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Relationship Between Vitamin D Status and Bone Mineralization, Mass, and Metabolism in Children With Osteogenesis Imperfecta: Histomorphometric Study

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ABSTRACT

The effect of low vitamin D levels in children with bone fragility disorders has not been examined in detail. In this study, we evaluated the relationship between vitamin D status and parameters of skeletal mineralization, mass, and metabolism in a group of pediatric osteogenesis imperfecta (OI) patients. This retrospective study consisted of 71 patients with a diagnosis of OI type I, III, or IV (ages 1.4 to 17.5 years; 36 girls) who had not received bisphosphonate treatment before iliac bone biopsy. Serum 25-hydroxyvitamin D [25(OH)D] levels ranged from 13 to 103 nmol/L and were less than 50 nmol/L in 37 patients (52%). None of the OI patients had radiologic signs of rickets or fulfilled the histomorphometric criteria for the diagnosis of osteomalacia (ie, elevated results for both osteoid thickness and mineralization lag time). Serum 25(OH)D levels were negatively correlated with age and serum parathyroid hormone levels but were not correlated with any parameter of bone mineralization (ie, osteoid thickness, mineralization lag time, or bone-formation rate per bone surface) or bone mass (ie, lumbar spine areal bone mineral density, iliac bone volume per tissue volume, or iliac cortical width). We found no evidence that serum 25(OH)D levels in the range from 13 to 103 nmol/L were associated with measures of bone mineralization, metabolism, or mass in children with OI. © 2011 American Society for Bone and Mineral Research.

KEY WORDS: BONE MINERALIZATION; BONE HISTOMORPHOMETRY; OSTEOGENESIS IMPERFECTA; OSTEOMALACIA; VITAMIN D

Introduction

Vitamin D plays an essential role in calcium homeostasis and in the development and maintenance of the skeleton.[1] Severe vitamin D deficiency can lead to impaired mineralization at the level of growth plate cartilage (a condition called *rickets*) and at the level of bone tissue matrix (*osteomalacia*). The effects of less severe degrees of vitamin D deficiency are less obvious, but there is a well-known negative correlation between serum levels of 25-hydroxyvitamin D [25(OH)D] and parathyroid hormone (PTH).[2]

According to Parfitt, four stages of hypovitaminosis D osteopathy (HVO) can be distinguished by dynamic histomorphometric analysis of iliac bone.[3,4] The mildest form (HVOIa) is characterized by increased bone turnover caused by secondary hyperparathyroidism and a normal mineralization process. In histomorphometric terms, this form is characterized by an elevated bone-formation rate and a normal thickness of osteoid seams. In the next stage (HVOIb), a mild mineralization defect is discernible, but each remodeling site still mineralizes completely. In histomorphometric terms, bone-formation rate decreases whereas osteoid thickness increases in this stage. Despite complete mineralization, HVOIa and HVOIb (sometimes called *preosteomalacia*) could be associated with bone loss as a consequence of elevated PTH levels.

The next two stages of hypovitaminosis D osteopathy (HVOII and HVOIII) represent full-blown osteomalacia, which occurs when remodeling sites fail to undergo complete mineralization. The histomorphometric hallmark of osteomalacia is the simultaneous occurrence of increased osteoid thickness and increased mineralization lag time. Patients are subdivided into those who retain some tetracycline double labels (HVOII or *early osteomalacia*) and those with no double labels (HVOIII or *late osteomalacia*).

One could hypothesize that children with underlying bone fragility should be especially vulnerable to the additional challenge of a low vitamin D status. A recent study by Bowden and colleagues found that a large proportion of children with bone fragility disorders have serum 25(OH)D concentrations that are below currently recommended levels.[5] It was speculated

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that these low vitamin D levels may contribute to low bone mass or worsen the primary bone disease. However, the effect of low vitamin D levels in children with bone fragility disorders has not been examined in detail.

