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Bone Mineral Density Measured by Quantitative Computed Tomography in Pre-pubertal/Early Pubertal Children with Hypovitaminosis D

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Abstract

Background: Clinically significant vitamin D deficiency is associated with decreased bone mineral density (BMD) and increased fracture risk in adults. A similar association is suggested to be present in children. In this study we analyzed serum vitamin D_3 level and corresponding BMD in pre-pubertal/early pubertal children.

Methods: 272 healthy pre-pubertal/early pubertal schoolchildren (9.74 ± 0.6 years of age) were involved in the study. Serum 25(OH) D_3 was measured by HPLC and were correlated to volumetric BMD (vBMD) which was assessed by peripheral quantitative computer tomography (pQCT).

Results: 25(OH)D_3 levels (samples taken in the autumn) were below 20 ng/ml in 37.1% of the subjects and in the range of 20-30 ng/ml in 26.2%. Therefore, the vitamin D status proved to be insufficient in a significant percentage of children. The vBMD was found to be moderately decreased: Z-score less than -1.0 was found in 10.3% of the children while values "low for age" (Z-score less than -2.0) were extremely rare (0.74 %). Total and trabecular densities, considered together, indicated a trend of decreasing vBMD (Z-score less than -1.0) in only 6.6% of the subjects.

Conclusion: Comparison of the two parameters (vitamin D_3 and vBMD) allows to conclude that, in a cross-sectional study of the pre-pubertal/early pubertal age group, 25(OH)D_3 levels at present considered as low are usually not associated with significantly low vBMD.

Keywords

25(OH)D_3, Hypovitaminosis D, vBMD, pQCT, Pre-puberty, Early puberty

Introduction

Severe childhood vitamin D deficiency is a key factor in the development of rachitis. The disease has become quite rare among Caucasians living in developed countries [1], although there are a number of studies showing that the vitamin D status of children is significantly less than the optimal level all over the world [1]. On the other hand, rachitis is not necessarily manifest even at definitely low vitamin D levels [2]. Pediatric reports on the relationships between vitamin D status and bone health tend to be quite contradictory. Clinically significant vitamin D deficiency is associated with decreased bone mineral density (BMD) and increased fracture risk in adults. It is hypothesized that similar relationship exist in children.

The majority of recent publications in this field are reviews, and many of the authors consider that further studies are necessary [1,3-6]. As part of an extensive screening program of pre-pubertal/early pubertal schoolchildren, we measured the serum level of calcium, phosphorus, alkaline phosphatase, osteocalcin, β-crosslaps and 25(OH)D_3 as well as determined volumetric bone mineral density (vBMD) by peripheral quantitative computed tomography (pQCT). The aim of this publication is to present the unexpected results of the comparative evaluation of 25(OH)D_3 and vBMD.

Subjects and Methods

These examinations were performed in pre-pubertal and early pubertal white European children living in one of the inner Buda districts of Budapest. This region of the city is populated by slightly more well-to-do families than the average. The selection was made in two schools. All parents of studied children provided written informed consent, enrollment included more than 90% of children in the chosen classes. To avoid the effects of pubertal changes as far as possible, the upper age-limit was 11 years (88.8% were pre-pubertal). The screening examinations (including blood sampling) were conducted in November. The study was approved by the Ethics Committee of the Children's Hospital.

Those having a result for 25(OH)D_3 (294 children) were aged 9.741 ± 0.598 years with a gender distribution of 153 boys and 141 girls. 272 of this group were also subjected to vBMD examination (age: 9.737 ± 0.608 years; 140 boys, 132 girls).

Serum concentration of 25(OH)D_3 was measured in an HPLC (Bio-Rad) apparatus equipped with a UV detector, using '25-OH Vitamin D_3 by HPLC' kits (Bio-Rad, Hercules, CA, USA, Cat. No.: 195-6530). The intra-assay CV of the method is 2.07–5.69% (depending on the concentration range), and the inter-assay CV is 3.59-5.15%. The validated normal range of the performing laboratory is 20-60 ng/ml.

