Molecular Genetics and Metabolism 105 (2012) 566–570

Contents lists available at SciVerse ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Relationships between lumbar bone mineral density and biochemical parameters in phenylketonuria patients

Martijn J. de Groot [a,b,*], Marieke Hoeksma [a,b], Margreet van Rijn [c], Riemer H.J.A. Slart [d], Francjan J. van Spronsen [a,b]

[a] Beatrix Children's Hospital, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
[b] GUIDE, Graduate School for Drug Exploration, Groningen, The Netherlands
[c] Section of Metabolic Diseases, Beatrix Children's Hospital, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
[d] Department of Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

ARTICLE INFO

Article history:
Received 8 January 2012
Accepted 8 January 2012
Available online 16 January 2012

Keywords:
Phenylketonuria
PKU
Bone mineral density
BMD
Dual-energy X-ray absorptiometry
DXA

ABSTRACT

Background: The etiology of reduced bone mineral density (BMD) in phenylketonuria (PKU) is unknown. Reduced BMD may be inherent to PKU and/or secondary to its dietary treatment.
Materials and methods: Lumbar BMD was measured by dual-energy X-ray absorptiometry in 53 early and continuously treated PKU patients (median age 16, range 2–35 years). First, Z-scores of BMD were correlated to age group, clinical severity of PKU, mean phenylalanine (Phe) concentration and Phe variation in the year prior to DXA scanning, as well as to blood vitamin, mineral, and alkaline phosphatase concentrations. Second, parameters were compared between subjects with reduced BMD (Z-score < -2 SD) and subjects with normal BMD.
Results: BMD was significantly reduced in our cohort ($p = 0.000$). Z-scores of BMD were neither significantly correlated to age group, nor clinical severity of PKU. Both mean Phe concentration and Phe variation in the year prior to DXA scanning did not significantly correlate with Z-scores of BMD. Higher blood calcium concentrations were significantly associated with lower BMD ($r^2 = -0.485$, $p = 0.004$). Other biochemical parameters, including vitamin B12 availability markers, did not show significant correlations with Z-score of BMD. Subjects with reduced BMD had significantly higher blood phosphorus concentrations than subjects with normal BMD ($p = 0.009$). No other significant differences were found between both BMD groups.
Conclusion: Reduced BMD in PKU is present from early age onward and does not progress with age. Therefore, BMD deserves attention from early age onward in PKU patients. Our findings are consistent with increased bone turnover in PKU. It remains unclear whether reduced BMD is inherent to PKU and/or secondary to its dietary treatment.

1. Introduction

In phenylketonuria (PKU, OMIM 261600; phenylalanine hydroxylase (EC 1.14.16.1) deficiency), reduced bone mineral density (BMD) has been reported by several authors [1–11]. Accordingly, subjects with PKU may have an increased fracture risk compared to healthy individuals [12]. The pathophysiology of reduced BMD in PKU is unknown. Therefore, it is unclear whether reduced BMD in PKU is the consequence of the disease itself and/or its dietary treatment.

Results on the relationship between BMD and biochemical markers in PKU are inconsistent. Some authors report a negative relationship between BMD and blood Phe concentrations [7,8,10,11,13], suggesting that reduced BMD is the consequence of inadequately treated PKU. However, other authors do not find this correlation [2,3,5,9]. Moreover, some studies report a relationship between reduced BMD and insufficient trace element intake [3,9], suggesting that reduced BMD in PKU may be secondary to dietary treatment. Comparison of results is hindered by differences in measurement technique and site, as well as by heterogeneity of the populations studied. The aim of our study was to investigate the relationships between Z-scores of lumbar BMD and age, clinical severity of PKU, mean Phe concentration and Phe variation of the year prior to DXA scanning, as well as blood concentrations of vitamins, minerals, and alkaline phosphatase. In particular, the relationship between vitamin B12 deficiency and BMD was investigated. PKU subjects are at risk for vitamin B12 deficiency [14–16], which is associated with reduced BMD [17–20] and increased fracture risk [21,22].

Abbreviations: PKU, phenylketonuria; Phe, phenylalanine; BMD, bone mineral density; DXA, dual-energy X-ray absorptiometry.

* Corresponding author at: Beatrix Children's Hospital, University Medical Center Groningen, CA80, P.O. Box 30.001, 9700 RB Groningen, The Netherlands. Fax: +31 503614235.
E-mail address: m.j.de.groot@umcg.nl (M.J. de Groot).

1096-7192/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.ymgme.2012.01.006

2. Materials and methods

2.1. Study subjects

A retrospective cohort study was performed in a population of 53 Dutch PKU patients (25 male; median age 16, range 2–35 years), using data obtained between January 2003 and January 2007. Most (51/53) subjects were Caucasian. All participants were free of concomitant diseases. Patients were diagnosed by the Dutch neonatal screening program on PKU, introduced in the Northern part of the Netherlands in 1967. A Phe-restricted diet was started within the first three weeks of life and continued since then in all patients. Using pre-treatment Phe concentrations, patients were classified as follows: 43% mild HPA, 17% mild PKU, 10% moderate PKU, 30% severe PKU. An alternative classification system is based on Phe tolerance, i.e. the amount of Phe (mg/kg/day) that can be consumed with blood Phe concentration remaining within target range. Phe tolerance at age 24 months was used, which reliably predicts Phe tolerance later in life [23]. Based on Phe tolerance at this age, four categories were defined: mild HPA (75–100% of age-matched recommended natural protein intake), mild PKU (intake 50–74%), moderate PKU (intake 25–49%), and severe PKU (intake<25%). According to this system, patients were classified as follows: 0% mild HPA, 16% mild PKU, 72% moderate PKU, 12% severe PKU.

