Bone Health in Adults with Phenylketonuria: A Cross-Sectional Study

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Abstract

Available literature documenting BMD in patients with PKU is mostly reported among heterogeneous populations including adults and children. We aim to describe the bone health status among adults (aged >18 years) affected with Phenylketonuria (PKU) and to evaluate the effect of diet and exercise on bone mineral density (BMD). Sample size of the study population was 27. Enrolled patients underwent multi-site Dual-energy X-ray absorptiometry (DXA) scan and laboratory tests. Nutritional and physical activity records were obtained on each subject to ascertain bone health. BMD in patients with PKU was low normal. 14% of the study subjects were found to have osteoporosis in at least one measured skeletal site. 70% had low BMD in one or more of the measured skeletal sites. BMD score was lowest at radius. Moderate correlation was observed between femoral and radial BMD and serum calcium level. Dietary intake of vitamin A was moderately correlated with BMD T-scores in femur. Our results indicate that BMD in patients with PKU is low normal with better BMD with vitamin A intake, trend towards better bone health with physical exercise and Sapropterin intake.

Keywords: Bone mineral density, PAH deficiency, PKU, Phenylketonuria, Phenylalanine.

Introduction

Phenylketonuria (PKU) (MIM#261600) is an autosomal recessive metabolic disorder caused by deficiency of the enzyme phenylalanine hydroxylase (PAH) (EC 1.14.16.1). PAH deficiency presents a spectrum of severity. Most severe are individuals with 'classical PKU' who have a complete or near-complete enzyme deficiency that typically results in untreated blood phenylalanine (PHE) levels of >1,200 µmol/l.

The American College of Medical Genetics and Genomics recommends The ACMG recommends lifelong treatment of all individuals with untreated blood phenylalanine levels greater than 360 µmol/L with a goal treatment range of 120–360 µmol/L [1]. The foundation of treatment in individuals with PKU is natural protein restriction. Despite the obvious benefits of dietary restriction, it has been conjectured that the dietary restriction may result in poor bone health. Other authors have postulated an inherent bone defect in patients with PAH deficiency that results in low bone mineral density (BMD) [2]. Recently, based on a study in Pah^{enu2} mouse model, it was suggested that hyperphenylalaninemia has a negative impact on differentiation mesenchymal stem cells into bone [3].

There is paucity of data on bone health among adults with PKU. Available literature documenting poor bone health has usually been reported among heterogeneous populations including both adults and children [4]. We evaluated bone health in adult patients with PKU including bone density measurement at several sites, biochemical parameters, bone turnover markers (BTM), detailed nutritional intake and physical activity assessment.

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Methods

Study Design

The study protocol was approved by the institutional ethics committee. A cross-sectional observational study was conducted from July 2010 to December 2011.

Subjects

Study Subject Recruitment: Adults affected with PKU followed at McGovern Medical School, The University of Texas Health Science Center, Houston, were invited to participate in the study. Announcements were posted on the PKUTexas Facebook group and PHEearless (Adult retreat Facebook group) to recruit additional subjects. IRB approved flyers were distributed to metabolic clinics in the state of Texas. Patients who were 18 years of age or older and diagnosed with PKU at birth were enrolled. Peri-menopausal, menopausal and pregnant women were excluded because of increased bone loss related to hormonal mediated factors and the risk of radiation during Dual-energy X-ray absorptiometry (DXA) scan in pregnancy, respectively. Women who had been pregnant or who had breastfed within one year of study enrollment were excluded because of associated rapid bone loss thus serving to confound the data. Patients taking bisphosphonates were also excluded as the drug alters BMD. Individuals were eligible for the study regardless of their intake of vitamin and mineral supplements, metabolic medical foods, large neutral amino acids and Sapropterin (KUVAN®, BioMarin Pharmaceutical Inc., Novato, CA). Enrollment into the study occurred over a period of 18 months. Eligible individuals were enrolled after an informed consent was obtained.

Data Collection

All enrolled participants were evaluated in the Center for Clinical and Translational Sciences Clinical Research Unit (CRU) at Memorial Hermann Hospital TMC, Houston, TX. History, physical examination, lab tests and DXA was performed on day of the visit. Age, sex, ethnicity and anthropometric data were recorded. Ethnicity data was collected as there are differences in bone health between ethnic groups in both men and in women [5].

