Serum 24,25-Dihydroxyvitamin D Concentrations in Osteogenesis Imperfecta: Relationship to Bone Parameters

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Background: Several studies suggest that 24,25-dihydroxyvitamin D [24,25$(OH)_2$D] may have an effect on bone mass and metabolism.

Objective: We evaluated the relationship between serum 24,25$(OH)_2$D levels and bone density and bone metabolism in children with a primary bone disorder—osteogenesis imperfecta (OI).

Materials and Methods: The study included 132 patients (age, 1.1 to 17.9 yr; 67 girls) with OI types I, III, or IV who had not received bisphosphonate treatment at the time of analysis.

Results: Serum 24,25$(OH)_2$D levels were significantly higher in OI type III than in OI type I or IV. Serum 24,25$(OH)_2$D concentrations were positively correlated with serum 25-hydroxyvitamin D (25OHD) levels and negatively correlated with serum PTH levels, and were not correlated with serum 1α,25-dihydroxyvitamin D [1,25$(OH)_2$D]. The ratio between serum 24,25$(OH)_2$D and 25OHD was negatively correlated with age and was independent of serum 25OHD concentrations. Regression analysis revealed that OI severity ($P = 0.04$), serum 25OHD levels ($P < 0.001$), and serum PTH concentrations ($P = 0.045$), but not age, gender, or serum 1,25$(OH)_2$D, were independent predictors of serum 24,25$(OH)_2$D levels. No correlation was found between serum 24,25$(OH)_2$D levels or the ratio between serum 24,25$(OH)_2$D and 25OHD and lumbar spine bone mineral density z-scores or bone marker levels (serum osteocalcin and urinary collagen type I N-telopeptide) after adjusting for OI type, age, and gender.

Conclusion: Patients with more severe OI type had higher 24,25$(OH)_2$D serum levels and higher serum 24,25$(OH)_2$D to 25OHD ratios, suggesting an increased 25OHD-24-hydroxylase activity. **(*J Clin Endocrinol Metab* 97: 1243–1249, 2012)**

Vitamin D plays an essential role in calcium homeostasis and the development and maintenance of the skeleton. Vitamin D undergoes two successive hydroxylation reactions: first in the liver where a hydroxyl group is added on carbon 25 by the activity of the vitamin D-25 hydroxylase, leading to the formation of 25-hydroxyvitamin D (25OHD); and second, in kidneys where the 25OHD-1α hydroxylase drives the addition of a hydroxyl group on carbon 1, leading to the formation of 1α,25-dihydroxyvitamin D [1,25$(OH)_2$D], the active hormonal form of vitamin D (1).

25OHD is hydroxylated at carbon 24 by the 25OHD-24-hydroxylase enzyme (CYP24A1), leading to the formation of 24,25-dihydroxyvitamin D [24,25$(OH)_2$D], a metabolite that circulates in the bloodstream at a concentration that is about 50-fold higher than that of 1,25$(OH)_2$D (2). The ratio between the serum concentrations of 24,25$(OH)_2D_3$ and of $25OHD_3$ can be regarded as an indicator of CYP24A1 activity (3).

CYP24A1 plays an important role in vitamin D metabolism, as is evident from the recent observation that

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Abbreviations: LS-aBMD, Lumbar spine areal bone mineral density; NTX, N-telopeptide of type I collagen; 25OHD, 25-hydroxyvitamin D; 1,25$(OH)_2$D, 1α,25-dihydroxyvitamin D; 24,25$(OH)_2$D, 24,25-dihydroxyvitamin D; OI, osteogenesis imperfecta.

a lack of this enzyme leads to infantile hypercalcemia (4, 5). Nevertheless, the physiological role of circulating $24,25(OH)_2D$ has not been clearly elucidated. Early studies concluded that $24,25(OH)_2D$ is a degradation metabolite that is not essential for development (6–8), but subsequent data suggested that $24,25(OH)_2D$ also exerts distinct effects on calcium and phosphorus homeostasis (9), cartilage (10, 11), and bone (12–14).