Therapeutic Phe concentration aims were 200–500 μmol/l for all ages up to 2002. From 2002 onwards, therapeutic Phe concentration aims were age-related: 120–360 μmol/l for subjects <12 years, and 120–600 μmol/l for subjects ≥12 years. Therapeutic aims for total protein intake were 1.0–1.8 g of total protein/kg body weight, decreasing with age.

2.2. Bone mineral density measurements

Lumbar BMD was measured by dual-energy X-ray absorptiometry (DXA), using a Hologic Discovery A densitometer (Hologic Inc., Bedford, MA, USA). DXA radiation doses were below 0.01 mSv, comparable to two days of background radiation. BMD values were expressed as Z-scores of BMD, i.e. BMD relative to sex- and age-matched healthy controls. Reference values for Z-score calculation were obtained from the United States National Health and Nutrition Examination Survey [24]. We used Z-scores rather than T-scores (i.e. the relative value of bone density compared to mean peak bone mass, reached at 25–30 years of age), since the use of T-scores in children leads to false-positive diagnoses of reduced BMD [25]. Measured Z-score of BMD in children is influenced by height, weight, and body mass index. In subjects with low height, weight and/or body mass index for age, measured Z-score of BMD may falsely suggest low BMD for age [26–28]. To analyze whether height, weight, and body mass index biased measured Z-score of BMD, correlations between Z-score of BMD and these growth parameters were studied.

2.3. Blood phenylalanine concentration

Blood spots were sampled on filter paper (type 2992, Scheier and Schuell, Den Bosch, the Netherlands) at home, once every two to four weeks. Blood Phe concentrations were determined in eluates of 1/8 disks punched from dried blood spots. Disks were eluted at room temperature for 30 min in 150 μl TCA solution (6.6% w/v TCA in H_2O) containing norvaline as internal standard, after which the debris was precipitated by centrifugation for 5 min at 14,000 rpm. Ten microliters of supernatant was transferred to a clean reaction vial and prepared for HPLC analysis by the AccQ-Tag® method, according to the manufacturer's protocol (Waters, Breda, the Netherlands).

Mean individual blood Phe concentrations of the year prior to the most recent DXA-scan were calculated, including the blood Phe concentration obtained at DXA-scanning when available. The proportion of the number of Phe concentrations below target range to the total number of Phe measurements was calculated. Mean cumulative variation of successive blood Phe concentrations was calculated using the formula of Barat et al. [8]. This method consists of calculating the differences between each two consecutive blood Phe measurements, summing these differences, and dividing the sum of differences by the number of measurements minus one.

Clinical severity of PAH deficiency was determined by assessing Phe tolerance, using the definitions described above.

2.4. Vitamin and mineral measurements

Data on blood concentrations of calcium, phosphorus, magnesium, vitamin D, total alkaline phosphatase, vitamin B12, methylmalonic acid (MMA), and total homocysteine (tHcy) concentrations were obtained. Vitamin D was measured as 1,25-dihydroxy-vitamin D (calcitriol), the biologically active form. Blood MMA and tHcy concentrations may be used as markers of functional vitamin B12 deficiency. Vitamin B12 is required to convert methylmalonyl-CoA to succinyl-CoA and homocysteine to methionine. In functional vitamin B12 deficiency, these reactions are impaired, resulting in elevated blood concentrations of tHcy and the methylmalonyl-CoA metabolite MMA. Thus, MMA and tHcy seem to be more suited than blood vitamin B12 concentration measurements to detect functional vitamin B12 deficiency [29,30]. Vitamin and mineral concentrations were measured once, preferably on the day of DXA-scanning, and were acquired at regular outpatient department visits. If biochemical data were unavailable for the DXA-scanning day, the most recent data prior to that day were used.

2.5. Study design

Data were analyzed in three ways. First, results of the following three age groups were compared: 0–9.9 years, 10.1–19.9 years and ≥20.0 years. This division into age groups was made to assess whether bone mineral density is reduced during a specific age period. Mean Z-scores of BMD per age group were calculated and compared to the mean Z-score of BMD that a healthy reference population would theoretically have (i.e. mean $Z = 0$). In addition, the proportion of subjects with Z-scores of BMD < -2 SD was calculated for each age group.

Second, analyses on the relationship between Z-score of lumbar BMD and parameters of interest were performed in all subjects as one group. Z-scores of BMD were plotted to the following parameters: mean individual Phe concentrations, cumulative Phe variation, Phe tolerance, and concentrations of vitamins, minerals, and bone metabolism markers. To investigate whether reduced Z-score for BMD is associated with Phe concentrations below target range, we correlated Z-scores of BMD with the absolute and relative number of measurements in which Phe was below target range.

Third, data of subjects with Z-scores of BMD < -2 SD were compared with data of subjects with Z-scores of BMD ≥ -2 SD. The rationale for this cut-off is that a Z-score of BMD < -2 SD indicates low bone density for age [25].

2.6. Statistical analyses

Testing for normality was done using the Kolmogorov–Smirnov test. Homogeneity of variance between groups was analyzed using Levene's test. In case of normally distributed variables and homogeneous variances, groups were compared with Student's *t*-test and one-way ANOVA. Otherwise, groups were compared with the Mann–Whitney *U* test and Kruskal–Wallis test. A one-sample *t*-test was used to compare the mean Z-score of BMD of different groups to the theoretical mean value of a healthy reference population (i.e. $Z = 0$). Correlation analyses were performed using Pearson's correlation test for normally distributed variables and Spearman's rank