density is within normal range in most adult phenylketonuria patients

Charlotte M. A. Lubout[1] | Francisco Arrieta Blanco[2] | Katarzyna Bartosiewicz[3] | François Feillet[4] | Maria Gizewska[3] | Carla Hollak[5] | Johanna H. van der Lee[6] | François Maillot[7] | Karolina M. Stepien[8] | Margreet A. E. M. Wagenmakers[9] | Mendy M. Welsink-Karssies[1] | Francjan J. van Spronsen[10] | Annet M. Bosch[1]

[1]Department of Pediatrics, Emma Children's Hospital, Amsterdam UMC – Location AMC, Amsterdam, The Netherlands

[2]Servicio de Endocrinología y Nutrición, Hospital Universitario Ramón y Cajal, Madrid, Spain

[3]Department of Pediatrics, Endocrinology, Diabetology, Metabolic Diseases and Cardiology of the Developmental Age, Pomeranian Medical University in Szczecin, Szczecin, Poland

[4]Department of Pediatrics, Hôpital d'Enfants Brabois, CHU Nancy, Nancy, France

[5]Department of Internal Medicine, Division of Endocrinology and Metabolism, Amsterdam UMC – Location AMC, Amsterdam, The Netherlands

[6]Pediatric Clinical Research Office, Woman-Child Center, Amsterdam UMC – Location AMC, Amsterdam, The Netherlands

[7]Service de Médecine Interne, CHRU de Tours, Université de Tours, UMR INSERM, Tours, France

[8]Mark Holland Metabolic Unit, Adult Inherited Metabolic Disorders, Salford Royal NHS Foundation Trust, Salford, United Kingdom

[9]Center for lysosomal and metabolic disease, Department of Internal Medicine, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

[10]Section of Metabolic Diseases, Beatrix Children's Hospital, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

Correspondence
Annet M. Bosch, Amsterdam UMC – Location AMC Department of Pediatrics, Division of Metabolic Disorders, Room H7-270, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.
Email: a.m.bosch@amsterdamumc.nl

Present address
Charlotte M. A. Lubout, Section of Metabolic Diseases, Beatrix Children's Hospital, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands.

Communicating Editor: Robin Lachmann

Abstract

Low bone mineral density (BMD) as a risk factor for fractures has been a long-standing concern in phenylketonuria (PKU). It is hypothesised that the disease itself or the dietary treatment might lead to a low BMD. Previous studies show conflicting results of BMD in PKU due to differences in age, techniques to assess BMD and criteria used. To assess the prevalence of low BMD and define possible risk factors in a large number of adult, early treated PKU (ETPKU) patients. European centres were invited for a survey, collecting retrospective data including results of dual-energy X-ray absorptiometry (DXA) scans of adult ETPKU patients. BMD of 183 adult ETPKU patients aged 18-46 (median age 28, all females premenopausal) years was lower than in the general population at most skeletal sites but the frequency of low BMD (Z-score ≤ -2) was at maximum 5.5%. No risk factors for low BMD in PKU patients could be identified. Low BMD occurs only

---

Abbreviations: BMD, bone mineral density; BMI, body mass index; DXA, dual-energy X-ray absorptiometry; ETPKU, early treated PKU; FNBMD, femoral neck bone mineral density; ISCD, International Society for Clinical Densitometry; LBMD, lumbar bone mineral density; PAH, phenylalanine hydroxylase; Phe, phenylalanine; PKU, phenylketonuria; RBMD, radius bone mineral density; TBMD, total body bone mineral density; TFBMD, total proximal femur bone mineral density.

in a small subset of PKU patients. DXA scans should be considered for well controlled patients from age 35-40 years and up and on indication in those PKU patients considered to be at increased risk for fractures.