The role of $24,25(OH)_2D$ has been evaluated in several human diseases. In X-linked hypophosphatemic rickets, treatment with $24,25(OH)_2D$ corrected bone lesions of rickets and osteomalacia (15). A study on predialysis renal insufficiency patients treated with either $1,25(OH)_2D$ alone or in combination with $24,25(OH)_2D$, supported a direct role of $24,25(OH)_2D$ in bone mineralization (16).

Several preclinical studies suggest that $24,25(OH)_2D$ may have a beneficial effect on bone mass. Treatment with high doses of $24,25(OH)_2D$ increases bone mass in vitamin D-replete rats (17), rabbits (18), and dogs (18). In a model for human X-linked hypophosphatemic rickets, treatment with $24,25(OH)_2D$ was associated with a dose-dependent increase in bone formation without inducing excessive bone resorption (19).

To our knowledge, the relationship of $24,25(OH)_2D$ serum levels with bone mass and bone metabolism has not been examined in children with bone fragility disorders. Osteogenesis imperfecta (OI) is the most frequent primary bone fragility disorder in children and adolescents (20). Four clinical types are commonly distinguished according to the traditional Sillence classification (21). OI type I comprises patients who do not have major bone deformities and whose height is within or close to the reference range. OI type II is usually lethal in the perinatal period. OI type III is the most severe form in children surviving the neonatal period. These patients are of very short stature and have limb and spine deformities secondary to multiple fractures. Finally, patients with mild to moderate bone deformities and variable short stature are classified as OI type IV. The clinical severity of the phenotype in patients surviving the neonatal period can be graded according to fracture rate, bone deformities, and stature in the order OI type I $<$ OI type III $<$ OI type IV (20).

In the present study, we determined the relationship between serum $24,25(OH)_2D$ status and bone parameters in 132 children with moderate to severe OI.

Patients and Methods

Patients

This retrospective study included OI patients followed at the Shriners Hospital for Children in Montreal. Inclusion criteria were: a diagnosis of OI type I, III, or IV; the availability of $24,25(OH)_2D$ serum levels at the time of the first evaluation at the Shriners Hospital (so that results would not be influenced by the treatments received at this institution); and age of 1 to 18 yr. Patients who had received bisphosphonate treatment before their first evaluation at the Shriners Hospital were excluded because bisphosphonates have a major influence on bone metabolism and bone density (22).

A total of 132 patients (67 females and 65 males) were included in the study. The age of these patients ranged from 1.1 to 17.9 yr. In 105 patients (80%), lumbar spine areal bone mineral density (LS-aBMD) measurements were available at the time of $24,25(OH)_2D$ analyses.

Results of sequence analysis of *COL1A1* and *COL1A2*, the genes coding for collagen type I, were available in 116 patients and revealed disease-causing mutations in 102 patients (88%). In the 14 patients where *COL1A1* and *COL1A2* sequence analyses were negative and in the 16 patients without DNA analysis, the diagnosis of OI was clinically asserted by the association of frequent fractures, low bone mass, and blue sclera or dentinogenesis imperfecta. The study was approved by the Shriners Hospital Institutional Review Board, and informed consent was obtained from legal guardians.

Anthropometric measurements

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). 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 (23).

Biochemical measurements

Serum total calcium, phosphorus, and alkaline phosphatase were measured using colorimetric methods (Monarch; Instrumentation Laboratories Inc., Lexington, MA). Serum active intact PTH concentrations (fragment 1–84) were determined by RIA (Diasorin Inc., Stillwater, MN).