Osteogenesis imperfecta (OI), a heritable condition that is usually caused by mutations in collagen type I–encoding genes, is the most prevalent pediatric bone fragility disorder.[6] OI also was the single most frequent diagnosis in the study by Bowden and colleagues.[5] In this study, we therefore evaluated the relationship between vitamin D status and parameters of skeletal mineralization, mass, and metabolism in a group of pediatric OI patients. Since precise evaluation of the bone mineralization process requires direct analysis of bone tissue, this study focuses on the relationship between serum 25(OH)D levels and histomorphometric parameters.

Subjects and Methods

Patient population

This study included patients with a diagnosis of OI type I, III, or IV who had undergone iliac bone biopsy at the Shriners Hospital for Children in Montreal and in whom 25(OH)D levels were determined at the time of biopsy. OI types I, III, and IV are the most common types of OI, and mutations in collagen type I–encoding genes usually can be found.[7]

The data of this study were obtained by retrospective chart review. Patients were included in the analysis only if they had had a bone biopsy before any treatment with a bisphosphonate compound was started because bisphosphonates have a profound effect on histomorphometric parameters.[8] Most of the bone biopsies were performed as part of the baseline evaluation in children who subsequently took part in a bisphosphonate treatment study.[8] Biopsies were not performed on patients with a body weight below 10 kg or who presented an elevated risk for anesthesia.

Iliac bone samples suitable for histomorphometric analysis were obtained in 112 patients with a diagnosis of OI type I, III, or IV. In 71 of these patients, serum levels of 25(OH)D had been determined at the time of biopsy. The age at biopsy of these patients ranged from 1.4 to 17.5 years. Results of sequence analysis of *COL1A1* and *COL1A2*, the genes coding for collagen type I were available in 65 of these patients and revealed disease-causing mutations in 63 patients. In the two patients with negative molecular testing and in the six patients without DNA analysis, diagnosis of OI was asserted clinically by the association of frequent fractures, low bone mass, and blue sclera. The study was approved by the Shriners Hospital Institutional Review Board, and informed consent was obtained from legal guardians.

Clinical evaluation

Height was measured using a Harpenden stadiometer (Holtain, Crymych, UK). Weight was determined using digital electronic scales for infants and mechanical scales for older children and adults (Healthometer, Bridgeview, IL, USA). Height and weight measurements were converted to age- and sex-specific *Z*-scores on the basis of reference data published by the Centers for Disease Control and Prevention.[9]

Biochemical measurements

Serum total calcium, phosphate, and alkaline phosphatase were measured using colorimetric methods (Monarch; Instrumentation Laboratories, Inc., Lexington, MA, USA). Serum active intact PTH (fragment 1–84) was determined by radioimmunoassay (IRMA, Diasorin, Stillwater, MN, USA). Serum 25(OH)D and 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] were measured with radioimmunoassays (Osteo SP; Incstar Corp., Stillwater, MN, USA).

Urinary creatinine concentration was determined colorimetrically. Urinary cross-linked *N*-telopeptides of type I collagen (NTX) were quantified by ELISA (Osteomark; Ostex International, Inc., Seattle, WA, USA) using the second-void sample of the morning. Results for urinary NTX/creatinine ratios in OI patients were expressed as a percentage of age-specific mean values using published reference data.[10] Patients were fasting at the time of urine sampling.

Radiographic analysis

Patients were evaluated for the presence of rickets at the time of bone biopsy by evaluating radiographs of wrists and/or knees. Both wrist and knee X-rays were available in 51 patients, wrists X-rays alone were available in 10 patients, and knee X-rays alone were available in 4 patients. Growth plates were fused in a 17-year-old boy. Thus a total of 64 patients could be assessed for the presence of rickets. Each radiograph was independently reviewed by two pediatric bone specialists who were blinded to the patients' histomorphometric and serum 25(OH)D data. Radiographic changes of rickets were scored using the radiographic scoring method developed by Thatcher and colleagues.[11]

Dual-energy X-ray absorptiometry

Dual-energy X-ray absorptiometry (DXA) was performed in the anteroposterior direction at the lumbar spine (L_1–L_4) using a Hologic QDR 2000W or 4500 device (Hologic, Inc., Waltham, MA, USA). Lumbar spine areal bone mineral density (LS aBMD) results were transformed to age-specific *Z*-scores combining reference data from Salle and colleagues[12] and data provided by the densitometer manufacturer.