1 | INTRODUCTION

Phenylketonuria (PKU, ORPHA79254, MIM 261600) is an inborn error of metabolism in which deficiency of the enzyme phenylalanine hydroxylase (PAH; EC 1.14.16.1) causes high phenylalanine (Phe) concentrations which lead to intellectual disability if left untreated. Since the 1960's-1970's, patients are detected early by newborn screening and treatment with a Phe-restricted diet is started immediately. This results in a near normal outcome of development. However, as the first early treated PKU (ETPKU) patients become older, new concerns about long term consequences of PKU and its treatment arise.[1-4] Most attention is given to the neurocognitive issues and psychosocial functioning, but also bone health has been a long-standing concern in PKU patients.[3,4] It is hypothesised that the Phe restricted diet or the disease itself might lead to a low bone mineral density (BMD) in PKU. However, previous studies show conflicting results due to differences in assessing BMD and different criteria for defining low BMD.[4-13] Furthermore, most studies included a small number of patients. In 2015, the meta-analysis by Demirdas and colleagues showed that low BMD for chronological age, defined as a Z-score ≤ -2 (in agreement with the International Society for Clinical Densitometry (ISCD) criteria) is expected in approximately 10% of ETPKU patients.[8,14] However, most of the studies included in this meta-analysis focused on BMD in both adult and paediatric patients, with only one small study focusing solely on adult patients.[15] Including the results of BMD in paediatric patients might distort the information about adult patients for several reasons. Factors like bone development, growth, puberty, and body composition all affect (assessment of) BMD.[16,17] In addition peak bone mass is attained during late adolescence/early adulthood.[18] Therefore, there is a need for the assessment of BMD in a large cohort of adult ETPKU patients. Insight in BMD of adults with PKU will determine further need for follow up.

The aim of the presented multicentre survey study was to collect retrospective data for assessment of the prevalence of low BMD and to define possible risk factors for low BMD in a large number of adult, ETPKU patients.

2 | METHODS

Seventeen European centres specialised in treating adult PKU patients were invited to participate in a survey study collecting retrospective data of individual adult ETPKU patients. Participating centres were asked to complete a questionnaire for all early treated (start treatment <4 weeks of age), adult (≥18 years) PKU patients of whom data of dual-energy X-ray absorptiometry (DXA) was available. Exclusion criteria were chronic conditions affecting bone health; chronic malabsorption disease, chronical use of steroids (>5 mg of prednisolone daily for 3 months or longer), rheumatoid arthritis, immobility, hypogonadism, organ transplantation, type 1 diabetes mellitus, thyroid disorders, chronic obstructive pulmonary disease as well as a postmenopausal state.[14,18-20]

The questionnaire included deindentified data involving demographics, anthropometrics, diet, and supplements, mean Phe (μmol/L) during the year before the recent DXA scan (one centre included mean Phe of the 2 years before the recent DXA scan), use of sapropterin dichloride, low vitamin D concentration (defined as 25-OH vitamin D <50 nmol/L),[21] (number of) fractures, smoking or ex-smoker status, and alcohol consumption.

The (most recent) DXA results in Z-score were collected for different skeletal sites; lumbar (LBMD), femoral neck (FNBMD), total proximal femur (TFBMD), radius (RBMD), and total body (TBMD). According to the ISCD criteria Z-scores and not T-scores were used as the studied patients involved premenopausal women and men <50 years of age. A BMD Z-score ≤ -2 SD was defined as below the expected range for age (low BMD), and a Z-score > -2 was defined as within the expected range for age.[14]

Data was collected in Castor EDC, a good clinical practice compliant data management system.

The medical ethics committee of the Amsterdam UMC, AMC, Amsterdam, The Netherlands, confirmed that for this survey study collecting retrospective data the Medical Research Involving Human Subjects Act (WMO) did not apply and informed consent was not required. This article does not contain any studies with human or animal subjects performed by any of the authors.

2.1 | Statistical analysis

Sample size calculation by the Lehr's formula[22] revealed that a sample size of at least 80 patients (after correction for

TABLE 1 Characteristic of included patients

Continuous data	N	Median (range)	
Age at data collection (years)	183	32 (19-53)	
Age most recent DXA (years)	183	28 (18-46)	
Recent BMI	179	24.9 (17.5-49)	
Mean Phe the year before the most recent DXA (μmol/L)	168	775 (61-1816)	
Categorical data	**N**		**%**
Gender	183	Male	42
Disease severity based on Phe before treatment[1,2]	87	Classic PKU (Phe ≥1200)	68
		Mild PKU (Phe >600 and <1200)	22
		Mild hyperphe (Phe ≤600)	10
Natural protein intake	183	Missing or not adherent to a diet	16
		Severe protein restriction (≤10 g/day)	24
		Moderate protein restriction (>10 to 20 g/day)	21
		Mild protein restriction (>20 to 40 g/day)	16
		Protein intake > recommended intake (>40 g/day)[a]	23
Sapropterin dichloride use	167	Yes	14
		No (not tested or unresponsive)	86
Low vitamin D concentration (25-OH vitamin D <50 nmol/L)	173	Yes	32
Vitamin D supplementation	162	Yes	26
Calcium supplementation	161	Yes	19
(ex) Smoker status	106	Yes	22
Alcohol consumption (on average >2 units/day)	106	Yes	26

Abbreviations: BMI, body mass index; DXA, dual-energy X-ray absorptiometry; PKU, phenylketonuria.