Quantification of vitamin D metabolites in serum samples

Serum vitamin D metabolites were extracted with acetonitrile, loaded on C18 cartridges, and eluted. 25OHD, $24,25(OH)_2D$, and $1,25(OH)_2D$ were fractionated using silica cartridges (Waters Inc., Mississauga, Ontario, Canada), from which they were eluted using hexane and propanol in varying percentages (24). The samples were then dried out and redissolved in 95% ethanol. The collected 25OHD, $24,25(OH)_2D$, and $1,25(OH)_2D$ fractions were quantified by RIA. The 25-hydroxyvitamin D ^{125}I RIA Kit (Diasorin Inc.) was used to determine 25OHD and $24,25(OH)_2D$. This kit uses an antibody that has 100% cross-reactivity for $25OHD_2$, $25OHD_3$, $24,25(OH)_2D_2$, and $24,25(OH)_2D_3$ (25) and information provided by the manufacturer. When applied to the 25OHD fraction, this kit therefore determines the total of $25OHD_2$ and $25OHD_3$. When applied to the $24,25(OH)_2D$ fraction, the total of $24,25(OH)_2D_2$ and $24,25(OH)_2D_3$ is measured. The $24,25(OH)_2D$ used to produce the standard curve to quantify $24,25(OH)_2D$ with this kit was kindly provided by Hoffmann-La Roche Inc. (Basel, Switzerland). The $1,25(OH)_2D$ ^{125}I RIA Kit (Diasorin Inc.) was used to quantify $1,25(OH)_2D$. Recovery of the three vitamin D metabolites was assessed using a β-counter, by spiking a sample with tritiated 25OHD,

$1,25(OH)_2D$, and $24,25(OH)_2D$ (Amersham Bioscience, Piscataway, NJ), respectively.

Osteocalcin was quantified with an immunoradiometric assay (N-tact OsteoSP; DiaSorin). Urine creatinine concentration was determined colorimetrically. Urinary cross-linked N-telopeptide of type I collagen (NTX), a marker of bone resorption, was quantified by ELISA (Osteomark; Ostex International Inc., Seattle, WA) 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 reference values using published reference data (26). Patients were fasting at the time of urine sampling.

Dual-energy x-ray absorptiometry

LS-aBMD was determined in the anterior-posterior direction at the lumbar spine (L1–L4) using a Hologic QDR 2000W or 4500 dual-energy x-ray absorptiometry device (Hologic Inc., Waltham, MA). During the transition from the QDR 2000 device to the QDR 4500 device, 30 pediatric patients underwent LS-aBMD measurements with both devices. This showed that results differed by less than 1.5% on average, which is negligible when compared with the wide interindividual variation in the study population. Therefore, results obtained with the two devices were grouped together. Results were transformed to age-specific z-scores combining reference data from Salle *et al.* (27) and data provided by the densitometer manufacturer.

Statistical analyses

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. Differences between two groups were tested for significance using the unpaired Student's *t* test. ANOVA was used to compare more than two groups, and Bonferroni's adjustment was used to adjust for multiple testing in *post hoc* comparisons.

Associations are given as Pearson correlation or Spearman rank correlation, as appropriate. Multiple regression analysis was used to assess potential predictors of serum $24,25(OH)_2D$ levels and the ratio between serum $24,25(OH)_2D$ and 25OHD. Age, gender (coding, male = 1; female = 2), OI severity (coding, OI-I = 1; OI-IV = 2; OI-III = 3), and biochemical parameters [serum PTH, $1,25(OH)_2D$, and 25OHD levels] were introduced as independent variables. The relationship between $24,25(OH)_2D$ levels and the serum $24,25(OH)_2D$ to 25OHD ratio and serum osteocalcin, urinary NTX, or LS-aBMD was evaluated in a multiple regression model after accounting for OI severity, age, and gender. The effect of potential predictor variables was assessed in the stepwise mode.

All tests were two-tailed, and throughout the study $P < 0.05$ was considered significant. These calculations were performed using the PASW Statistics software version 18.0 (SPSS Inc., Chicago, IL).

Results

Clinical and biochemical characteristics were similar between sexes, and therefore results of girls and boys were analyzed as a single group. As expected in an OI population, average height, weight, and LS-aBMD z-scores were very low overall, and were higher in patients with OI type I than in OI types III and IV (Table 1) (28).