Bone biopsy and histomorphometry

Labeling was performed prior to biopsy using demeclocycline (15 to 20 mg/kg per day taken orally during two 2-day periods separated by a 10-day free interval). Transiliac bone samples were collected 4 or 5 days after the labeling. Biopsy preparation and histomorphometric analyses were performed as described previously.[13] Measurements were carried out using a digitizing table with OsteoMeasure software (Osteometrics, Inc., Atlanta, GA, USA). Nomenclature and abbreviations follow the recommendations of the American Society for Bone and Mineral Research.[14]

In histomorphometric terms, *osteomalacia* is defined as the simultaneous occurrence of increased osteoid thickness and increased mineralization lag time.[15] The criteria used for abnormality in these parameters depend on the source of reference data. We used a value of 2 SD above the mean of

the age-specific reference range to distinguish "normal" from "elevated" values.[16] This results in cut-off values of 9 μm for osteoid thickness and 25 days for mineralization lag time.[13] When both values are above these cut-offs, osteomalacia is said to be present. Since osteoid thickness and mineralization lag time do not vary between 2 and 20 years of age, these criteria can be applied throughout this age range.[13]

Statistical analysis

Raw results were transformed to age- and sex-specific *Z*-scores from the average result in the reference population using the published reference data cited in the description of each measurement technique. To compare histomorphometric measures between groups, results of each patient were expressed as a percentage of the published age-specific mean value.[13]

To evaluate seasonal variability, biochemical and histomorphometric data collected in the months with least sunlight in Montreal (November to April) were compared with those collected in the months with the most sunlight (May to October).

Variables were tested for normal distribution using the Kolmogorov-Smirnov test. Normally distributed data were expressed as mean and standard deviation (SD). Geometric means and geometric SDs were calculated for non–normally distributed variables. These variables were log transformed for tests that require normal distribution.

Differences between two groups were tested for significance using the unpaired Student's *t* test or the Mann-Whitney *U* test, as appropriate. Analysis of variance (ANOVA) or the Kruskall-Wallis test was used to compare more than two groups, and Bonferroni's adjustment was used to adjust for multiple testing. Group differences in dichotomous variables were tested for significance using the chi-square test. For values that were expressed as a percentage of the mean value of the reference range, the significance of the difference from 100% was calculated by the one-sample *t* test.

Associations are given as Pearson correlations or Spearman rank correlations, as appropriate. Logistic regression analysis was used to evaluate the relationship between serum 25(OH)D levels and histomorphometric parameters after adjusting for confounders. Age, gender, OI severity (coding OI-I = 1; OI-IV = 2; OI-III = 3), and serum 25(OH)D levels were introduced as confounders in models predicting bone metabolic indices (ie, osteoid thickness, mineralization lag time, or bone-formation rate per bone surface) and bone mass indices (ie, LS aBMD, bone volume per tissue volume, and cortical width). The effect of potential predictor variables was assessed in enter and stepwise multivariate modes. Results were expressed as odds ratios (ORs) with 95% confidence intervals (CIs).

All tests were two-tailed, and throughout the study, $p < .05$ was considered significant. These calculations were performed using the PASW Statistics software Version 18.0 (SPSS, Inc., Chicago, IL, USA).

