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SHORT REPORT

Jadwiga Ambroszkiewicz · Joanna Gajewska
Teresa Laskowska-Klita

A study of bone turnover markers in prepubertal children with phenylketonuria

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To investigate the effect of low-phenylalanine diets on bone mineralisation status, we compared biochemical bone formation and resorption markers in prepubertal children with phenylketonuria with those of age-matched healthy controls.

Dietary phenylalanine (Phe) restriction is the only known strategy of preventing neurological impairment and mental retardation in patients with phenylketonuria (PKU). In general, most phenylketonuric patients on a low-Phe diet can achieve normal growth and intellectual development. It is suggested, however, that this form of diet may influence bone metabolism, especially in childhood and adolescence when growth and bone turnover are at their most intensive. Some authors have described decreased bone mineral density and osteopenia in patients suffering from PKU [1, 2, 3,5]. Measurements of bone mineral density (BMD) reflect only bone mineral status but not the dynamics of bone turnover. Non-invasive biochemical markers which show global skeletal activity have lately been developed and validated for the assessment of bone formation and bone resorption processes. Among them, osteocalcin (OC), bone alkaline phosphatase (BALP) and collagen type 1 cross-linked C-telopeptide (CTX) are considered to be clinically useful. Recently, the novel cytokine osteoprotegerin (OPG), belonging to the tumour necrosis factor receptor family has been established as an endogenous inhibitor of osteoclastogenesis and resorption processes.

Our study population consisted of 37 children with PKU under surveillance at the Department of Paediatrics at the Institute of Mother and Child in Warsaw. Patients were divided into two groups. Those in group A ($n = 12$; median age 4.5 years; range 3–10 years) followed their therapeutic diet strictly and had mean serum Phe concentrations close to the reference range (189.4 ± 64.2 μmol/l) and those in group B ($n = 25$; median age 6.0 years; range 3–10 years) did not adhere to their diet and had increased Phe concentrations (649.2 ± 140.6 μmol/l). The mean serum Phe concentration was calculated for the last 3 years of the life of each patient. Children with PKU were fed with an aminoacid mixture PAM, Milupa-PKU 2 or Phenyl-free. These patients have normal values for calcium and phosphate. Healthy children sent to our laboratory for routine analytical control ($n = 27$; median age 5.9 years; range 4–9 years) were the reference group. The whole group of investigated children was ethnically homogenous. Venous blood samples were collected after an overnight fast, centrifuged and serum levels of Phe, calcium and phosphate were determined. Remaining serum samples were frozen and collected for measurement of BALP, OC, CTX and OPG. Phe was assayed fluorometrically according to McCaman and Robbins; calcium and phosphate by standard procedures with a Cobas Integra analyser (Roche, Switzerland). Serum OC and CTX were measured by immuno-enzymatic ELISA assays (Osteometer, Denmark). For determination of BALP, the Alkphase-B kit from Metra Biosystems (USA) and for OPG, the kit from Biomedica (Austria) were used. The differences were evaluated by ANOVA with Bonferroni correction. The significance was set at $P < 0.05$.

We observed lower levels of bone formation and bone resorption markers in group A than in group B, but these differences are not statistically significant (Table 1). However, OC, CTX and OPG concentrations were significantly lower in both groups of PKU children in comparison to the healthy age-matched controls.

There are only a few papers presenting values of bone turnover markers in PKU subjects. Perez-Duenas et al. [6] observed the same levels of OC and BALP in a group of younger patients and significantly lower serum BALP

J. Ambroszkiewicz (✉) · J. Gajewska · T. Laskowska-Klita
Department of Biochemistry,
Institute of Mother and Child,
ul Kasprzaka 17a, 01-211
Warsaw, Poland
E-mail: biochem@imid.med.pl
Tel.: +48-22-3277260
Fax: +48-22-3277280

Table 1 Serum levels (expressed as median values and ranges) of OC, BALP, CTX and OPG in PKU patients versus controls

	Group A ($n=12$)	Group B ($n=25$)	Controls ($n=27$)
Gender (F/M)	9/3	17/8	18/9
Age (years)	4.5 (3–10)	6.0 (3–10)	5.9 (4–9)
OC (μg/l)*	67.1 (42.9–140.5)	80.0 (43.9–148.6)	102.8 (79.6–121.8)
BALP (U/l)	93.8 (75.0–141.8)	102.5 (74.5–145.1)	110.2 (89.0–129.7)
CTX (ng/l)**	1322 (1017–2871)	1685 (1096–2762)	2030 (1363–2815)
OPG (pmol/l)***	3.58 (2.32–4.59)	3.33 (2.37–5.01)	4.46 (2.34–5.64)

* $P<0.05$ for group A versus controls and group B versus controls
** $P<0.001$ for group A versus controls and $P<0.01$ for group B versus controls
*** $P<0.01$ for group A versus controls and $P<0.001$ for group B versus controls

activity in older subjects (>18 years of age). Hillman et al. [4] reported results, similar to ours, that bone formation markers (BALP, OC) were significantly lower in prepubertal PKU patients than in controls. Our results concerning the formation as well as resorption markers suggest a decreased bone turnover rate in prepubertal children with PKU compared to controls. Therefore these patients may not achieve an optimal bone mineral content and may be at risk of bone abnormalities. Further studies of bone turnover markers and BMD in children with PKU in different age-groups are needed in order to prevent osteopenia and osteoporosis in later life.

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