Serum $24,25(OH)_2D$ concentrations in patients with OI

Serum $24,25(OH)_2D$ levels were significantly higher in patients with OI type III than in patients with OI type I, or in patients with OI type IV (Table 1). There were no other significant differences between OI types with regard to serum calcium, phosphorus, 25OHD, $1,25(OH)_2D$, or PTH levels, but serum osteocalcin levels were significantly higher in OI type I. The proportion of patients with serum

TABLE 1. Clinical, biochemical, and bone densitometric results

Variable	n	OI type I	n	OI type III	n	OI type IV	*P*
Gender (male/female)	42	21/21	27	15/12	63	32/31	0.85
Age (yr)	42	7.0 (4.2)	27	6.7 (4.8)	63	7.5 (4.6)	0.73
Height (z-score)	42	−1.2 (0.9)[a,b]	26	−7.9 (2.9)[c]	63	−3.9 (2.5)	<0.001
Weight (z-score)	41	−1.1 (1.4)[a,b]	26	−5.1 (3.2)[c]	63	−2.6 (2.3)	<0.001
LS-aBMD (z-score)	32	−3.4 (1.1)[a,b]	21	−5.9 (0.8)	52	−5.2 (1.3)	<0.001
Total calcium, mmol/liter (norm, 2.25–2.63)	42	2.38 (0.11)	27	2.40 (0.17)	63	2.40 (0.12)	0.85
Phosphate, mmol/liter (norm, 1.23–1.62)	42	1.57 (0.20)	27	1.61 (0.27)	63	1.59 (0.20)	0.70
Alkaline phosphatase, IU/liter (norm, <300)	42	314 (108)	27	298 (98)	63	291 (110)	0.55
Osteocalcin, nmol/liter (norm, 1.7–5.2)	35	5.8 (2.5)[a,b]	25	2.6 (1.5)	51	3.3 (2.4)	<0.001
PTH, pmol/liter (norm, <10)	42	7.2 (3.1)	27	7.1 (2.6)	63	6.9 (2.2)	0.82
Urinary NTX/creatinine (% reference mean)	35	125 (67)	24	120 (45)	56	133 (77)	0.70
25OHD, nmol/liter (norm, 34–91)	42	59 (22)	27	65 (21)	63	59 (24)	0.47
$1,25(OH)_2D$, pmol/liter (norm, 65–134)	42	100 (43)	27	111 (72)	63	92 (49)	0.31
$24,25(OH)_2D$, nmol/liter (norm, 3.2–5.1)	42	4.9 (3.5)[a]	27	7.2 (3.4)[c]	63	4.7 (3.5)	0.007
$24,25(OH)_2D$:25OHD ratio	42	0.08 (0.03)[a]	27	0.12 (0.06)[c]	63	0.08 (0.05)	0.001

Values are expressed as mean (SD). *P* values represent the significance of the difference between the three groups (ANOVA).

[a] Significantly different between type I and type III OI groups.

[b] Significantly different between type I and type IV OI groups.

[c] Significantly different between type III and type IV OI groups.

25OHD levels below 50 nmol/liter, the level considered insufficient (29), was similar in OI type I (13 of 42 patients; 31%), OI type III (eight of 27 patients; 30%), and OI type IV (25 of 63 patients; 40%) ($P = 0.53$, χ^2 test).

In the whole study population, serum 24,25(OH)$_2$D concentrations were positively correlated with serum 25OHD levels and negatively correlated with serum PTH levels, and were not correlated with serum 1,25(OH)$_2$D (Fig. 1). The ratio between serum 24,25(OH)$_2$D and 25OHD was negatively correlated with age and was independent of serum 25OHD concentrations (Fig. 2). As previously described, serum 25OHD levels were inversely correlated with age in children with OI ($r = -0.39$; $P < 0.001$) (30).

Predictive factors of serum 24,25(OH)$_2$D levels

Multivariate stepwise regression analysis was performed to evaluate which putative determinants (age, gender, OI severity, 25OHD, 1,25(OH)$_2$D, and PTH) were independently associated with 24,25(OH)$_2$D levels. This revealed that OI severity ($P = 0.04$), serum 25OHD levels ($P < 0.0001$), and PTH ($P = 0.045$), but not age, gender, or serum 1,25(OH)$_2$D, were significant independent predictive factors of serum 24,25(OH)$_2$D levels. In this model, the regression equation was: serum 24,25(OH)$_2$D (nmol/liter) = 0.02 + [0.09 × serum 25OHD levels (nmol/liter)] + [0.73 × OI severity] − [0.20 × serum PTH (nmol/liter)].