Results

There were no significant sex differences in the clinical and biochemical characteristics presented in Table 1 or in bone histomorphometric results (Table 2). Therefore, results of girls and boys were analyzed as a single group. Average height and LS aBMD *Z*-scores were very low, as expected in a population with moderate to severe OI (Table 1).[17] In accordance with this, histomorphometric parameters of bone structure were clearly lower than in the reference population (Table 2). Bone surface–based parameters of bone formation (ie, osteoid surface, osteoblast surface, mineralizing surface, and bone-formation rate) and of bone resorption (ie, osteoclast surface and eroded surface) all were elevated, reflecting the increased bone remodeling activity that is typical for children and adolescents with OI.[18]

Serum 25(OH)D levels ranged from 13 to 103 nmol/L and were less than 15 nmol/L in one patient (1%), less than 30 nmol/L in 7 patients (10%), less than 50 nmol/L in 37 patients (52%), and less than 80 nmol/L in 67 patients (94%). The mean (±SD) serum 25(OH)D level was 49 (14) nmol/L in OI type I, 47 (23) nmol/L in OI type III, and 53 (20) nmol/l in OI type IV ($p = .53$ for the significance of the difference between OI types, ANOVA). In the

Table 1. Clinical Characteristics and Biochemical Results of the Study Population

	n	Result	Reference range
Sex (girls/boys)	71	36/35	—
OI type (I/III/IV)	71	29/12/30	—
Age (years)	71	8.1 (4.5)	—
Height (*Z*-score)	66	−3.6 (3.2)	−2 to +2
Weight (*Z*-score)	68	−1.6 (1.6)	−2 to +2
LS aBMD (*Z*-score)	68	−4.6 (1.3)	−2 to +2
Total serum calcium (mmol/L)	71	2.34 (0.14)	2.25 to 2.63
Serum phosphorus (mmol/L)	71	1.60 (0.25)	1.23 to 1.62
Serum alkaline phosphatase (U/L)	71	294 (119)	<300
Serum 25(OH)D (nmol/L)	71	50 (18)	34 to 91
Serum $1,25(OH)_2D_3$ (pmol/L)	68	105 (58)	65 to 134
Serum PTH (pmol/L)	66	7.5 (2.6)	2.5 to 10
Urinary NTX/creatinine (% reference mean)	65	117 (60)	

Note: Results are expressed as mean (±SD). Percent reference mean represents results expressed as a percentage of the mean of the age-specific reference range.

Table 2. Iliac Bone Histomorphometric Results in the Study Population

	n	Raw result	% of mean	*p*
Structural parameters				
Core width (mm)	58	4.2 (1.7)	63 (23)	<.001
Cortical width (mm)	69	493 (276)	58 (29)	<.001
Bone volume per tissue volume (%)	62	12 (5)	58 (22)	<.001
Parameters of cancellous bone formation				
Osteoid thickness (μm)	71	5.0 (1.2)	83 (19)	<.001
Osteoid surface per bone surface (%)	71	45 (14)	158 (56)	<.001
Osteoblast surface per bone surface (%)	70	22 (11)	275 (144)	<.001
Osteoid volume per bone volume (%)	71	4.7 (2.6)	164 (114)	<.001
Mineralizing surface per bone surface (%)	68	25 (9)	199 (72)	<.001
Mineralizing surface per osteoid surface (%)	68	59 (21)	132 (52)	<.001
Mineral apposition rate (μm/d)	71	0.73 (0.18)	77 (19)	<.001
Mineralization lag time (d)[A]	68	12.6 (1.6)	81 (1.6)	<.001
Bone-formation rate per bone surface ($\mu m^3/\mu m^2$/year)	68	70 (32)	157 (76)	<.001
Parameters of cancellous bone resorption				
Eroded surface per bone surface (%)	70	22 (9)	144 (56)	<.001
Osteoclast surface per bone surface (%)	69	1.6 (1.1)	146 (108)	<.001

Results are expressed as mean (SD). [A]Values are geometric mean (geometric SD). '% of mean' represents results expressed as a percentage of the mean of the age-specific reference range. P values represent the significance of difference of the % of mean value from 100% (one sample t-test).

whole study cohort, serum 25(OH)D was negatively correlated with age and serum PTH levels (Fig. 1) but was not correlated with serum alkaline phosphatase or the urinary NTX/creatinine ratio. Serum levels of $1,25(OH)_2D_3$ ranged from 15 to 279 pmol/L (reference range 65 to 134 pmol/L) and were below the reference range in 15 patients (22%). No seasonal variability in 25(OH)D serum concentrations was found.