[a]An amount of >40 g was based on the safe amount of protein of 0.8 g/kg/day according to FAO/WHO/UNU with some patients weighing around 50 kg.

unequal sized groups[23]) was sufficient to yield 80% power to detect a difference of at least 7.6% in prevalence of low BMD in ETPKU patients compared to the general population with a type I error of 5%, based on the available data by Demirdas et al.[8]

Analysis was performed using IBM SPSS statistics 23. Normally distributed data was compared by parametric tests (one sample t test or unpaired t test), non-normally distributed data was analysed by non-parametric tests (Wilcoxon signed rank test or Mann-Whitney U test). Categorical data was analysed

TABLE 2 Mean, median BMD Z-scores, and % low BMD measured at several skeletal sites

Skeletal site	N	Mean Z-score (±1 SD)	Median Z-score (range)	Compared to general population[b]	Number (%) low BMD[c]
Lumbar	181	−0.527 (± 1.030)	−0.600 (−2.8;2.6)	$P < .0000$*	10 (5.5)
Femoral neck	111	−0.324 (± 0.913)	−0.400 (−2.4;2.0)	$P = .0003$*	4 (3.6)
Total proximal femur	128	−0.262 (± 0.925)	−0.300 (−2.4;2.9)	$P = .002$*	2 (1.6)
Radius	55	−0.298 (± 1.176)	−0.400 (−3.2;1.8)	$P = .065$	3 (6)
Total body[a]	88	-	−0.400 (−5.9;2.7)	$P = .002$*	4 (5)

Abbreviation: BMD, bone mineral density.

[a]Not normally distributed.

[b]Mean Z-score= 0.

[c]Defined as Z-score ≤−2.[14]

*Significantly different from the general population ($P < .05$) after post-hoc adjustment of P value by Bonferroni method (multiplying P value by the number of tests (5)).

TABLE 3 Comparison of possible risk factors between patients with low BMD (Z-score ≤−2) and patients with BMD within normal range (Z-score >−2)

Continuous data		Low BMD		Normal BMD		P (Mann-Whitney U test)
		N	Median	N	Median	
Age most recent DXA (year)		10	27.5	171	28.0	.864
Recent BMI		10	21.7	167	24.9	.053
Mean Phe the year before the recent DXA (μmol/L)		10	800	156	775	.573
Categorical data		**Low BMD N (%)**		**Normal BMD N (%)**		**P (Fisher's Exact test)**
Gender M		8 (80)		68 (40)		.018*
Sapropterin dichloride use yes		1 (11)		22 (14)		>.99
Low vitamin D concentration yes		5 (50)		50 (31)		.294
Vitamin D supplementation yes		3 (38)		39 (26)		.434
Calcium supplementation yes		3 (38)		27 (18)		.174
(ex) Smoker status yes		0		23 (23)		.574
Alcohol consumption (on average >2 units/day) yes		2 (50)		25 (25)		.272
Natural protein intake	Missing or not adherent to a diet	3 (30)		27 (16)		.588
	Severe protein restriction (≤10 g/day)	1 (10)		42 (25)		
	Moderate protein restriction (>10 to 20 g/day)	1 (10)		35 (21)		
	Mild protein restriction (>20 to 40 g/day)	2 (20)		28 (16)		
	protein intake > recommended intake (>40 g/day)[a]	3 (30)		39 (23)		

Abbreviations: BMD, bone mineral density; BMI, body mass index; DXA, dual-energy X-ray absorptiometry.

[a] An amount of >40 g was based on the safe amount of protein of 0.8 g/kg/day according to FAO/WHO/UNU with some patients weighing around 50 kg.

*Not significant (P ≥ .05) after post-hoc adjustment of P value by Bonferroni method (multiplying P value by the number of tests (11)).

by a χ^2 or Fishers Exact test. In case of multiple hypothesis testing, a post-hoc analysis was done by the Bonferroni method (multiplying P-value by the number of tests). A P-value <.05 was considered statistically significant.[22,24,25]

In case of multiple DXA scans in one patient, the most recent was selected for analysis. Mean BMD (Z-score) was assessed for the different skeletal sites and compared to the expected mean Z-score (0) in the general population. Frequency of low BMD was calculated.