Weight was measured using a Detecto® scale and height was measured using an Accustat Genentech stadiometer. All study subjects underwent a multi-site DXA scan by Lunar Prodigy Advance DXA system (analysis version: 11.40). BMD was measured at AP spine (L1-L4), bilateral femur (neck and total) and bilateral radii (radius ultra-distal, forearm radius 33%, radius total). BMD was defined using the World Health Organization T-score which is the number of standard deviations compared to a reference normal young population (NHANES ages 20-30 years)/ USA (ages 20-40 years). A T-score ≤−2.5 was interpreted as osteoporosis, a T-score between −2.5 and −1 as low bone mass (previously osteopenia) and a T-score ≥-1 as normal. Although T-score are generally used for BMD estimation in adults, we also used Z-scores since they are more accurate as they compare to same age and sex. A Z-score ≤−2 was interpreted as low bone density. Fasting blood and urine samples were collected at the time of the CRU visit. Laboratory studies included blood chemistry (kidney and liver function, electrolytes, levels of vitamin A, 25-hydroxy vitamin D (25(OH) D), 1-25-dihydroxy vitamin D (1-25$(OH)_2$ 2D), intact parathyroid hormone (iPTH), BTM (including bone–specific alkaline phosphatase (bsALP), osteocalcin, n-telopeptide), plasma amino acids, copper, zinc, magnesium, manganese, ferritin and hemoglobin. Information about Sapropterin intake was collected for each subject. Nutritional assessment of study subjects was performed using a single three-day diet record and the diet was analyzed using MetabolicPro (https://www.metabolicpro.org/). Physical activity was assessed using the National Institutes of Health questionnaire called *Check Up on Your Bones* that included sex, age, smoking habits, medical and family history, sun exposure and amount of exercise. Physical activity of the study subjects was reported in terms of days per week.

Statistical Analysis

The statistical program Stata (v.14, College Station, TX, USA) was used. Categorical variables are expressed as frequencies (with percentages) and comparisons across groups was performed using Chi-square or Fisher exact tests. Continuous variables were assessed for normality using the Shapiro-Wilk test. Continuous variables are expressed as mean ± standard deviation (SD) or median and interquartile range (IQR), for normally and not normally distributed data, respectively. Comparison of the means of quantitative variables across groups was done using the Student-t test or ANOVA (with *post-hoc* Tukey test) if they followed normal distribution and using the Mann–Whitney U test or Kruskal-Wallis (with *post-hoc* Dunn's test) if not. A p-value of <0.05 was considered statistically significant. Pearson or Spearman correlation coefficients (ρ) was calculated to assess correlations between the continuous demographic variables (age, height, weight, and body mass index (BMI) and bone-specific BMD.

Results

A total of 27 subjects (aged 28 ±7.5 years) including 16 females and 11 males met study inclusion criteria and consented to participate in the study. Study participants included non-Hispanic white (n=25), Hispanic (n=1) and African American (n=1) individuals. The mean height and weight of the study subjects was 1.62 ±0.09 meters and 66.6 ± 15.8 kg respectively.

Bone mineral density

Mean BMD T-score for the study population was low normal at AP spine (-0.34±1.14) and femur (-0.64±1.10) and osteopenic at radius (-1.24±0.90) (Table 1). The BMD T-scores at each of the four anatomical sites had statistically significant moderate to strong correlation with the BMD T-scores at the other sites (ρ=0.56 – 0.92, p<0.005) (Table 2). The BMD T-score values were significantly lower at the radius than the other three sites (p<0.001). Although the BMD T-score values were slightly higher in the AP spine compared to the femur and femoral neck, the difference was not statistically significant (p=0.09 and p=0.12, respectively). Four subjects (14%) in our study population were found to have osteoporosis on at least one site. Nineteen (70%) of the subjects had decreased BMD (osteoporosis/ osteopenia) in one or more of the measured skeletal sites. Six patients (22%) had decreased BMD (osteoporosis/ osteopenia) at all three sites. One patient had osteoporosis and seven (26%) had low bone mass at AP spine. Z-scores showed similar findings to T-scores with one patient who had a Z-score < -2.0 at AP spine (Z-scores were only available rounded to 1 decimal space, so a value of -2.0 rather than -1.96 was used to signify two standard deviations from the mean). Z-score results indicated that one patient had osteoporosis and seven had low bone mass at femur (dual femur total mean BMD). Additionally, one patient had Z-score <-2 at femur, two had osteoporosis (one in radius ultra-distal and one in radius forearm) and 14 (52%) had low bone mass in radius (radius total BMD). Seven patients had Z-score <-2 at radius. One patient had osteopenia at femur and osteoporosis at radius. This patient has a history of post traumatic pelvic fracture 15 years prior to the study. Another patient with past history of traumatic fracture had normal bone density at all three sites.