As to histomorphometric indicators of bone mineralization, OI patients, on average, had a lower mineralization lag time and osteoid thickness than the reference population[13] (Table 2). Osteoid thickness ranged from 2.9 to 8.7 μm. Mineralization lag time ranged from 5 to 41 days and was more than 25 days in seven patients. Thus none of the patients fulfilled the criteria for the diagnosis of osteomalacia (which in children requires both an osteoid thickness of greater than 9 μm and a mineralization lag time of more than 25 days). The radiographic score for rickets was 0 (corresponding to absence of rickets) in all 64 patients in whom wrist and/or knee radiographs were available.

Serum 25(OH)D levels were not correlated with bone-formation rate, osteoid thickness, or mineralization lag time (Fig. 2). To study the relationship between 25(OH)D levels and bone parameters in more detail, patients were divided into a group with sufficient vitamin D levels and a group with insufficient levels. The level above which 25(OH)D concentrations should be considered sufficient is a matter of ongoing debate, but the current conservative consensus is that serum 25(OH)D levels should be at least 50 nmol/L.[1,19] Therefore, a cut-off value of 50 nmol/L was used to distinguish vitamin D sufficiency from vitamin D insufficiency.

The distributions of OI types and of the sexes were similar between the two groups, but patients with serum 25(OH)D levels of less than 50 nmol/L were significantly older than patients with higher 25(OH)D levels (Table 3). In the insufficiency group, average serum levels of total calcium, phosphorus, and

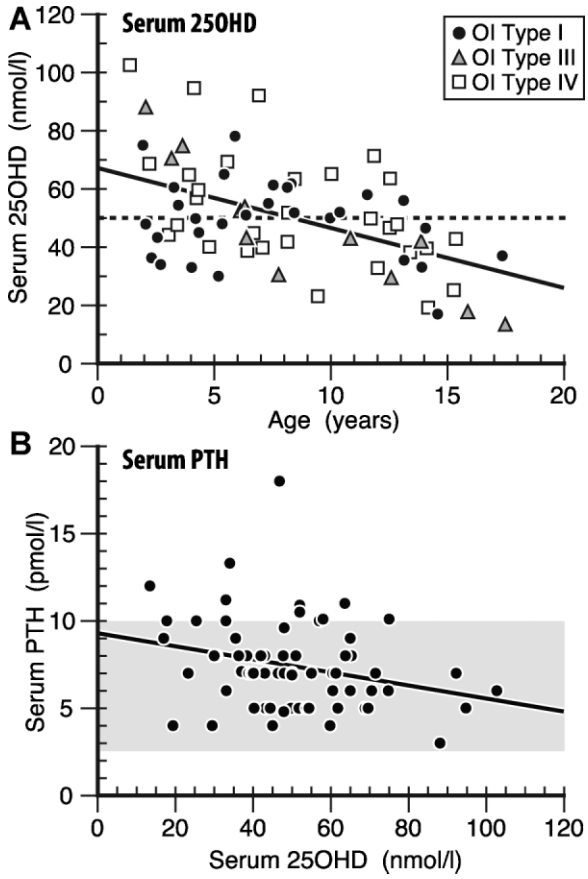

Fig. 1. (*A*) Relationship between age and serum levels of 25(OH)D ($r = -0.51$, $p < .001$ for the entire population). (*B*) Relationship between serum levels of 25(OH)D and serum PTH ($r = -0.27$, $p = .03$).