vBMD measurements were performed by mobile pQCT set up at the schools. We chose the Stratec XCT 2000 (XCT 2000 Bone Scanner, Stratec Medizintechnik GmbH, Pforzheim, Germany), the pQCT apparatus most commonly used in pediatric applications [7].
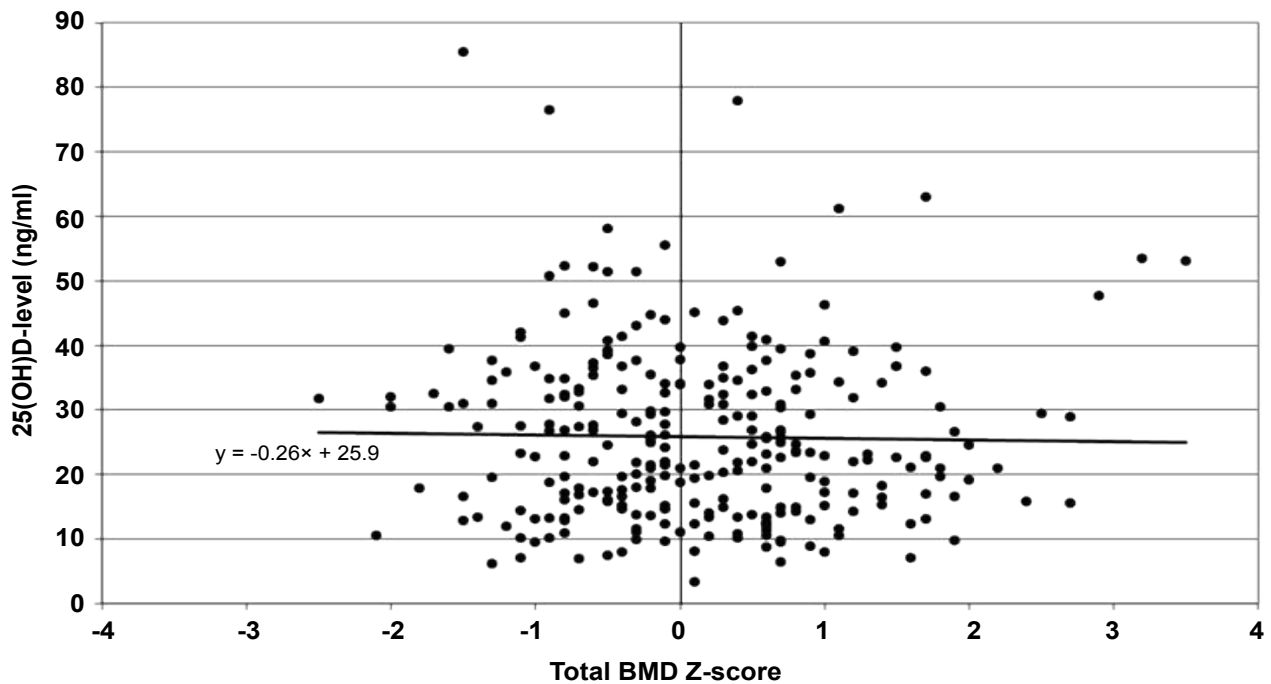
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Figure 1: Vitamin D$_3$ values as a function of total vBMD Z-scores.

The chart shows the equation $y = -0.26 \times + 25.9$

Table 1: Distribution of serum 25(OH)D concentration values.

Serum concentration	Case of 25(OH)D	
ng/ml	number	%
> 40	37	12.6
30.1 - 40.0	71	24.1
20.1 - 30.0	77	26.2
10.1 - 20.0	91	31.0
< 10	18	6.1

Table 2: Comparison of 25(OH)D$_3$ values and total vBMD Z-scores < -1.0.

25(OH)D$_3$ concentration		vBMD (total)
ng/ml	number of subjects	Z-score < -1.0 number of subjects/%
10	18	2/11.1
10.1-20.0	88	9/10.2
20.1-30.0	70	3/4.3
30.1-40.0	64	9/14.0
> 40	32	3/9.4

pQCT was employed to determine total and trabecular vBMD on the non-dominant forearm at the 4% position of the distal radial, following routine procedures, especially in screening studies [8]. The lower arm is considered an ideal site for the assessment of fracture risk in children, as this site is the most common location of pre-pubertal and adolescent bone fractures [1,9].

The designation "quantitative" means that measurement is made in three dimensions in a given volume (vBMD), expressed in units of g/cm^3. The radiation exposure associated with the test is < 2 µSievert. Its accuracy is characterized by a margin of error of 0.3-2.0 %.