The spine and hip region are the sites of preference to measure BMD.[14,19] Several possible risk factors were taken into consideration when comparing patients with a low BMD at the spine level and those with a BMD within normal range. They included; gender, age at time most recent DXA, recent Body Mass Index, natural protein intake, use of calcium and vitamin D supplements, use of sapropterin dichloride, mean Phe the year before the recent DXA scan, low vitamin D concentration, and life style factors (smoking and alcohol consumption).[4,7,10-13,18,19,26] Not for all patients with a statement of normal diet the exact amount of natural protein intake was available. Before statistical analysis was performed, natural protein intake was categorised according to the following groups; (a) missing or not adherent to a diet, (b) severe protein restriction ($\leq$10 g/day), (c) moderate protein restriction (>10 to 20 g/day), (d) mild protein restriction (>20 to 40 g/day), (e) protein intake > recommended intake (>40 g/day). An amount of >40 g was based on the safe amount of protein of 0.8 g/kg/day according to FAO/WHO/UNU with some patients weighing around 50 kg.[3]

Frequency of fractures was compared to the estimated age-standardised fracture prevalence as described for England.[27]

3 | RESULTS

A total of 8 (out of 17 invited) European metabolic centres, situated in France, Poland, Spain, the Netherlands, and the United Kingdom, participated in the study. Among 9 other centres, 3 could not participate for various reasons and 6 did not respond.

For most centres all of the patients were included. In 2 centres not all patients could be included within the inclusion period. Therefore, a random selection of patients was made to prevent bias (selection on alphabetical order or order of appearance at the outpatient clinic).

Data of a total of 216 patients were received. Thirty-three patients were excluded for the following reasons: no early treatment N = 11, most recent DXA scan <age 18 years $N = 11$, or significant co-morbidity that might affect BMD $N = 11$, resulting in a total number of 183 to be analysed.

The characteristics of the included patients are presented in Table 1. Based on the Phe concentration before diagnosis and/or the natural protein intake, most of the included

patients have severe PAH deficiency. The mean individual Phe concentration the year before the recent DXA was performed showed a wide range, with a median for the group of 775 μmol/L.[2-4]

Most BMD results were available for the spinal level. Mean BMD Z-scores were significantly lower in PKU patients compared to the reference population for all skeletal sites except the radius. The lowest result was seen for the spine with a mean Z score of −0.527 ($\pm$1.030). Frequency of low BMD (defined as a Z-score $\leq$−2) was observed in 1.6%-5.5% with the maximum being observed at the spinal level (Table 2).

No statistically significant differences were found in possible risk factors between patients with low BMD and patients with a BMD within normal range (Table 3).

Fractures were described in 30 patients (16.4%), which is significantly lower than the estimated age-standardised fracture prevalence of 38.2% ($P < .000$) as described for England.[27] Of those patients who had experienced a fracture, 7 sustained more than 1 fracture. There was insufficient data on which fractures were fragility fractures. There was no significant difference in mean LBMD Z-score between patients with fractures and patients without known fractures ($P = .97$).

4 | DISCUSSION

This multicentre study of BMD in adult ETPKU patients shows that, although the mean BMD in ETPKU patients is significantly lower compared to the general population, BMD is within normal range in most patients. The prevalence of low BMD is at most observed at the lumbar spine in 5.5% of patients, which is lower than described in the meta-analysis by Demirdas et al and other previous studies.[7,8,10-13]

This difference could be explained by the fact that most studies included in the meta-analysis focused on a combination of both paediatric and adult PKU patients. Assessing BMD in children is hampered by several factors and it is known that low BMD might be overestimated in children with a chronic illness.[16,17] Also in the study of De Groot et al, low BMD was described in only 6% of adult patients while the percentage tended to be higher in children.[7] The only previous study which focused exclusively on BMD in adult PKU patients, used other definitions (osteopenia and osteoporosis; not according to the current ISCD definition) but a Z-score $\leq$−2.5 was detected in 6.5%.[15] In addition, more recent studies that focused on BMD in both children and adults found low BMD defined as Z-score $\leq$−2 in 4.5% to 7.4% of the total group of PKU patients.[28,29] As the present study focuses on adults who are expected to have achieved their peak bone mass,[18] this is probably a more reliable reflection of BMD in PKU.