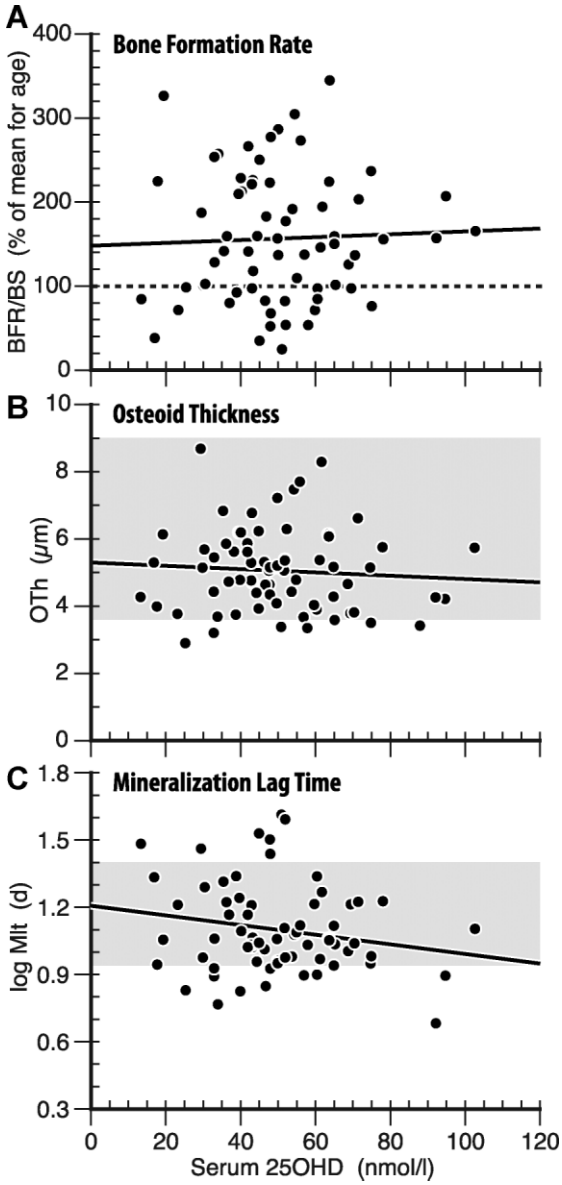

Fig. 2. Relationship between 25(OH)D serum levels and histomorphometric parameters. (*A*) Bone-formation rate ($r = 0.04$, $p = .75$) expressed as a percentage of the mean value of the age-specific reference range. The average result expected in a healthy population (100%) is shown as a dotted line. (*B*) Osteoid thickness ($r = -0.07$, $p = .55$). (*C*) Log-transformed mineralization lag time ($r = -0.19$, $p = .13$). The upper and lower limits of the reference range for osteoid thickness and mineralization lag time are indicated by the gray zone for each parameter.

$1,25(OH)_2D_3$ tended to be lower, and those of serum PTH were 13% higher than in the sufficiency group, but none of these differences reached statistical significance owing to the wide variability of results.

LS aBMD *Z*-scores and biochemical markers of bone turnover were very similar between the two groups (Table 3). In accordance with this, histomorphometric parameters of bone structure, as well as of bone formation and resorption, did not reveal significant group differences (Table 4). In particular, bone-formation rate, osteoid thickness, and mineralization lag time were very similar between groups (Table 4).

When gender, age, OI severity, and 25(OH)D levels were entered into a multivariate stepwise logistic regression model, none of these parameters emerged as a significant independent predictive factor of bone metabolic indices (ie, osteoid thickness, mineralization lag time, or bone-formation rate per bone surface), whereas age and OI severity emerged as significant independent predictive factors of bone mass indices (ie, LS aBMD *Z*-score, bone volume per tissue volume, and cortical width).

Discussion

In this study on children with moderate to severe OI, we found that more than half the patients had 25(OH)D serum levels below what is currently considered vitamin D sufficiency. However, none of the patients had signs of rickets or osteomalacia or other histomorphometric signs of hypovitaminosis D osteopathy. We also did not detect a relationship between serum 25(OH)D levels and indicators of bone mass.

