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Sex differences in body composition and bone mineral density in phenylketonuria: A cross-sectional study

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ABSTRACT

Background: Low bone mineral density (BMD) and subsequent skeletal fragility have emerged as a long-term complication of phenylketonuria (PKU).
Objective: To determine if there are differences in BMD and body composition between male and female participants with PKU.
Methods: From our randomized, crossover trial [1] of participants with early-treated PKU who consumed a low-phenylalanine (Phe) diet combined with amino acid medical foods (AA-MF) or glycomacropeptide medical foods (GMP-MF), a subset of 15 participants (6 males, 9 females, aged 15–50 y, 8 classical and 7 variant PKU) completed one dual energy X-ray absorptiometry (DXA) scan and 3-day food records after each dietary treatment. Participants reported lifelong compliance with AA-MF. In a crossover design, 8 participants (4 males, 4 females, aged 16–35 y) provided a 24-h urine collection after consuming AA-MF or GMP-MF for 1–3 weeks each.
Results: Male participants had significantly lower mean total body BMD Z-scores (means ± SE, males = −0.9 ± 0.4; females, 0.2 ± 0.3; $p = 0.01$) and tended to have lower mean L1–4 spine and total femur BMD Z-scores compared to female participants. Only 50% percent of male participants had total body BMD Z-scores above −1.0 compared to 100% of females ($p = 0.06$). Total femur Z-scores were negatively correlated with intake of AA-MF ($r = -0.58$; $p = 0.048$). Males tended to consume more grams of protein equivalents per day from AA-MF (means ± SE, males: 67 ± 6 g, females: 52 ± 4 g; $p = 0.057$). Males and females demonstrated similar urinary excretion of renal net acid, magnesium and sulfate; males showed a trend for higher urinary calcium excretion compared to females (means ± SE, males: 339 ± 75 mg/d, females: 228 ± 69 mg/d; $p = 0.13$). Females had a greater percentage of total fat mass compared to males (means ± SE, males: 24.5 ± 4.8%, females: 36.5 ± 2.5%; $p = 0.047$). Mean appendicular lean mass index was similar between males and females. Male participants had low-normal lean mass based on the appendicular lean mass index.
Conclusions: Males with PKU have lower BMD compared with females with PKU that may be related to higher intake of AA-MF and greater calcium excretion. The trial was registered at www.clinicaltrials.gov as NCT01428258.

1. Introduction

PKU (PKU; OMIM 261600) is an autosomal recessive genetic disease that results in a deficiency of phenylalanine hydroxylase (PAH; EC 1.14.16) to hydroxylate Phe to tyrosine (Tyr), using tetrahydrobiopterin as a cofactor [2]. Early identification of PKU with newborn screening and initiation of a low-Phe diet within the first weeks of life are essential to prevent severe cognitive impairment caused by the neurotoxicity of high Phe concentrations in the brain [3,4]. Primary treatment for PKU involves lifelong adherence to a low-Phe diet, restricted in protein from natural foods, in combination with low-Phe amino acid medical foods (AA-MF) or glycomacropeptide

Abbreviations: AA-MF, Amino acid medical foods; ALM, Appendicular lean mass; BMD, Bone mineral density; DXA, Dual-energy X-ray absorptiometry; GMP-MF, Glycomacropeptide medical foods; MF, Medical foods; PAH, Phenylalanine hydroxylase; PE, Protein equivalent; Phe, Phenylalanine; PKU, Phenylketonuria; PRAL, Potential renal acid load; RDN, Registered Dietitian Nutritionist; TBS, Trabecular bone score; Tyr, Tyrosine

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medical foods (GMP-MF) to meet daily protein and micronutrient needs [4,5].

Skeletal fragility, characterized by low bone mineral density (BMD) and increased fracture risk, is a long-term complication of PKU for which incidence, etiology and prevalence are poorly understood. Approximately 40–50% of adults and 33% of children with PKU, treated with AA-MF lifelong, sustain fragility fracture [6,7]. Additionally, femora from $PKU^{enu2/enu2}$ mice have lower BMD, and biomechanical analysis indicates that they fracture with less force than wild type littermate control mice [8]. However, it is unclear whether there are differences in indicators of bone health between males and females with PKU [9–18], and often, comparisons for males and females are not pursued [6,7,19–26].

We recently conducted a randomized, controlled crossover trial to investigate the safety and efficacy of Phe-free AA-MF and low-Phe GMP-MF in 30 participants with early treated PKU and concluded that GMP-MF did not significantly increase plasma Phe concentrations [1]. From this clinical trial, two sub-studies were conducted: 1) A cross-sectional study in which one dual energy X-ray absorptiometry (DXA) scan was obtained from 15 participants with PKU; 2) A crossover pilot study in which 24-h urine collections and food records were obtained from 8 participants with PKU consuming AA-MF and GMP-MF to determine the impact of dietary acid load on excretion of renal net acid and minerals [9]. In our recent crossover pilot study, we demonstrated that ingestion of high-acid AA-MF significantly increased urinary excretion of renal net acid, calcium, and magnesium and concluded that this may negatively affect bone health in PKU [9]. Unexpectedly, we identified that 2 of 8 participants (both males) had low BMD-for-age based on DXA. We hypothesize that higher intake of AA-MF needed to support an intense pubertal growth spurt, may increase urinary calcium excretion and reduce bone accretion in males with PKU. Our objective was to investigate whether there are differences in BMD and body composition between males and females with PKU.