4.1 | Risk factors for low BMD

In our study population no risk factors for low BMD could be identified. This is in agreement with other studies.[8,15] The prediction model described by Coakley et al, although based on a small number of patients, found dietary factors like compliance, medical food prescriptions, and vitamin D intake to be of influence.[28] In the present study, it was not possible to draw conclusions on the exact amount of natural protein intake and medical food protein but an association with non-adherence to diet, low vitamin D concentration, or vitamin D supplementation was not found. Some studies show that deficiencies in essential fatty acids can be seen in PKU, but there is limited evidence that this might be related to BMD in PKU.[29,30] Our study did not investigate this.

4.2 | Fracture risk

Fracture prevalence in our population was lower than the age-standardised fracture prevalence as described for the population of England.[27] It is reasonable to consider that this is more or less comparable with the prevalence of fractures in Europe. The low fracture rate found in this study could be explained by several reasons. Most importantly, in view of the retrospective design of this study, the number of fractures may have been underestimated. Other reasons might be a difference in participation in high fracture risk activities such as specific sports between ETPKU patients and the general population[27] which was not evaluated in this study. Previous studies focusing on fractures in PKU reported a comparable risk to the general population as well as a higher fracture risk above age 8 detected in a very young group of PKU patients measured by parent or self-report.[29,31]

BMD is not the only factor that determines the risk of fractures, to assess the fracture probability in the general population other risk factors should be taken into account.[14,19,20] The Fracture Risk Assesment Tool (Frax) is available for fracture risk assessment above age 40 years.[19]

4.3 | Recommendations

Altogether, most ETPKU patients have a BMD within normal range, with only a maximum of 5.5% having a low BMD. As mean BMD is lower compared to the general population, the prevalence of low BMD might be higher in PKU patients >35-40 years of age, when bone mass is known to decline.[18]

With the present data it can be argued that a DXA scan should be requested in PKU patients who have an age >35-40 years or in case of additional risk factors for fractures like prolonged immobility, hypogonadism, chronic use of steroids, malabsorption, alcohol abuse, previous fragility fractures, or other conditions that cause an increased fracture risk.[14,18,19] The European PKU guideline advices (best practice based) to perform a first DXA scan in adolescence.[3,4] Based on the present study it is a consideration to perform the first DXA scan in a well regulated PKU patient without further risk factors for fractures not in adolescence but at an age >35-40 years.

4.4 | Strengths and limitations

The strength of the present study is the relatively large number patients, the fair proportion of patients with an age in their fourth decade, and the participation of centres from different European countries, making the study population representative of the European ETPKU population. In addition, the study population included many patients with severe PKU (based on Phe before start of treatment or natural protein intake).[2-4]

However, all patients were seen in a centre specialised in PKU, making this a relatively well monitored population. Therefore the results might not be applicable to less regulated patients.

Most participating centres routinely performed a DXA scan in all their patients, some centres only made a DXA scan in a selection of patients. This might have caused bias in the prevalence of low BMD. However, it is more likely that only the patients who are thought to be at risk for low BMD will be selected for a DXA scan. In that case, the reported prevalence of low BMD is an overestimation.

Only the most recent DXA scans were selected for analysis as BMD was thought to be more relevant in older patients. It could be argued that in patients with multiple DXA scans, a result of low BMD would result in a change of treatment influencing the most recent DXA. However, no factors of influence could be identified, making less likely that interventions like vitamin D supplementation or a change in diet directed by an earlier DXA scan resulted in an improved BMD.

Due to the low prevalence of low BMD, the total number of patients with low BMD was 10, hampering additional analysis focusing on risk factors. Because of this small number a multiple regression model was not considered as a most suitable test.

As dietary factors were derived from patient charts, the data were not complete enough to draw conclusions on total protein and medical food protein. Preferably dietary diaries filled in by patients should be used to correlate BMD with dietary factors.

The described fracture prevalence was based on chart studies which are less reliable than an observational study or assessment of patient reported fracture risk.

Although the study included some of the oldest ETPKU patients >40 years of age, predictions on BMD in older age

cannot be made. As bone loss occurs with advancing age[18,19] future studies in elderly, ETPKU patients are warranted to assess how often low BMD is seen in advancing age and whether these patients have an increased fracture risk.