Our observation that many children with OI have serum 25(OH)D concentrations below currently recommended levels is in accordance with a recent report by Bowden and colleagues.[5] Indeed, the proportion of patients with 25(OH)D levels less than 50 nmol/L was higher in this study population (52%) than in the previous study population (21%). The higher incidence of vitamin D insufficiency in our population may be attributable to geographic factors—presumably most of our patients lived at a more northern latitude than the children described by Bowden and colleagues. It is also possible that the more severe bone fragility and hence decreased mobility in our cohort limited the time they spent in the sun. Similar to Bowden and colleagues, we did not find a seasonal variation in 25(OH)D levels, which also may reflect limited sun exposure in this patient group. Our observation that serum 25(OH)D levels were lower in teenagers than in younger children mirrors the general situation in North American youths.[1,20]

We observed the expected inverse relationship between serum 25(OH)D and PTH concentrations. The average PTH level in our vitamin D insufficiency group [mean 25(OH)D level 37 nmol/L] was 13% higher than in the vitamin D sufficiency group [mean 25(OH)D level: 65 nmol/L]. When the mean 25(OH)D levels in these two groups are entered into the 25(OH)D-PTH regression model established in healthy French adults, a 12% difference in PTH concentrations is predicted.[21] The actually observed difference in mean serum PTH concentrations between these two groups thus was remarkably close to the predicted value, indicating that children with OI have the same PTH response to decreasing 25(OH)D levels as healthy adults.

The observation that none of the patients in our population had a mineralization defect (rickets or osteomalacia) is not unexpected. In an Australian study on 121 adults with osteoporosis who had a similar distribution of serum 25(OH)D levels as our patient group (range 12 to 159 nmol/L, median 56 nmol/L), Need and colleagues also did not find a case of osteomalacia.[22] Similar observations have been made recently in a study on 24 children with fractures who were evaluated for suspected primary osteoporosis.[23] However, at first sight, our

Table 3. Anthropometric, Densitometric, and Biochemical Results of OI Patients According to Vitamin D Status

Variable	*n*	25(OH)D < 50 nmol/L	*n*	25(OH)D ≥ 50 nmol/L	*p*
Age (years)	37	9.5 (5.0)	34	6.7 (3.3)	.009
Gender (male/female)	37	18/19	34	17/17	.91
OI type (type I/III/IV)	37	14/7/16	34	15/5/14	.83
Height (*Z*-score)	34	−4.3 (3.4)	32	−2.8 (2.9)	.07
Weight (*Z*-score)	34	−1.8 (1.4)	34	−1.5 (1.7)	.43
LS aBMD (*Z*-score)	34	−4.5 (1.3)	34	−4.6 (1.4)	.78
Total serum calcium (mmol/L)	37	2.32 (0.14)	34	2.37 (0.13)	.09
Serum phosphorus (mmol/L)	37	1.55 (0.29)	34	1.64 (0.20)	.12
Serum alkaline phosphatase (UI/L)	37	283 (118)	34	306 (121)	.42
Serum 25(OH)D (nmol/L)	37	37 (10)	34	65 (13)	<.001
Serum 1,25$(OH)_2D_3$ (pmol/L)	37	93 (49)	31	120 (64)	.05
Serum PTH (pmol/L)	35	7.9 (2.9)	31	7.0 (2.1)	.17
Urinary NTX/creatinine (% reference mean)	36	119 (66)	29	115 (52)	.80

Note: Values are mean (±SD). Values of *p* represent the significance of the difference between the two groups (Student's *t* test).

findings seem to be at odds with a recent large histomorphometric study in subjects who had died from accidents and sudden disease.[24] In that study, it was concluded that a large proportion of subjects with low 25(OH)D levels had a mineralization defect. However, this conclusion was based on an analysis of osteoid volume per bone volume—a parameter that does not allow distinguishing between a mineralization defect and elevated bone turnover.[3,4] This autopsy study in fact showed that the overwhelming majority of subjects with serum 25(OH)D levels below 20 nmol/L had normal osteoid thickness, demonstrating that they actually did not have osteomalacia.[24] This is entirely consistent with the observations in this study.