2. Methods

2.1. Study design and protocol

As stated in the introduction, this manuscript presents data from two sub-studies of our previously reported randomized, controlled, crossover trial [1]. First, utilizing a cross-sectional study design, we obtained one whole-body DXA scan from 15 of 30 participants who completed our randomized, controlled, crossover trial where participants consumed their typical low-Phe diet in combination with average intake of 0.74–0.76 g protein equivalents/kg/day from AA-MF or Glytactin™ GMP-MF for 3-wk each [1]. Intakes of medical foods composed of primarily elemental amino acids are described as protein equivalents. Participants reported lifelong intake of AA-MF prior to the trial. Thus, DXA scans reflect intake with AA-MF.

Second, a crossover pilot study was conducted in 8 of 30 particpants with early treated PKU [9]. Participants consumed a low-Phe diet in combination with a Glytactin™ GMP-MF with a low potential renal acid load and an AA-MF with a high potential renal acid load for 1–3 weeks each. Participants provided one 24-h urine collection for each dietary treatment and two-three 24-h food records before and during the 24-h urine collection. Food records for all studies were analyzed using Food Processor SQL (version 10.12.0, ESHA) [1,9].

Briefly, for the crossover pilot study, the nutrient intakes of the low-Phe diet with AA-MF and GMP-MF treatments were generally similar except for the dietary protein source of medical foods, such that amino acids were consumed with AA-MF and primarily intact protein with GMP-MF. Mean intakes of total energy (2266–2566 kcal/d), total protein (79–81 g/d), and protein equivalents from medical foods (55-57 g protein equivalents/d) were similar between dietary treatments [9]. Despite similar intakes of total calcium (1745–1898 mg/d) and magnesium (568–684 mg/d), participants excreted more urinary calcium and magnesium with AA-MF than GMP-MF [9]. Consistent with the greater potential renal acid load (mEq/d) with AA-MF compared to Glytactin™ GMP-MF (means ± SE, AA-MF, 39 ± 5; GMP-MF, −43 ± 6; $p < 0.0001$), excretion of renal net acid was 3-fold high with ingestion of AA-MF compared with GMP-MF [9].

Inclusion criteria included PKU diagnosis that was early-treated with medical food, a current prescribed diet providing > 50% of daily protein needs from AA-MF, and enrollment or completion of our clinical trial at the Waisman Center site [1]. Classical and variant PKU were defined based on genotype and response to sapropterin dihydrochloride [1]. The University of Wisconsin-Madison Health Sciences review board approved the study protocol. All participants provided written informed consent. The trial was registered at www.clinicaltrials.gov as NCT01428258.

2.2. Clinical measurements

Bone mineral density (BMD) and body composition were measured using a single GE-Healthcare Lunar iDXA densitometer (Madison, WI, USA) [9]. DXA scans were obtained and analyzed using enCORE software version 13.31 or 13.6. Weight-adjusted BMD Z-scores were derived using the manufacturer's sex-specific normative database. Spine trabecular bone scores (TBS) were obtained using Medimaps Group TBS inSight software version 2.0.0.1 or 2.1.0.0. (Mérignac, France) [27]. The appendicular lean mass index (ALMI) was calculated as the sum of lean mass of arms and legs (kg) divided by height squared (m^2) [28]. ALMI Z-scores were analyzed using enCORE software version 17.0 with the USA NHANES 1999–2004 reference population for participants over 20 years of age. Mean DXA parameters for male and female participants and the statistical comparisons evaluating differences related to sex and PKU genotype are herein reported for the first time. Detailed methods related to the analysis of the 24-h urine collections have been previously reported [9]. Potential renal acid load was calculated to predict dietary acid load from AA-MF, using the following equation: Potential renal acid load (mEq/d) = (2 × (0.00503 × mg Met/d)) + (2 × (0.0062 × mg Cys/d)) + (0.037 × mg phosphorus/d) + (0.0268 × mg chloride/d) − (0.021 × mg potassium/d) − (0.026 × mg magnesium/d) − (0.013 × mg calcium/d) − (0.0413 × mg sodium/d) [9,29,30].

2.3. Statistical analysis

All statistical analyses were performed using SAS version 9.4 and assumptions of normality and equal variance were tested. Most analyses used PROC MIXED (SAS Institute Inc.). Participant characteristics and DXA scan data were analyzed using ANOVA with effects for sex and genotype (classical or variant PKU). The Kruskal-Wallis test was used to test for differences due to diet or genotype, if data was skewed. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Participants

Fifteen (6 males, 9 females) participated in this sub-study, including 12 adults (aged 19–50 y) and 3 adolescents (aged 15–17 y). Participant characteristics are summarized in Table 1. Of the 8 participants categorized with classical PKU, all were adults (4 males, 4 females). Of the 7 participants categorized with variant PKU, 4 were adults (1 male, 3 females) and 3 were adolescents (1 male, 2 females). Although we tended to have more females than males with variant PKU, BMD or BMD Z-scores were similar between participants with classical and variant PKU (Supplemental Table 1). Two participants (both female adolescents) used a consistent dose of sapropterin dihydrochloride throughout the study.