5 | CONCLUSION

Mean BMD in early treated adult PKU patients is lower than in the general population but the frequency of low BMD is at maximum 5.5%. DXA scans should be done in well controlled PKU patients >35-40 years of age and in those PKU patients considered to be at increased risk for fractures.

CONFLICT OF INTEREST

C.M.A.L. has received a speaker fee from the Recordati Rare Disease Foundation.

M.G. has been a member of scientific advisory boards for PKU (supported by Merck Serono, BioMarin, and Nutricia International). She has received honoraria as consultant and speaker for Merck Serono, BioMarin, Nutricia International, Danone, Mead Johnson, and Vitaflo.

C.H. is involved in pre-marketing studies with Sanofi, Takeda, and Idorsia in the field of lysosomal storage disorders.

F.M. has been a member of scientific advisory boards for PKU (supported by Merck Serono and BioMarin). He has received honoraria as consultant for BioMarin, and as speaker for BioMarin, Nutricia International, and Vitaflo. He has received research grants from Merck Serono and BioMarin

M.A.E.M. Wagenmakers received funding for an independent research proposal from the Nutricia metabolic Research Fund.

F.J.v.S. is a member of scientific advisory boards for defects in amino acid metabolism of APR, Arla Food International, BioMarin, Eurocept Int, Lucana, Moderna TX, Nutricia, Rivium, and SoBi, has received research grants from Alexion, BioMarin, Codexis, Nutricia, SoBi, and Vitaflo, has received grants from patient organisations ESPKU, Metakids, NPKUA, Stofwisselkracht, Stichting PKU research, and Tyrosinemia Foundation, and has received honoraria as consultant and speaker from APR, BioMarin, MendeliKABS, and Nutricia.

A.M.B. has been a member of the advisory board of BioMarin in 2017 and had received a speaker fee from Nutricia in 2017.

F.A.B., K.B., F.F., J.H.v.d.L., K.M.S., and M.M.W.-K. declare that they have no conflict of interest.

AUTHOR' CONTRIBUTIONS

Design of the study and interpretation of the data was done by C.L., F.J.S., A.B. C.L., F.A., K.B., F.F., M.G., C.H., F.M., K.S., M.W., A.B. contributed to the data collection. C.L. and M.W.K. were involved in constructing the database. C.L. performed the statistical analysis with help of J.H.L. C.L., F.J.S., A.B. were involved in drafting the manuscript. All authors were involved in revising the manuscript. All authors have read and approved the final manuscript.

DATA ACCESSIBILITY

Original data will be made available to the editors if requested

ORCID

Charlotte M. A. Lubout 🔟 https://orcid.org/0000-0002-8058-440X

REFERENCES

1. Blau N, van Spronsen FJ, Levy HL. Phenylketonuria. *Lancet*. 2010;376:1417-1427.
2. Regier DS, Greene CL. Phenylalanine hydroxylase deficiency. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *GeneReviews ((R))*. Seattle, WA: University of Washington, Seattle; 1993. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved.
3. van Spronsen FJ, van Wegberg AM, Ahring K, et al. Key European guidelines for the diagnosis and management of patients with phenylketonuria. *Lancet Diabetes Endocrinol*. 2017;5:743-756.
4. van Wegberg AMJ, MacDonald A, Ahring K, et al. The complete European guidelines on phenylketonuria: diagnosis and treatment. *Orphanet J Rare Dis*. 2017;12: 162-017-0685-2.
5. Adamczyk P, Morawiec-Knysak A, Pludowski P, Banaszak B, Karpe J, Pluskiewicz W. Bone metabolism and the muscle-bone relationship in children, adolescents and young adults with phenylketonuria. *J Bone Miner Metab*. 2011;29:236-244.
6. Choukair D, Kneppo C, Feneberg R, et al. Analysis of the functional muscle-bone unit of the forearm in patients with phenylketonuria by peripheral quantitative computed tomography. *J Inherit Metab Dis*. 2017;40:219-226.
7. de Groot MJ, Hoeksma M, van Rijn M, Slart RH, van Spronsen FJ. Relationships between lumbar bone mineral density and biochemical parameters in phenylketonuria patients. *Mol Genet Metab*. 2012;105:566-570.
8. Demirdas S, Coakley KE, Bisschop PH, Hollak CE, Bosch AM, Singh RH. Bone health in phenylketonuria: a systematic review and meta-analysis. *Orphanet J Rare Dis*. 2015;10: 17-015-0232-y.
9. Geiger KE, Koeller DM, Harding CO, Huntington KL, Gillingham MB. Normal vitamin D levels and bone mineral density among children with inborn errors of metabolism consuming medical food-based diets. *Nutr Res*. 2016;36:101-108.