While the lack of osteomalacia in our OI patient population is in line with previous studies, one might have expected that some patients with low 25(OH)D levels have signs of milder hypovitaminosis D osteopathy, such as increased bone turnover, or differences in structural parameters of cortical and cancellous bone, as have been found in adults without OI.[3,4,22,23,25] A possible explanation for the lack of correlation between 25(OH)D serum levels and these histomorphometric parameters in our patients is that bone tissue in children does not react to low 25(OH)D levels in the same manner as adult bone. Another possibility is that weak OI osteoblasts are unable to respond to increasing PTH levels in a normal fashion. In any case, this study shows that bone remodeling activity is elevated in OI regardless of 25(OH)D levels. This may have made the detection of any PTH effect on bone remodeling activity more difficult.

The fact that we did not find a relationship between 25(OH)D and indicators of bone mass is consistent with the report by Bowden and colleagues, who also did not find a relationship

Table 4. Histomorphometric Parameters of Patients With OI According to Vitamin D Status

	n	25(OH)D < 50 nmol/L	*n*	25(OH)D ≥ 50 nmol/L	*p*
Structural parameters					
Core width (%)	31	65 (24)	27	61 (23)	.57
Cortical width (%)	35	59 (32)	34	57 (26)	.85
Bone volume per tissue volume (%)	33	59 (25)	29	57 (20)	.79
Parameters of cancellous bone formation					
Osteoid thickness (%)	37	82 (18)	34	84 (21)	.72
Osteoid surface per bone surface (%)	37	165 (58)	34	150 (54)	.29
Osteoid volume per bone volume (%)	37	178 (136)	34	149 (83)	.29
Osteoblast surface per bone surface (%)	37	287 (158)	33	263 (128)	.50
Mineralizing surface per bone surface (%)	36	201 (78)	32	196 (67)	.78
Mineralizing surface per osteoid surface (%)	36	129 (54)	32	136 (50)	.62
Mineral apposition rate (%)	37	77 (18)	34	77 (21)	.95
Mineralization lag time (%)[a]	37	82 (1.6)	32	80 (1.6)	.72
Bone-formation rate per bone surface (%)	36	158 (77)	32	155 (77)	.89
Parameters of cancellous bone resorption					
Eroded surface per bone surface (%)	37	144 (59)	33	144 (54)	.98
Osteoclast surface per bone surface (%)	37	153 (117)	32	139 (99)	.62

Note: Values are mean (±SD). All results are expressed as a percentage of the mean of the age-specific reference range.[13]
[a]Values are geometric mean (± geometric SD). Values of *p* represent the significance of the difference between the two groups (Student's *t* test).

between serum 25(OH)D and LS aBMD.[5] It should be noted that even randomized, controlled trials have struggled to find an effect of vitamin D supplementation on bone density outcomes in healthy children.[26] It is therefore not surprising that the present cross-sectional analysis in an OI population with a wide intersubject variability in bone density failed to reveal a relationship between serum 25(OH)D levels and LS aBMD.

The fact that we did not observe a relationship between 25(OH)D levels and bone metabolism or bone mass indicators does not mean that 25(OH)D is not important in children and adolescents with OI. For example, the start of intravenous bisphosphonate treatment usually leads to a decrease in serum calcium levels.[6] Inadequate vitamin D levels might exacerbate the risk of posttreatment hypocalcemia.

Conclusion

We found no evidence that serum 25(OH)D levels in the range from 13 to 103 nmol/L were associated with measures of bone mineralization, metabolism, or mass in children with OI.

Disclosures

All the authors state that they have no conflicts of interest.

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