BMD correlations with demographics

Statistically significant correlations were identified between various demographic parameters and BMD T and Z-scores at various anatomical sites (Table 3). Age was negatively correlated to BMD (declining BMD with advancing age), while weight and BMI were positively correlated with BMD (increasing BMD with increasing body weight). The correlation between height and BMD was weak (Table 3). There was no statistically significant correlation between BMD scores and ethnicity or gender of the study subjects.

Table 1. Mean BMD T-scores and Z-scores at various sites in the study population

	BMD T-scores		BMD Z-scores	
	mean	Standard deviation	mean	Standard deviation
AP Spine	-0.34	1.14	-0.25	1.10
Femur*				
Left	-0.60	1.10	-0.53	0.89
Right	-0.65	1.13	-0.44	0.99
Total	-0.64	1.10	-0.56	0.90
Femoral neck*				
Left	-0.50	1.23	-0.62	0.84
Right	-0.70	1.17	-0.65	0.86
Total	-0.67	1.14	-0.64	0.84
Radius*				
Ultra-distal	-0.71	1.23	-0.74	1.22
Forearm 33%	-0.90	0.83	-0.93	0.88
Total	-1.24	0.90	-1.27	0.92

Table 2. Correlation coefficients between BMD T-scores at various sites

	AP Spine	Femur	Femoral neck
Femur	0.70		
Femoral neck	0.56	0.92	
Radius	0.59	0.76	0.71

Table 3. Demographic distribution of study sample and correlation with total BMD

	Mean (SD)	Correlation with Total BMD T-scores			
		Femoral	Femoral Neck	Radial	AP Spine
Age, years	28.0 (7.5)	-0.45 *	-0.40 *	-0.29	-0.19
Height, m	1.62 (0.09)	-0.21	-0.13	-0.17	-0.44 *
Weight, kg	66.62 (15.84)	0.55 **	0.61 ***	0.53 **	0.15
BMI	25.45 (6.02)	0.65 ***	0.67 ***	0.62 ***	0.38

*Statistically significant at p<0.05 to p>=0.01
**Statistically significant at p<0.01 to p>=0.001
***Statistically significant at p<0.001

Biochemical markers and BMD

Correlation analysis showed a statistically significant correlation between femoral and radial BMD T-scores with serum calcium levels (mg/dl) (ρ = 0.44 for both) and moderate correlation of radial BMD T-score and pre-albumin level (ρ = 0.40). The mean values of serum biochemical markers among patients with BMD T-scores ≤-1 were not significantly different from those with BMD T-scores >-1. Mean PHE level among the study population was 702±375 µmol/l (11.6±6.2 mg/dl). Most patients had normal serum biochemical markers except for serum 25 (OH)vitamin D (deficiency in 2 and insufficiency in 7 patients), serum copper (low in 8 patients), bone specific ALP (high in 7 patients) and serum tyrosine levels (low in 8 patients) (Table 4). Vitamin D deficiency was defined as a 25(OH) D below 20 ng/ml, and vitamin D insufficiency as a 25(OH)D of 21–29 ng/ml [1].

Bone turnover markers and BMD

Mean (SD) serum osteocalcin and bsALP in the study population were 33.4±77.3 ng/ml (nl=3.2-39.6) and 15.8±7.03 ug/L (nl=0.0-20.1) respectively. Urinary n-telopeptide/creatinine ratio was 54.6±27.2 nM BCE/mM Cr (nl=5-65). Serum or urinary BTM did not correlate significantly with BMD at different anatomical sites (Table 5). The mean values of BTM among patients with BMD T-scores ≤-1 were not different from those with BMD T-scores >-1.

Sapropterin intake and BMD

Among the study group, 7/27 (26%) patients were on Sapropterin therapy at the time of evaluation. The femoral and femoral neck BMD T-scores were slightly higher in patients on Sapropterin therapy, but the difference did not reach statistical significance (Table 6).