A nation-wide survey to establish vBMD reference values, as assessed by mobile pQCT, was carried out previously in a well-balanced population of Hungarian children using measurements [10]. In this previous study, randomization of the studied children (2237 healthy school children/1068 boys/age 9.5-15.5 years) was performed according to the localization of the schools (Budapest, East-Hungary, West-Hungary), type of settlements (capital, size of town or village), sex and age (altogether six categories) to gain statistically correct and representative sampling. Total and trabecular vBMD values were grouped according to the sex and age groups [10]. In the present study measured vBMD values were compared to this reference data, which have similar g/cm^3 values compared to German and British data [11,12] obtained by the same measurement technique.

Physical examination for height, body weight and pubertal development as well as the routine laboratory parameters were studied; the possible modifying influence of Tanner stages of puberty and BMI on the bone health was also analyzed in this cohort.

Results

There was a slight but significant difference in the ages between the tested boys and girls (boys: 10.18 ± 0.31, girls: 9.27 ± 0.46 years; p < 0.001), although all the children were attending the same year at school. The mean ± SD 25(OH)D$_3$ level of the 294 serum samples was 26.25 ± 13.09 ng/ml (range: 3.3 and 85.5). There was no gender difference the vitamin D levels: boys, 27.17 ± 13.50; girls, 25.25 ± 12.59 ng/ml (p = 0.208).

The distribution of 25(OH)D$_3$ concentrations is summarized in (Table 1). When a lower limit of normal of 20 ng/ml was used, 37.1% of the samples fall into the range of hypovitaminosis D; when the cut-off is moved to 30 ng/ml, nearly two-thirds of the values (63.3%) will rate as low.

BMD Z-score values below -2.0 were found in four cases (two total- and two trabecular vBMD). No child had both Z-scores (total and trabecular) below -2.0. Total Z-scores were in 26 (9.6 %) trabecular vBMD Z-scores were between -1.0 and -2.0 in 40 (14.7 %) children, while for both parameters were between -1.0 and -2.0 in 18 (6.6 %) cases. All the other Z-score values were above -1.0.

The serum calcium, phosphorus, alkaline phosphatase, osteocalcin and β-crosslaps levels were in the normal range.

Graphic representation of the individual scores does not reveal any specific trend or any correlation between the two parameters (25(OH)D$_3$ and total vBMD Z-score); the majority of the values below either 20 or 30 ng/ml fall into the normal range of vBMD (Figure 1); Analyzing patients with vBMD Z-scores below -1.0 also does not show a particular trend in Vitamin D levels (Table 2).

In another approach, the vitamin D levels obtained were listed in decreasing order and compared to the associated density Z-scores of those falling into the top and bottom quarter, fifth and eighth. With this statistical technique there is some tendentious parallelism between the two parameters (Table 3). In these extreme cohorts the higher or lower vitamin D mean values are associated with higher or lower mean vBMD Z-scores, but in spite of this trend, the differences

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Table 3: Vitamin D3 and vBMD values in the highest (top)and lowest (bottom) cohorts (N = number of subjects).

Cohorts (N)	Vitamin D_3 (ng/ml)		vBMD (Z-score)	
Top				
Eighth (34)	50.4 ± 11.2		0.24 ± 1.2	
Fifth (54)	45.7 ± 10.8		0.19 ± 1.1	
Quarter (68)	43.5 ± 10.6		0.15 ± 1.1	
Bottom		p < 0.0001		p = 0.43
Quarter (68)	11.6 ± 2.7		0.084 ± 0.9	
Fifth (54)	10.7 ± 2.4		0.039 ± 0.9	
Eighth (34)	9.4 ± 2.0		0.035 ± 0.9	

of the Z-score values are statistically non-significant. In addition, the bone density in the cohort with low mean vitamin D levels (9.4-11.6 ng/ml) was found to be in the normal range (mean Z-scores: 0.035-0.084).

33 children (11.2%), among them 21 girls had already entered puberty at the time of the examination (Tanner II, except one case of one girl, Tanner III). The magnitude of their 25(OH)D_3 values showed a distribution identical with that of the pre-pubertal children. Four of them (12.1%) had vBMD Z-scores below -1.0.

The BMI of 27 children was above the 95th percentile. Their 25(OH)D_3 level was 25.17 ± 18.16 ng/ml, similar to the whole cohort (26.25 ± 13.09). The mean total vBMD Z-score was not significantly higher (+0.42 ± 1.03 in obese children versus +0.03 ± 1.13).

In summary, in this cross-sectional study the volumetrically determined total bone density of pre-pubertal and early pubertal children with higher or lower vitamin D_3 levels was within the normal range (Z-score > -2) with two exceptions; lower vitamin D levels were not associated with decreased bone densities.