10. Mendes AB, Martins FF, Cruz WM, da Silva LE, Abadesso CB, Boaventura GT. Bone development in children and adolescents with PKU. *J Inherit Metab Dis*. 2012;35:425-430.

11. Miras A, Boveda MD, Leis MR, et al. Risk factors for developing mineral bone disease in phenylketonuric patients. *Mol Genet Metab*. 2013;108:149-154.

12. Perez-Duenas B, Cambra FJ, Vilaseca MA, Lambruschini N, Campistol J, Camacho JA. New approach to osteopenia in phenylketonuric patients. *Acta Paediatr*. 2002;91:899-904.

13. Zeman J, Bayer M, Stepan J. Bone mineral density in patients with phenylketonuria. *Acta Paediatr*. 1999;88:1348-1351.

14. International Society for Clinical Densitometry (ISCD) (2015) Official Positions-adult and pediatric 2015. https://www.iscd.org/official-positions/2015-iscd-official-positions-adult/.

15. Modan-Moses D, Vered I, Schwartz G, et al. Peak bone mass in patients with phenylketonuria. *J Inherit Metab Dis*. 2007;30: 202-208.

16. Bachrach LK. Osteoporosis in children: still a diagnostic challenge. *J Clin Endocrinol Metab*. 2007;92:2030-2032.

17. Gafni RI, Baron J. Overdiagnosis of osteoporosis in children due to misinterpretation of dual-energy x-ray absorptiometry (DEXA). *J Pediatr*. 2004;144:253-257.

18. Weaver CM, Gordon CM, Janz KF, et al. The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations. *Osteoporos Int*. 2016;27:1281-1386.

19. Hernlund E, Svedbom A, Ivergard M, et al. Osteoporosis in the European Union: medical management, epidemiology and economic burden. A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). *Arch Osteoporos*. 2013; 8: 136-013-0136-1.

20. Kanis JA. Diagnosis of osteoporosis and assessment of fracture risk. *Lancet*. 2002;359:1929-1936.

21. Fuleihan G, Bouillon R, Clarke B, et al. Serum 25-hydroxyvitamin D levels: variability, knowledge gaps, and the concept of a desirable range. *J Bone Miner Res*. 2015;30:1119-1133.

22. Petrie A, Sabin C. *Medical Statistics At a Glance*. Oxford, UK: Blackwell Science Ltd.; 2000.

23. Whitley E, Ball J. Statistics review 4: sample size calculations. *Crit Care*. 2002;6:335-341.

24. De Vocht A. Basishandboek SPSS 23. IBM SPSS Statistics. *The Netherlands: Bijleveld Press*; 2015.

25. Van Geloven N, Dijkgraaf MGW, De Haan RJ, De Borgie CAJM, Corten I, Opmeer BC. *E-Learning Practical Biostatistics: Course Handouts*. Amsterdam, The Netherlands: AMC Clinical Research Unit; 2012.

26. Barat P, Barthe N, Redonnet-Vernhet I, Parrot F. The impact of the control of serum phenylalanine levels on osteopenia in patients with phenylketonuria. *Eur J Pediatr*. 2002;161:687-688.

27. Donaldson LJ, Reckless IP, Scholes S, Mindell JS, Shelton NJ. The epidemiology of fractures in England. *J Epidemiol Community Health*. 2008;62:174-180.

28. Coakley KE, Douglas TD, Goodman M, Ramakrishnan U, Dobrowolski SF, Singh RH. Modeling correlates of low bone mineral density in patients with phenylalanine hydroxylase deficiency. *J Inherit Metab Dis*. 2016;39:363-372.

29. Demirdas S, van Spronsen FJ, Hollak CEM, et al. Micronutrients, essential fatty acids and bone health in phenylketonuria. *Ann Nutr Metab*. 2017;70:111-121.

30. Lage S, Bueno M, Andrade F, et al. Fatty acid profile in patients with phenylketonuria and its relationship with bone mineral density. *J Inherit Metab Dis*. 2010;33(Suppl 3):S363-S371.

31. Greeves LG, Carson DJ, Magee A, Patterson CC. Fractures and phenylketonuria. *Acta Paediatr*. 1997;86:242-244.