Nutritional intake and BMD

The mean dietary intake of vitamin A was 1052.7 RAE while calcium was 1429 mg, which was higher than the dietary reference intake (DRI). The dietary intake of vitamin A was positively correlated with BMD T-scores in the femur (ρ=0.56) and femoral neck (ρ=0.63); p<0.005 for both sites. Other nutritional parameters especially 24-hr dietary calcium and vitamin D intake did not correlate significantly with BMD T-scores (Table 7).

Physical activity and BMD

Half of the study subjects performed physical activity for 2-5 days/week and 15% of the patients performed physical exercise for more than 5 days a week (Table 8). Weight bearing exercise was performed by a significantly smaller proportion of subjects (46% none; 35% 1-5 days a week; 19% >5 days/week) (Table 9). The median BMD T-score by skeletal site and frequency of physical activity and frequency of weight bearing exercise is shown in Table 8 and Table 9, respectively. The BMD T-scores for the femoral neck were moderately correlated with both physical activity (Spearman correlation coefficient, ρ=0.48, p=0.013) and weight bearing exercise (ρ=0.45, p=0.028). None of the other anatomical sites demonstrated any correlation between the BMD correlation between the BMD T-scores and activity or exercise. In addition, both frequency of physical activity and frequency of weight bearing exercises were strongly correlated (ρ=0.74, p<0.001). This trend of increasing BMD T-scores with increasing frequency of activity or exercise was also evident after coding of the frequencies into categorical groups. Physical activity was stratified into low (0-1 days per week), moderate (2-4 days per week) and high (at least 5 days per week). BMD T-scores were significantly higher in the high activity group (median: -0.3, IQR: -0.8 to 1.1) than in the low activity group (median: -1.3, IQR: -2.15 to -0.65) (Dunn's test p-value = 0.009).

Table 4. Mean serum concentrations of biochemical markers and correlations with BMD T-scores

	Serum concentratio, mean (SD)†	Reference range	# Outside normal range	Correlation with Total BMD			
				Femoral	Femoral Neck	Radial	AP Spine
Total protein, g/dl	7.2 (0.4)	6.1-8.1	0	0.27	0.22	0.27	0.23
Albumin, g/dl	4.6 (0.3)	3.6-5.1	0	0.02	0.02	0.02	-0.31
Bone-specific alkaline phosphatase, µg/L	71.5 (22.0)	4.7-29.3	7	-0.02	0.08	0.08	0.06
Calcium, mg/dl	9.6 (0.3)	8.6-10.3	1	0.44*	0.28	0.44*	0.28
Phosphorus, mg/dl	3.3 (0.6)	2.5-4.5	2	0.28	0.34	0.25	0.23
Intact parathyroid hormone (iPTH), pg/ml	30.0 (8.8)	14-64	0	-0.08	-0.01	0.00	-0.11
25-OH vitamin D, ng/mL	38.6 (18.2)	30-100	12	0.00	-0.04	0.02	-0.20
Copper, mcg/dl	121.9 (52.7)	70-175	8	0.13	0.09	0.29	0.30
Zinc, mg/dl	97.7 (16.8)	9-14.7	0	-0.14	-0.23	0.04	-0.29
Magnesium, mg/dl	2.1 (0.2)	1.5-2.5	0	0.01	0.14	-0.33	-0.23
Vitamin A, mcg/dl	62.0 (16.9)	38-98	1	0.06	0.04	0.32	0.25
Pre-albumin, mg/dl	30.3 (5.9)	17-43	2	0.18	0.13	0.40 *	0.22
Phenylalanine, µmol/L	706.0 (374.7)	40-74	0	-0.18	-0.22	0.04	0.03
Tyrosine, µmol/L	63.5 (39.5)	38-96	8	0.26	0.32	0.23	0.20

† SD = standard deviation; sample size is 27 for each analyte except for iPTH and Vitamin A (n=25) and Copper and Zinc (n=26)
* Statistically significant at $p<0.05$

Table 5. Mean concentrations of bone turnover markers and correlations with BMD T-scores

	n	Concentration		Correlation with Total BMD T-scores			
		mean	SD	Femoral	Femoral Neck	Radial	AP Spine
Serum							
Osteocalcin, ng/ml, 8-38	25	33.42	77.32	-0.03	-0.01	0.15	-0.21
Bone-specific alkaline phosphatase, µg/L	26	15.85	7.03	-0.26	-0.08	-0.30	-0.35
Urine							
n-telopeptide, nmol	25	438.60	363.03	0.00	0.10	-0.10	-0.16
n-telopeptide/creatinine ratio	24	54.63	27.20	-0.26	-0.15	-0.15	-0.28

Table 6. BMD T-scores and Sapropterin therapy.

