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Hormonal and biochemical parameters correlated with bone densitometric markers in prepubertal Hungarian children ☆

Violetta Csakvary [a], Tamas Puskas [b], Gyorgy Oroszlan [a], Peter Lakatos [c], Bernadette Kalman [d], Gabor L. Kovacs [e,f], Erzsebet Toldy [g,h,*]

[a] Department of Pediatrics, Markusovszky Teaching Hospital, 9700 Szombathely, Markusovszky St. 5, Hungary
[b] Department of Radiology, Markusovszky Teaching Hospital, 9700 Szombathely, Markusovszky St. 5, Hungary
[c] 1st Department of Internal Medicine, Semmelweis University, Budapest, 1083 Budapest, Hungary
[d] Deputy Director of Sciences, Markusovszky Teaching Hospital, 9700 Szombathely, Markusovszky St. 5, Hungary
[e] Institute of Diagnostics, University of Pécs, 7624 Pécs, Ifjúság St. 13, Hungary
[f] Insitute of Laboratory Medicine and Szentagothai Research Centre, University of Pécs, 7624 Pécs, Ifjúság St. 13, Hungary
[g] Central Laboratory, Markusovszky Teaching Hospital, 9700 Szombathely, Markusovszky St. 5, Hungary
[h] Department of Practical Diagnostics, Faculty of Health Science, University of Pécs, 9700 Szombathely, Markusovszky St. 5, Hungary

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ABSTRACT

Background: The conditions that define bone development in prepuberty profoundly influence bone health later in life. We aimed to reveal important determinants of bone mass in Tanner stage I.
Methods: We studied 84 healthy children (43 girls and 41 boys) aged 7 to 11 years. Serum estradiol (E2), 25-hydroxyvitamin D3-vitamin [25(OH)D3], intact parathyroid hormone (PTHi), osteocalcin (OC) and β-crosslaps (CTXs) were longitudinally analyzed (Roche Diagnostics System). Total and spine bone mineral content (tBMC and LBMC) and density (tBMD and LBMD) were assessed, and total fat body mass index (FBMi) was calculated (DXA Lunar Prodigy).
Results: The serum PTHi, OC and LBMD values were significantly higher in girls than in boys. The mean $25(OH)D_3$ level was lower but not significantly in girls compared to boys. Significant negative correlation was found between PTHi and $25(OH)D_3$ levels ($r=-0.28$; $p=0.011$) when tested in all subjects, but no correlation was detected when the gender groups were separately tested. There was a trend for higher E2 levels in girls. Significant positive correlation ($r=0.32$; $p=0.042$) was detected between FBMi and E2 concentration in girls only. A significant negative correlation was found between E2 and $25(OH)D_3$ levels ($r=-0.37$, $p<0.05$) in girls with elevated (>3.6 pmol/l) PTHi and with suboptimal (<75 nmol/l) $25(OH)D_3$ levels. Furthermore, positive correlations were noted between E2 and CTXs and OC ($r=0.54$, $p<0.01$ and $r=0.39$, $p<0.03$) and a marginally significant positive correlation ($r=0.33$; $p=0.06$) was detected between OC and PTHi levels in girls. However, we detected no correlations when these markers were analyzed in boys. There was a significant correlation between E2 and all BMC and LBMD values in both genders. The tBMD, LBMD and tBMC values showed weak, but significant negative associations with $25OHD_3$ levels ($\beta=-0.44$ to -0.55; $p<0.001$) in girls only. All BMD and BMC values were positively predicted by OC levels, but not by CTXs, in both genders. Among the biochemical markers, E2 was the only factor correlating with all dependent variables (BMCs and BMDs) in both genders. Among all parameters analyzed, FBMi ($\beta=0.64$) showed the strongest influence on tBMC characteristically in girls only.
Conclusions: Our results support that 1.) E2 levels play a key role in defining bone turnover and bone mass in both genders already in prepuberty; 2.) high PTHi levels in childhood should be evaluated with caution, because the normal range for serum PTHi in different Tanner stage groups is not well established; and 3.) the negative