Discussion

25(OH)D_3 level

The serum 25(OH)D_3 concentration is generally accepted to be the best biomarker of the body's vitamin D supply [13]. Recently, however, a lively debate has developed on vitamin D supply and its significance. According to the official statement of a large international group of experts [14], 25(OH)D_3 levels above 20 ng/ml are sufficient for maintaining adequate bone health. The population in North-America in general falls therefore into the sufficient category. They considered the extra-skeletal effects of vitamin D, at this point, unproven and suggested that excess intake may actually have deleterious effects [14].

However, according to another group of experts [15], vitamin D deficiency is very common world-wide and there is no doubt about the existence of the numerous significant extra-skeletal effects of vitamin D. They suggest that the levels in the range of 20-30 ng/ml should be considered insufficient, while levels above 30 ng/ml are optimal. In order to achieve this threshold, daily doses exceeding 4000 IU are deemed permissible [15].

Similar difference of opinion exists also in the pediatric literature. Vitamin D_3 level was considered sufficient above 20 ng/ml by the Lawson Wilkins Pediatric Endocrine Society [16], the British Paediatric and Adolescent Bone Group [17] and experts of European pediatric gastroenterologist from eleven countries [18]. In a new "expert position statement" and other pediatric publications the 25(OH)D levels < 20 ng/ml are defined as deficiency, between 20 and 30 ng/ml as insufficiency, and only > 30 ng/ml as sufficiency [19-21].

The experts, however, also call attention to the significant inter-assay differences between 25(OH)D_3 determination methods, which have been corroborated by numerous investigators. In their opinion, HPLC and its versions supplemented with tandem mass spectrometry (MS/MS) may be the most reliable assays [22-24].

A mean 25(OH)D_3 level of 26.25 ± 13.09 ng/ml for nearly three hundred pre-pubertal/early pubertal children determined in the autumn can be considered as low, and the ratio of children with 25(OH)D_3 levels below the lower limit of 20 ng/ml (37.1%) is high. If we follow the school of thought that infers vitamin D_3 insufficiency from 25(OH)D_3 levels below 30 ng/ml, then only 36.7% of the healthy children aged ~10 years, living under relative good conditions in Budapest were optimally supplied with vitamin D at the time of the study. More severe deficiency was measured in 6.1% (< 10 ng/ml) and 0.37% (< 5 ng/ml) of the subjects. Similar data was found in another study on adolescents living in Pécs (Hungary), where the average 25(OH)D_3 level was 23.12 ng/ml [25].

BMD

The ideal test method for bone health of clinical relevance is the determination of fracture risk on the basis of longitudinal data [5]. The risk of fracture depends - among others - on skeletal fragility, which is affected by bone mass, size, geometry, micro architecture as well as bending strength [26].

The DXA apparatus developed for the measurement of bone mass is considered adequate for practical purposes. The majority of BMD determinations occur by DXA. However, this two-dimensional method measures areal BMD (aBMD in g/cm^2), and it is not or hardly suitable for studying bone size, geometry or quality. Elimination of this deficiency has been attempted in multiple ways, especially in children, who are still growing, using various geometric algorithms for correction. Alternatively, data measured are compared to the aBMD values of children matched for gender, Tanner stage, bone age, body height or Z-score rather than age [27]. Advantages of the DXA method in pediatrics are rather the much higher prevalence of devices of this type, and an enormous amount of reference data accumulated over the years [3,5,28].

pQCT, the method used in our work yields volumetric results in units of g/cm^3. It can provide information on bone size, geometry and quality, and in addition to total vBMD, it also gives the trabecular fraction separately. The measurement is faster than the one using DXA, it is associated with lower radiation exposure (DXA: 1.5–10.0 μSv; pQCT: < 2.0 μSv), and its accuracy is also acceptable [8]. In follow-up studies, results approaching the data given in the manual were obtained: within-subject CV < 2.7 % [29].

According to our results, only the total density of two subjects and the trabecular density in another two children were found under Z-score of -2.0.

25(OH)D level and vBMD parallel measured with pQCT

Publications comparing the results of simultaneously determined vBMD performed with pQCT on the lower arm of pre-pubertal children and the 25(OH)D levels of both genders are very sparse in the literature, as also demonstrated by the recently published reviews [1,3,5,6].