Total BMD T-scores mean (SD)	Sapropterin use		p-value
	Yes (n=7)	No (n=20)	
Femoral	-0.56 (1.63)	-0.67 (0.91)	0.829
Femoral Neck	-0.33 (1.78)	-0.79 (0.85)	0.368
Radial	-1.24 (1.3)	-1.25 (0.77)	0.996
AP Spine	-0.59 (1.64)	-0.26 (0.96)	0.521

Table 7. Nutritional Intake and BMD.

	Average Daily Intake		Correlation coefficients with Total BMD T-scores			
	mean	SD	Femoral	Femoral Neck	Radial	AP Spine
Protein, g	65.0	25.0	0.16	0.34	0.23	-0.14
Vitamin A, IU	7018.1	5802.7	0.56**	0.63**	0.37	0.29
Vitamin D, IU	574.2	492.7	0.05	0.12	0.21	-0.1
Phenylalanine, g	0.8	0.5	-0.20	-0.09	-0.09	-0.22
Tyrosine, g	5.4	2.4	0.06	0.17	0.14	-0.11
Arginine, g	4.2	2.0	0.17	0.32	0.23	-0.12
Calcium, mg	1429.5	691.9	0.16	0.26	0.31	-0.03
Phosphorus, mg	1557.0	611.4	0.11	0.21	0.26	-0.11
Magnesium, mg	417.6	188.9	0.04	0.09	0.09	-0.24
Manganese, mg	3.7	1.5	0.05	0.14	0.00	-0.02

**Statistically significant, $p < 0.005$

Table 8. BMD T-scores by skeletal site and frequency of physical activity.

Physical activity (days / week)	Number of patients†	Median (IQR) BMD T-scores *			
		Femoral	Femoral Neck	Radial	AP Spine
0	2	-1.2 (-2.5, 0.1)	-1.35 (-2.4, -0.3)	-1.25 (-2.4, -0.1)	-0.05 (-1.2, 1.1)
1	6	-1.5 (-1.9, -0.5)	-1.3 (-1.9, -1)	-1.8 (-2.1, -1.6)	-0.85 (-1.8, -0.4)
2	3	0.0 (-2, 0.6)	-0.4 (-2.2, 0.1)	-1.8 (-2, -0.7)	0.0 (-1.1, 1.3)
3	2	-0.35 (-0.7, 0)	-0.8 (-1.1, -0.5)	-1.15 (-2.1, -0.2)	-1.05 (-1.3, -0.8)
4	2	-0.3 (-0.5, -0.1)	0.25 (-0.4, 0.9)	-1.05 (-1.4, -0.7)	0.2 (-0.4, 0.8)
5	6	0.0 (-1.2, 0.4)	-0.6 (-0.8, 1.1)	-0.9 (-1.5, 0.2)	0.0 (-1.4, 0.7)
6	1	1.0 (1, 1)	1.6 (1.6, 1.6)	-0.4 (-0.4, -0.4)	0.4 (0.4, 0.4)
7	4	-0.65 (-0.85, -0.3)	-0.25 (-0.55, -0.15)	-0.85 (-1.35, -0.75)	-0.4 (-1.3, 0.35)
Correlation coefficients, n=26	0.25	0.48	0.31	0.13	
p-value		0.223	0.013	0.126	0.54

† Data on physical activity missing for one (1) patient; * IQR = interquartile range

Table 9. BMD T-scores by skeletal site and frequency of weight bearing exercise.