Abbreviations: $25(OH)D_3$, 25-hydroxy vitamin D_3; tALP, total alkaline phosphatase; BMCs, bone mineral contents; BMD, bone mineral density; BMDs, bone mineral densities; BMI, body mass index; Ca, calcium; Ca-Alb, corrected Ca with albumin; CTXs, serum beta crosslaps; DXA, dual-energy X-ray absorptiometry; DBP, D vitamin binding protein; E2, estradiol; FBMi, fat body mass index; FGF23, fibroblast growth factor 23; FSH, follicle-stimulating hormone; IOM, Institute of Medicine; PTHi, intact-parathyroid hormone; LBMC, lumbar spine bone mineral content; LBMD, lumbar spine bone mineral density; LH, luteinizing hormone; Mg, magnesium; OC, osteocalcin N-MID; OPG, osteoprotegerin; P1NP, procollagen propeptide; PHOS, inorganic phosphorus; Q, quartiles; RANKL, decreasing the receptor activator of nuclear factor kB ligand; rr, reference range; SD, standard deviation; tBMC, whole (total) bone mineral content; tBMD, whole (total) bone mineral density; TSH, thyrotropin stimulating hormone.

☆ The work was conducted at the Department of Practical Diagnostics of Institute of Diagnostics, University of Pécs, and Markusovszky Teaching Hospital, 9700 Szombathely, Markusovszky St. 5, Hungary.

* Corresponding author at: Department of Practical Diagnostics, University of Pecs, and Isotope Laboratory of Central Laboratory, Markusovszky Teaching Hospital, Szombathely, 9700 Szombathely, Markusovszky St. 5, Hungary. Fax: +36 94515598.
E-mail addresses: per.csak@chello.hu (V. Csakvary), puskastam@gmail.com (T. Puskas), oroszlan.gyorgy@markusovszky.hu (G. Oroszlan), lakatos.peter@med.semmelweis-univ.hu (P. Lakatos), kalman.bernadett@markusovszky.hu (B. Kalman), gabor.l.kovacs@aok.pte.hu (G.L. Kovacs), toldy.erzsebet@gmail.com (E. Toldy).

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correlation between 25(OH)D and E2 and the positive correlation between PTHi and OC suggest that estrogens regulate PTHi indirectly and cause lower circulating 25(OH)D_3 levels. We propose that the decreased levels of 25(OH)D3 reflect not the real vitamin supply, but may rather be the result of E2 regulation. Therefore, the actual serum 25OHD levels may underestimate the availability of factors supporting bone formation.

Introduction

The peak bone mass predicts the risk for osteoporosis in later life. During the modeling period of bone development, the bone mass significantly increases and reaches its peak. This process is under a strong genetic control but also influenced by nutritional factors and lifestyle [1]. The bone metabolism in children differs from that in adults, and reflects both skeletal growth and remodeling. During skeletal growth, new bone is formed at a site different from that of resorption. The assessment of bone health is routinely carried out by using densitometric analyses. However, these measurements do not reflect the dynamic nature of bone tissue composition in contrast to the biochemical markers of bone metabolism. Bone mineralization depends on adequate intakes and absorption of calcium (Ca) along with an optimal supply of vitamin D [2,3]. The vitamin D status can be estimated by measurements of the serum 25-hydroxyvitamin D [25(OH)D] produced in the liver. Presently, there is no consensus concerning the optimal plasma levels of 25(OH)D [4–8]. This lack of consensus can be explained, at least in part, by the different optimal levels of 25(OH)D needed to ensure skeletal health and proper development of other organs. Generally, levels of 25(OH)D above 75 nmol/l are defined as optimal regarding various health outcomes [5–9]. Some guidelines, including the Endocrine Society Clinical Practice Guideline [5], recommend higher than 75 nmol/l values for acceptable plasma levels of 25(OH)D, which maximally suppress the secretion of parathyroid hormone (PTHi) [7–11]. Given the limitations of the experimental data and the different goals of the IOM and The Endocrine Practice Guidelines Committee, the differences appear almost inevitable in their recommendations. Holick et al. [8] underscore, however, that the existing guidelines provide reasonable recommendations for clinical care at this time. Nevertheless, these guidelines, as well as the IOM recommendations, will require reconsideration in the future as additional data from ongoing longitudinal studies become available [8].