Similarly, multivariate stepwise regression analysis was performed to evaluate which putative determinants (age, gender, OI severity, 1,25(OH)$_2$D, and PTH) were independently associated with the ratio between serum 24,25(OH)$_2$D and 25OHD. This revealed that age ($P = 0.006$) and OI severity ($P = 0.004$), but not gender, PTH, or serum 1,25(OH)$_2$D, were significant independent predictive factors of the 24,25(OH)$_2$D to 25OHD ratio. In this model, the regression equation was: ratio of serum 24,25(OH)$_2$D to 25OHD = 0.073 + [0.017 × OI severity] − [0.002 × age (yr)].

Relationships between 24,25(OH)$_2$D levels and bone parameters

No correlation was found between serum 24,25 (OH)$_2$D levels and LS-aBMD (expressed as absolute values or z-scores) or bone marker levels (serum osteocalcin and urinary NTX/creatinine), either before or after adjusting for OI severity, age, and gender ($P > 0.1$ in each case). Similarly, the ratio between serum 24,25(OH)$_2$D and 25OHD was not correlated with serum osteocalcin ($P = 0.90$), urinary NTX/creatinine ($P = 0.75$), or LS-aBMD ($P = 0.43$), after adjustment for OI severity, age, and gender.

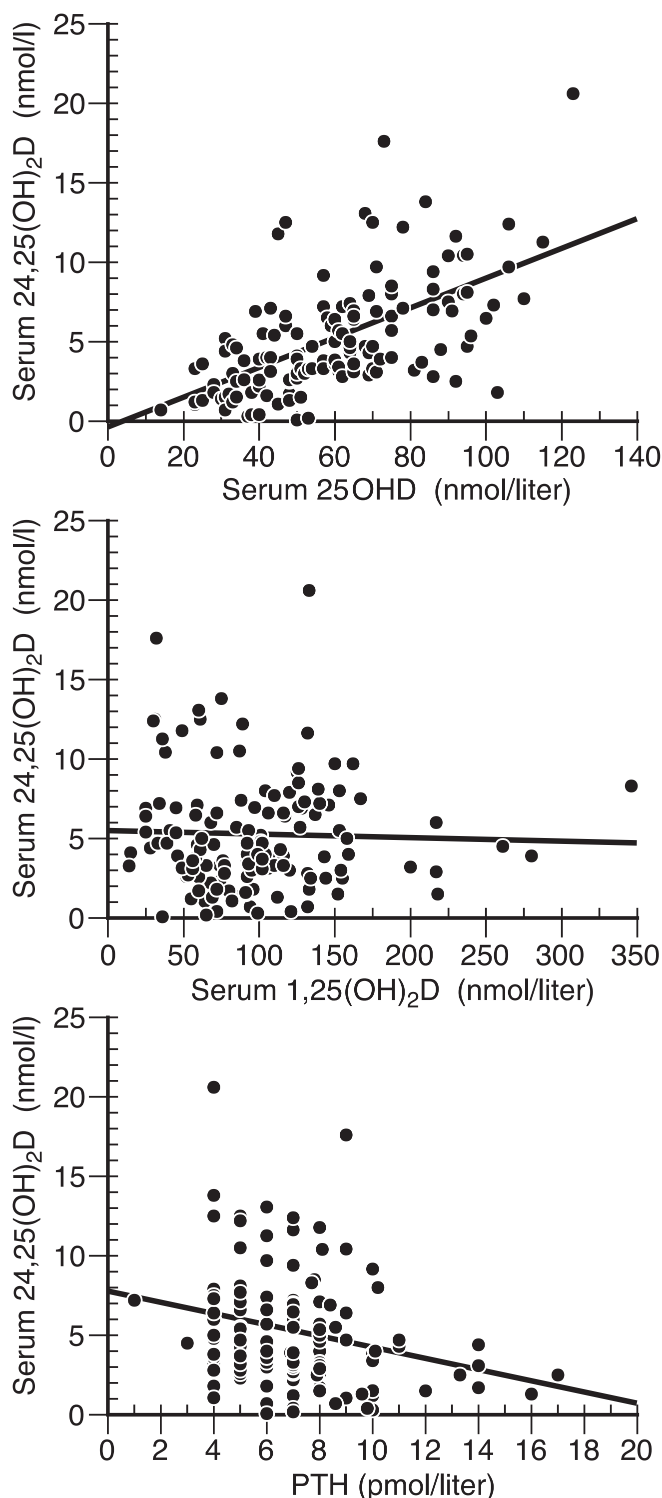

FIG. 1. Relationship between serum 24,25(OH)$_2$D levels and serum 25OHD levels ($r = 0.60$; $P < 0.0001$), serum 1,25(OH)$_2$D levels ($r = -0.03$; $P = 0.71$), and serum PTH levels ($r = -0.25$; $P = 0.004$).

Discussion

In this study, we found that the most severely affected group of young OI patients, those with OI type III, had higher 24,25(OH)$_2$D serum levels and higher serum 24,25(OH)$_2$D to 25OHD ratios than patients with other