Weight bearing exercise (days per week)	Number of patients†	Median (IQR) BMD T-scores*			
		Femoral	Femoral Neck	Radial	AP Spine
0	12	0.9 (2.05, 0.05)	-1.05 (-2, -0.35)	-1.65 (-2.25, -0.65)	-0.3 (-1.3, 0.75)
1	2	0.2 (0.7, 0.3)	-0.7 (-1.1, -0.3)	-2 (-2.1, -1.9)	-0.85 (-1.3, -0.4)
2	2	0.9 (0, 1.8)	0.55 (-0.5, 1.6)	-0.35 (-1.5, 0.8)	1.5 (0.7, 2.3)
3	1	0 (-)	-0.5 (-0.5, -0.5)	-0.2 (-0.2, -0.2)	-0.8 (-0.8, -0.8)
4	4	0.35 (0.8, 0.05)	-0.15 (-)	-1.15 (-)	-0.5 (-)
5	2	0.5 (0, 1)	0.4 (-0.8, 1.6)	-0.1 (-0.4, 0.2)	0.2 (0, 0.4)
6	0	-	-	-	-
7	1	0.7 (-)	-0.3 (-)	-0.7 (-)	-0.2 (-)
Correlation coefficients, n=24		0.28	0.45	0.35	0.09
p-value		0.186	0.028	0.095	0.69

† Data on physical activity missing for three (3) patients; * IQR = interquartile range

Similarly, BMD T-scores were higher in the high weight bearing exercise group (2 or more days per week; median T-score: -0.25, IQR: -0.5 to 0.9) than in the low weight bearing exercise group (0 to 1 days per week; median T-score: -1.05, IQR: -1.9 to -0.3) (Mann-Whitney ranked sums p=0.026).

Discussion

In this study, we measured BMD at different skeletal sites and assessed multiple factors that have been implicated in bone health. Twenty-five of the 27 subjects were diagnosed with PKU as neonates. All 27 subjects were on PHE-restricted diet. About a quarter of the patients were on Sapropterin therapy. Four subjects (14%) in our study population were found to have osteoporosis on at least one site. Nineteen (70%) of the 27 subjects had low BMD (osteoporosis/osteopenia) in one or more of the measured skeletal sites. Physical activity, importantly weight bearing exercise was significantly associated with improved femoral neck BMD. We noticed dietary vitamin A intake to have a positive impact on the femoral bone health in this patient population. Patients on Sapropterin therapy had slightly higher femoral BMD.

BMD

Our results demonstrate low bone density among the study group. We found low bone mass/osteoporosis in at least one site (femur/ AP spine/ radius) in 70% of our study population. 14% patients had osteoporosis on at least one site. Miras et al. measured BMD in spine and femur and found a reduced mineral bone disease (MBD) prevalence in PKU of 14% (osteoporosis in 2 and osteopenia in 4 patients) [6]. Modan-Moses et al. who studied BMD in spine and femur detected osteopenia (defined as Z-score between–1.0 and–2.5) in 38.7% of their adult PKU patients, while osteoporosis was detected in 6.5% (defined as Z-score<–2.5) [7]. Studies assessing a BMD in mixed group of pediatric and adult patients have also reported altered BMD in patients with PKU [2, 8–10]. A recent meta-analysis and systematic review on bone health in PKU by Demirdas et al. revealed that the mean total body, lumbar spine, and femoral hip BMD Z-scores in patients with PKU are lower than in their healthy peers, but well within the reference range for normal. They reported a projected 10% of patients have a BMD Z-score below -2; however, 90% of early treated patients with PKU are not at risk for low BMD [4]. The difference in numbers between our study and earlier studies largely appears to be contributed by the fact that none of these other studies measured radius BMD. A significant proportion of our patients had decreased BMD in the radius (63%).

Bone mineral density has been shown previously to decline with age. The observed decline happens in the fifth-sixth decade of life. We found a trend towards declining BMD with advancement in age and our population aged 28 ±7.5 years. This correlation was poor to moderate in magnitude. Perez-Duenas who studied a heterogeneous group of PKU patients (age range: 10-33 years) found no correlation between BMD and age [9, 11]. Despite a relatively young population with a mean age of 28 ±7.5 years, 2 patients (7%) among our study group had history of post-traumatic fractures. The femoral BMD T-scores in these patients was 0.1 and -2.4 respectively. Modan-Moses et al. [7] reported fracture incidence of 13% in their patients with PKU at a mean age of 25 ±5.3 years. Greeves et al. [12] have reported a significantly higher risk of fracture over the age of 8 years with a relative risk of 2.6 (95% CI 1.1-6.1). The low BMD and relatively higher fracture rate than the normal population emphasizes the importance of the need for promotion of bone health in this population early in life at the time of peak bone recruitment.