The investigators usually measured BMD by DXA in the postmenarcheal age group. This material is quite wide-range, but is not fully relevant for comparison. Recent reviews summarizing these results conclude that there is little evidence for a correlation between the 25(OH)D_3 level and areal or volumetric bone density in healthy children, and the data are decidedly inconsistent, which is also true for the relationship between low vitamin D status and fracture risk. The observations published also disagree in identifying the circumstances under which vitamin D supplementation improves bone density, although very low 25(OH)D_3 levels are probably one of those [6,30].

In a Finnish study, the cortical vBMD values of girls aged 10–12 years (pre-puberty, early puberty) radially measured by pQCT were significantly lower when 25(OH)D values were below 10 ng/ml than in the case of higher vitamin D level [31]. In a study of low subject number (21 subjects), total and trabecular radial vBMD values of African-American girls aged 12-14 years were measured by pQCT. No correlation was found between the very low vitamin D levels (5.0–16.3 ng/ml) and the good vBMD values. The authors explain the observation by the low number of subjects [32]. Another group

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of investigators found the total and trabecular vBMD Z-scores of postmenarcheal girls measured by the same technique to be in the normal range in spite of the very low $25(OH)D_3$ levels (mean level 7.2 ng/ml) both before and after vitamin D supplementation [30].

Recently $25(OH)D_3$ level was found positively related to subsequent cortical bone mass and predicted strength in children (7.6-11.8 years) measured by pQCT at the 50% mid tibia [33]. However, in a study from the US using pQCT also on tibia for the determination of the vBMD Z-scores of children aged 9-13 years, Z-scores were found to be higher in black children with lower vitamin D supply than in their white contemporaries who had higher vitamin D levels [34].

In summary, the total and trabecular radial vBMD Z-scores of nearly 300 children aged 8.5-11 years, measured by pQCT regularly fell into the normal range independently of vitamin D levels considered normal or low; the serum $25(OH)D_3$ values between 3.3-85.5 ng/ml did not show any correlation with vBMD.

Possible explanations

The continuously boosted effect of PTH enhances bone resorption over bone formation. The concept based on this physiological process suggests that, from the bone health point of view of, the biologically relevant lower limit of $25(OH)D_3$ is the one which does not yet induce the PTH level to rise [35]. Based on a very careful analysis of the relationship between of $25(OH)D_3$ and PTH levels, a recently published article [2] puts this PTH-inducing $25(OH)D_3$ level somewhere between 10.8 and 16.4 ng/ml, suggesting to be the lower limit value for the diagnosis of vitamin D deficiency (as a "biochemical definition"). In adults the corresponding range is higher: 15-30 ng/ml, as bone health needs less vitamin D in pre-puberty before the rapid pubertal growth.

The possibility of yet another explanation is opened by a concept developed a short time ago. According to Australian authors, "the key element of … rickets in children is a delay in mineralization. It can be resolved by normalization of plasma calcium and phosphate homeostasis *independently of vitamin D activity*" [36,37]. This opinion is mainly based on the results of *in vitro* experiments and animal models, which indicate that all the three main bone cell types (osteoblast, osteoclast, osteocyte) are capable of the expression of both the vitamin D receptor and the enzyme 25(OH)D 1α-hydroxylase (CYP27B1), making possible the synthesis of the active hormone $(1,25(OH)_2D)$ within the bone tissue. The kidneys can also enhance CYP27B1 enzyme activity e.g. under the effect of PTH, thus, according to observations made on humans with decreased $25(OH)D_3$ levels, synthesis of the active hormone [38] and preservation of bone quality are possible above a $25(OH)D_3$ level of ~8 ng/ml, if calcium supply is optimal.

In a recent case-control study from India, low dietary calcium intake was prevalent in nutritional rickets cases compared to the healthy controls, while low vitamin levels were not characteristic [39].

The main limitations of our study are the cross-sectional design rather than longitudinal, the analysis of total and trabecular density out of the possible vBMD parameters, and the lack of the serum PTH values. The schools where our studies were conducted were not "elite" schools, but a slight difference in living standard in this well-to-do area exists compared to the Hungarian average, therefore may represent a further limitation.

Conclusion

Vitamin D screening in healthy pre-pubertal/early pubertal schoolchildren living under favorable conditions in Budapest detected significant hypovitaminosis D. Simultaneous determination of vBMD by pQCT at the distal radius did not indicate decrease in bone density. Based on the analysis of these two parameters, low vitamin D supply is not associated with decreased vBMD in pre-pubertal children.

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