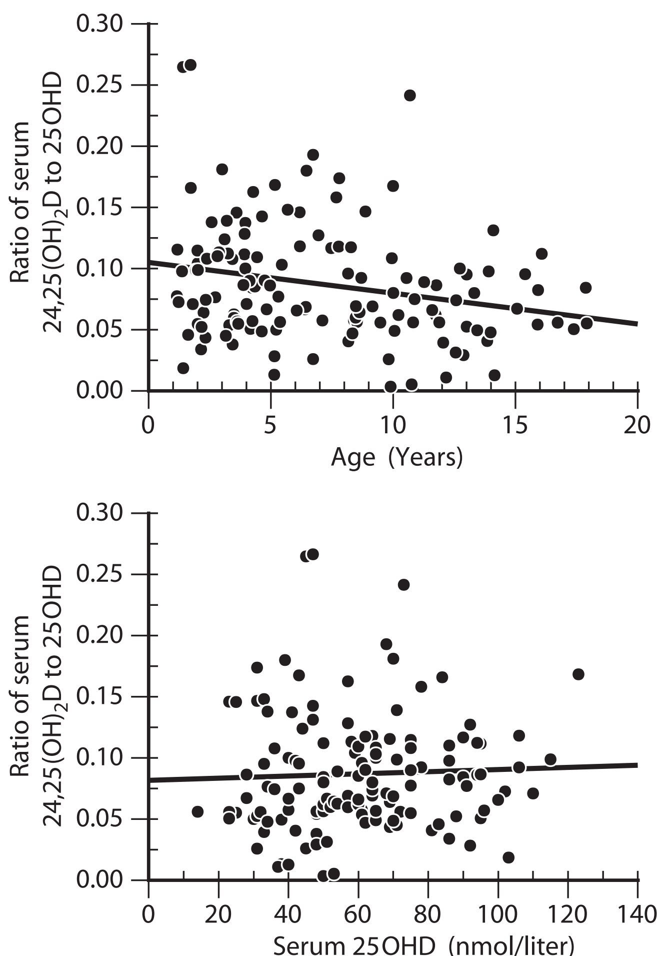

FIG. 2. Relationship between the ratio of serum $24,25(OH)_2D$ to 25OHD and age ($r = -0.23$; $P = 0.007$), and serum 25OHD levels ($r = 0.04$; $P = 0.62$).

OI types. The differences between OI types were not related to differences in bone mass or bone metabolism because no relationship was observed between serum $24,25(OH)_2D$ concentrations and LS-aBMD or biochemical bone markers. The group differences in $24,25(OH)_2D$ serum levels were also not explained by differences in 25OHD serum concentrations because 25OHD levels were similar between groups, and the differences in $24,25(OH)_2D$ serum levels between OI types persisted after adjustment for 25OHD concentrations.

These findings suggest that OI type III patients have increased CYP24A1 activity, which may have implications for the amount of vitamin D supplementation that is needed to achieve a given desired serum level of 25OHD. Indeed, a higher serum $24,25(OH)_2D$ to 25OHD ratio seems to be associated with a lower increase in serum 25OHD concentration after vitamin D supplementation (3). We previously observed that more than one fourth of OI patients have insufficient (<50 nmol/liter) 25OHD serum concentrations, and that more severely affected patients tend to have lower 25OHD levels (30). The higher serum $24,25(OH)_2D$ to 25OHD ratio suggests that OI type III patients may require higher doses of vitamin D to reach vitamin D sufficiency.