Many adults have very low 25(OH)D levels without evidence of increased production of PTHi, and 25(OH)D levels greater than 75 nmol/l do not always cause PTHi suppression. Most authors agree that an elevated PTHi level does not necessarily indicate inadequate vitamin D status in children [6]. In puberty, an adequate calcium intake with a high-normal PTHi and low-normal 25(OH)D level may stimulate periosteal bone formation and increases bone accrual [5,10]. Abrams et al. [10] investigated healthy children with suboptimal vitamin D status and Tanner stages II–III, and showed an inverse relation between PTHi and 25(OH)D, while the PTHi levels were below the upper cut off value (<3.8 pmol/l) in all cases. Preliminary evidence suggests that with adequate calcium intake, a high-normal PTHi and low-normal 25(OH)D level may result in greater bone size and mass during puberty [6,11]. However, it is also well known that estrogens play a key role in balancing bone health in several ways. Estradiol (E2) stimulates osteoblast proliferation, bone formation and differentiation by stimulating the expression of osteoprotegerin (OPG) and decreasing the production of the receptor activator of nuclear factor κB ligand (RANKL). However, estrogens inhibit the differentiation of osteoclast precursors while also enhance their apoptosis [12] and thus, contribute to a decreased bone resorption. In addition, E2 decreases PTHi synthesis in the parathyroid gland through an indirect mechanism which involves fibroblast growth factor 23 (FGF23) and prevents bone loss [12,13].

Numerous publications have reported the beneficial effect of E2 and the adverse effect of vitamin D insufficiency on bone mineralization (1,2,7, 9–13). Nevertheless, there are only a few data concerning the importance of vitamin D supply in prepubertal age [9,11,14].

Most data show the effects of serum calcium levels, vitamin D insufficiency and PTHi levels on bone metabolism in children over 12 years of age [2,10–12,14–19]. In contrast, only a few publications are available concerning children under 12 years of age and with a Tanner stage I [20,21]. In pre-pubertal age, there is no study analyzing the relations among serum PTHi, 25(OH)D, E2, bone biomarker levels [osteocalcin (OC), β-crosslaps (CTXs), procollagen type 1 N-terminal propeptide (PINP)] and bone accrual. For this reason, our aim was to test in pre-pubertal children with Tanner stage I the associations of these biochemical markers with bone mineral densities (BMDs) and contents (BMCs), taking also into consideration those lifestyle habits that can have an impact on the healthy bone formation (physical activity, daily calcium and vitamin D intake, soft drink and coke consumption).

Material and methods

Children were recruited from a primary school of our town (Szombathely, in West Hungary) from January to April in 2008. Inclusion criteria were: healthy children with Tanner stage I, without any vitamin supplementation and without other medications.

Information collection and measured parameters

General information on health history and lifestyle factors including diet (calcium and vitamin D intake, soft drink and coke consumptions) and physical activity was collected by using a locally generated questionnaire to be completed by the parents. The daily calcium and vitamin D intake was assessed by using data of the Hungarian National Food Composition Book [22]. Anthropometrical data were also measured. Pubertal stage was established using the criteria of Tanner based on physical examination of pubic hair and breast development (girls), and testicular volume determination by orchidometer (boys) [23].

Parents were previously informed about the details of the study and the research was conducted with their written consent. The Research Ethics Committee of Markusovszky Teaching Hospital approved the study.

For the accurate determination of fat mass of body composition (FBM) and for the evaluation of total (t) body and lumbar spine (L) bone mineral density (tBMD and LBMD, g/cm^2, respectively) and bone mineral content (tBMC and LBMC, g), dual-energy X-ray absorptiometry was used (DXA, Medical Systems Prodigy Lunar, GE Health Care USA pediatric CORE 2002 software, version 6.5, GE Health Care USA).

From weight and height, the value of body mass index (BMI), and from FBM and height, the value of FBMi were calculated. BMI Z-scores were calculated according to the National Growth Chart and Guide [24]. Children were categorized as thin with BMI Z-scores less than 2.0 SD, and obese with BMI Z-scores higher than 2.0 SD.

Sample collection and methods of biochemical parameters

The blood samples were drawn between 8 and 10 am and centrifuged in 1 h. The samples were analyzed for routine chemical parameters including total alkaline phosphatase (tALP) with an upper cut-off of