Skeletal growth proceeds through the coordinated action of bone deposition and resorption to allow bones to reach their adult form [13]. The process of bone modeling begins during fetal growth and continues until epiphyseal fusion, usually by the end of the second decade of life [14]. The bone mass achieved by young adulthood is a critically important determinant to lifelong bone health. The cause of decreased BMD in PKU patients is not established. It may be multifactorial including factors like diet restrictions as well as inherent bone mineralization and maturation defects [3]. Thus, discussions about means to improve skeletal health in patients with PKU should be initiated early.

We found BMI and radial BMD showing the best correlation at 0.67 (p<0.001).

BMD at radius vs. other sites

Interestingly, we found the BMD scores to be lowest at the radius as compared to femur and AP spine. Similar findings are reported in other studies. Most of these studies assessing BMD in patients with PKU used DXA AP spine and femur to assess BMD except Schwahn et al. [8] who used peripheral quantitative computed tomography (pQCT) to assess radial BMD. They found significant alterations of architecture or composition of trabecular bone. Recently Choukair et al. [15] also analyzed macroscopic bone architecture in the radius and forearm muscle size by pQCT and muscle strength by hand dynamometry. They found that radial bone is characterized by inadequately reduced bone strength in relation to muscular force, reduced cortical thickness, and reduced total BMD at the metaphyseal site [15]. Increased bone resorption and largely unchanged bone formation has been shown to result in the loss of bone mineral during space flight [16]. The cause of reduced radial BMD in PAH deficient patients may be due to compounded effect of lack of weight bearing to the primary alteration of bone metabolism in patients with PKU. More studies are needed to evaluate this finding. It will be interesting to study the frequency of fractures at various sites among individuals with PKU and compare them with the general population. The information learned may play a role in designing appropriate exercise regimens for patients with PKU.

Bone turnover markers

We measured bsALP and osteocalcin (markers of bone formation) N-telopeptide (marker of bone resorption). No abnormalities in the mean levels of BTM were identified in our study. Thus, alteration in bone turnover was not found in our study. Also, BMD did not correlate with BTM.

Imbalance between bone resorption and formation marker levels, together with increased urinary Ca, have been implicated as a cause for high risk for osteopenia or osteoporosis in later life in patients with PKU [17]. Earlier studies have found significant alterations in one or more BTMs [17, 18]. In a recent study in Pah^{enu2} mouse model, PAH deficient cells were found to have significantly reduced alkaline phosphatase activity [3]. Further studies may be needed to understand the role of altered bone turnover contributing to decreased BMD in patients with PKU.

Blood PHE levels and BMD

We found poor correlation between mean PHE levels and BMD at different sites (ρ= -0.22 to 0.04). Earlier studies (including adult PKU patients) that have investigated the correlation between PHE blood levels and BMD have not identified any correlations [2, 7, 9, 10, 19]. Barat et al. [19] however reported that variation in PHE levels may correlate with osteopenia (study population ranged 5-21 years). Recently, in a study on Pah^{enu2} mouse model, the authors found hyperphenylalaninemia down-regulates mineralization more significantly in PAH deficient cells [3].

A limitation to the extrapolation of our results is that the levels of PHE may fluctuate and a single measurement was used for purpose of this study. Using mean PHE levels over several months would be more reliable.

Vitamin D status in patients with PKU

Like our results, prior studies report no correlation between plasma 25-hydroxyvitamin-D and BMD at any site [7, 9, 10]. Miras et al. [6] found no statistical differences in calcium, phosphorus, fat intake, PHE, vitamin D and iPTH, in individuals with low and normal BMD. The results suggest that patients with PKU are not at an increased risk over the general population for poor bone health due to vitamin D. Normal levels should be maintained to avoid an additional factor that could contribute to decreased BMD in patients with PKU.

BMD and BH4

We found a trend towards better BMD at radius and femur with Sapropterin intake. But mean AP BMD was lower in Sapropterin group. The differences did not reach statistical significance at any of the sites. In contrast, in a previous study conducted by Miras et al.[6], none of the twelve PKU patients treated with tetrahydrobiopterin (27.9% of the study patients), for an average of 7.1 years, developed reduced MBD. Depending on response to tetrahydrobiopterin, a higher intake of natural protein may be tolerated. The protein intake was higher among our patients on Sapropterin (mean: 84.4± 23.4 grams) compared to those that were not on Sapropterin (mean: 59.0±22.7) (p=0.027). However, there was no correlation between protein intake and BMD at any of the four anatomical sites. Further long-term evaluation is needed to evaluate the effect of Sapropterin intake on bone health in these patients.