The origin of this increased CYP24A1 activity in OI type III patients is unclear at this point. One possibility is that the surplus production of $24,25(OH)_2D$ takes place in the skeleton. OI type III is characterized by a large number of fractures. Experiments in chicken have found that circulating levels of $24,25(OH)_2D$ increase during fracture repair and that chicken fed $24,25(OH)_2D$ had superior mechanical properties compared with chicken fed $1,25(OH)_2D$ alone (31, 32). Similarly, fracture healing is delayed in CYP24A1-null mice, and this defect can be corrected by exogenous administration of $24,25(OH)_2D$ (14). Thus, because OI type III patients frequently have active fracture calluses in one or more skeletal sites, this may increase their CYP24A1 activity. Prospective studies would be required to elucidate the effect of fracture repair on $24,25(OH)_2D$ levels in OI patients.

As previously described in healthy children and adults, we found that serum $24,25(OH)_2D$ levels were linearly associated with serum 25OHD concentrations (3, 33–35). The ratio between serum $24,25(OH)_2D$ and 25OHD remained constant across the 25OHD concentration range, suggesting that a relatively stable proportion of 25OHD is converted into $24,25(OH)_2D$. It is nevertheless interesting to note that the ratio between serum $24,25(OH)_2D$ and 25OHD decreased with age in our study population, although serum 25OHD levels are inversely correlated with age in children. This suggests that older study participants were converting 25OHD to $24,25(OH)_2D$ at a somewhat faster rate than younger children, which may be related to an increase in CYP24A1 activity by sex hormones during puberty (34).

Although CYP24A1 is inducible in all $1,25(OH)_2D$ target tissues, circulating $24,25(OH)_2D$ levels are associated with renal CYP24A1 activity (36). As previously described in healthy children and adults (33, 35), we found that serum $24,25(OH)_2D$ levels were negatively associated with serum PTH concentrations. This confirms *in vitro* studies reporting that PTH negatively regulates renal CYP24A1 activity, whereas a synergistic effect together with $1,25(OH)_2D$ is observed in osteoblasts (37, 38).

Several *in vitro* and *in vivo* studies have suggested a putative role for $24,25(OH)_2D$ in bone biology. One study suggested that $24,25(OH)_2D$ stimulates osteocalcin synthesis in human osteoblasts (39). However, we did not observe a relationship between serum $24,25(OH)_2D$ levels and serum osteocalcin or urinary NTX/creatinine, a marker of bone resorption. This is consistent with a previous clinical trial in X-linked hypophosphatemic rickets patients receiving $24,25(OH)_2D$ supplementation (15). A

positive association between serum 24,25(OH)$_2$D levels and vertebral bone mineral content has been reported in healthy men (35). However, in early postmenopausal women, no beneficial effects of 24,25(OH)$_2$D treatment on bone mineral density or bone loss were observed (40). In our study, we did not observe a relationship between physiological serum 24,25(OH)$_2$D levels and bone density.

This study has several limitations. Owing to the retrospective nature of this study, several potential modifiers of serum 24,25(OH)$_2$D levels are lacking (*e.g.* number and severity of fractures, pubertal status, and vitamin D intake from dietary, supplement, and drug sources). Moreover, besides PTH, other hormones, especially phosphate-regulating hormone fibroblast-like growth factor 23, may modify CYP24A1 activity (36), but their levels were not available in our patients. Consequently, we were not able to elucidate the origin and the clinical consequences of the increased CYP24A1 activity in OI type III patients. Prospective and longitudinal studies are required to delineate the determinants of vitamin D metabolism in such patients and to identify the clinical consequences of increased CYP24A1 activity.

Conclusion

This retrospective cross-sectional study provides some evidence that the group of young OI patients with the most severe skeletal phenotype have higher serum 24,25(OH)$_2$D levels and higher serum 24,25(OH)$_2$D to 25OHD ratios, independently of bone mass or bone metabolism.

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