Nutrition intake

We did not find correlation between bone health parameters and dietary protein, calcium or phosphorous intake in our cohort unlike some other studies [6, 11]. Instead vitamin A intake was associated with better bone health in our cohort. Because the finding is unique to our study, it needs to be prospectively assessed in future studies before making further recommendations. In general, vitamin A plays a role in bone health, but the strength of available evidence is not strong [20].

BMD and physical activity

We identified a statistically significant trend of increasing femoral neck BMD T-scores when compared across the three physical activity frequency groups (low, moderate, and high). The trend was driven by a significant difference between the low and high groups. Similar trends were also observed for femoral neck BMD T-scores and frequency of weight bearing exercises. However, interpretation of this result should be moderated by the fact that the sample size was limited in the individual strata. The small sample sizes in these groups increase the error present in the predicted estimates. However, although not statistically significant, the correlation coefficients for the femur and the radius did suggest that there might be a trend of slightly increasing BMD in an individual with increasing physical activity – a result that we may not have been able to identify due to a lack of power. Another limitation in the analysis was that there was no record of the duration, or intensity of physical activity performed each day. Miras et al. [6] found no statistical differences in physical activity level in individuals with low and normal BMD. Due to a lack of consistency across studies, small sample sizes and paucity of data in this population, these results on physical activity require further evaluation in larger cohorts to determine the true effect of physical activity on bone health in patients with PKU.

Bone modeling is sensitive to mechanical loading, emphasizing the importance of physical activity throughout growth [13]. A significant proportion of peak bone mass is acquired during adolescence [20]. In general, compliance to dietary restriction and other recommendations is usually more difficult to achieve in adolescence than in childhood [2]. To prevent osteoporosis in adulthood, adequate recommended nutritional intake and physical exercise are essential to achieve optimal peak bone mass. Thus, these parameters should be carefully assessed during each clinic visit in patients with PKU starting early in childhood and very closely during adolescence to prevent low bone mass later in life. Moderate physical exercise may be recommended in patients with PKU for at least 5 days a week. Weight bearing exercise forces

work against gravity. Some examples include weight training, walking, hiking, jogging, climbing stairs, tennis and dancing

In summary, our results indicate that BMD in patients with PKU is low normal with better BMD with vitamin A intake, trend towards better bone health with physical exercise and Sapropterin intake. The skeletal system is a dynamic organ with that experiences the process of bone remodeling that continues throughout life. Patients with PKU should be educated and counseled about bone health at each clinic visit starting early in life. Maintaining DRI of macronutrients and micronutrients and weight bearing exercise are crucial in maintaining the equilibrium of bone metabolism in balance in PKU population who are predisposed to low bone mass. Close monitoring is needed. The limitation of the study is that it was a snap-shot assessment rather than longitudinal evaluation.

Study Limitations

Since this is an observational cross-sectional study, it lacks the perspective on long term management of the patients. BMD is best correlated with the median of the Phe levels and not an isolated value. It would be good to consider long term disease management as that will unequivocally influence bone health. Additionally, the study covers patients on varying treatment options that could potentially influence bone health.

Author's Notes

This paper is dedicated to the memory of Mary Ruppe, MD.

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Authors' Contributions

AA Analysis and data interpretation, Manuscript writing, Critical revision, Final approval. DR Analysis and data interpretation, Critical revision, Final approval. HN Acquisition of Data, Critical revision, Final approval. MR Conception and design, Acquisition of Data. SH Analysis and data interpretation, Statistical analysis, Critical revision, Final approval. HS Conception and design, Acquisition of Data, Technical procedures, Manuscript writing, Critical revision, Final approval.

Declaration of Conflicting Interests

Hope Northrup is investigator in clinical trials sponsored by BioMarin Pharmaceutical Inc.

HN has participated in advisory boards and received consulting fees (BioMarin). HN provides presentations on Palynziq® for Symbiotix and is compensated.

Heather Saavedra is investigator and dietitian in clinical trials sponsored by BioMarin Pharmaceutical Inc. HS has participated in advisory boards and received consulting fees (BioMarin). HS provides presentations on Palynziq® for Symbiotix and is compensated.

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