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Effect of high-dose vitamin D supplementation on bone density in youth with osteogenesis imperfecta: A randomized controlled trial☆

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ABSTRACT

Osteogenesis imperfecta (OI) is a heritable condition characterized by fragile bones. Our previous studies indicated that serum 25-hydroxyvitamin D (25OHD) concentrations were positively associated with lumbar spine areal bone mineral density (LS-aBMD) in children and adolescents with OI. Here we assessed whether one year of high-dose vitamin D supplementation results in higher LS-aBMD z-scores in youth with OI. A one-year double-blind randomized controlled trial conducted at a pediatric orthopedic hospital in Montreal, Canada. Sixty patients (age: 6.0 to 18.9 years; 35 female) were randomized in equal numbers to receive either 400 or 2000 international units (IU) of vitamin D, stratified according to baseline bisphosphonate treatment status and pubertal stage. At baseline, the average serum 25OHD concentration was 65.6 nmol/L (SD 20.4) with no difference between treatment groups ($p = 0.77$); 21% of patients had results < 50 nmol/L. Vitamin D supplementation was associated with higher serum 25OHD concentrations in 90% of participants. The increase in mean 25OHD was significantly higher ($p = 0.02$) in the group receiving 2000 IU of vitamin D (mean [95% CI] = 30.5 nmol/L [21.3; 39.6]) than in the group receiving 400 IU (15.2 nmol/L [6.4; 24.1]). No significant differences in LS-aBMD z-score changes were detected between treatment groups. Thus, supplementation with vitamin D at 2000 IU increased serum 25OHD concentrations in children with OI more than supplementation with 400 IU. However, in this study where about 80% of participants had baseline serum 25OHD concentrations ≥ 50 nmol/L, this difference had no detectable effect on LS-aBMD z-scores.

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1. Introduction

Osteogenesis imperfecta (OI) is the most common primary bone fragility disorder affecting youth and is usually caused by mutations in one of the two genes coding for collagen type I [1]. Affected individuals frequently have fractures and areal bone mineral density (aBMD) is usually low at the lumbar spine. The severity of the disorder varies widely and is reflected in the Sillence classification, where OI type I represents the mildest form of OI, OI type II is the neonatal lethal form, OI type III is the most severe type of OI in survivors of the neonatal period, and OI type IV is 'moderately severe', with a phenotype that is intermediate between OI type I and III [2]. There is presently no cure to correct the disease-causing genetic defect in OI, but bisphosphonates are widely used to increase aBMD and to reduce the number of fractures [2].

It is well established that vitamin D plays an essential role in achieving adequate bone mineralization [3]. Vitamin D status is best measured by the serum concentration of 25-hydroxyvitamin D (25OHD) which accounts for both endogenous and exogenous sources of the vitamin [4]. General recommendations for the lower limit of target serum 25OHD concentrations range from 50 to 75 nmol/L but concentrations between 75 and 110 nmol/L appeared to best prevent fractures in adults without OI [4]. Many individuals may require oral vitamin D supplementation at a daily dose of 2000 international units (IU) to achieve serum 25OHD concentrations above 75 nmol/L, and this dose has been shown to be safe [5–7].

Even though vitamin D supplementation does not address the underlying genetic abnormality in OI, vitamin D could still have a beneficial effect in this disorder. For example, OI is associated with increased transforming growth factor beta signaling in bone [8]. Vitamin D seems to decrease transforming growth factor beta signaling in a number of conditions where transforming growth factor beta activity is increased [9,10]. Vitamin D can also have a positive effect on muscle, which indirectly may exert a beneficial influence on bone [11]. Even though OI is primarily thought of as a bone disease, muscle mass and function are also impaired and therefore any potential effect of vitamin D on muscle could be highly relevant for individuals with OI [12,13].

Previous studies have shown that many children and adolescents with OI have low serum 25OHD concentrations [14–18], which

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conceivably could have a negative impact on aBMD and fracture risk. Our previous studies on 71 children with OI failed to find a relationship between serum 25OHD concentrations and histomorphometric measures of bone microstructure and mineralization in iliac bone biopsy samples [16]. However, in a larger retrospective study on 282 children and adolescents with OI types I, III and IV we observed a positive correlation between serum 25OHD concentrations and lumbar spine aBMD (LS-aBMD) z-scores [17]. Regression analysis indicated that for every 1 nmol/L increase in serum 25OHD concentration, LS-aBMD z-score increased by 0.008 [17]. Although these retrospective data are suggestive, it is not clear whether serum 25OHD concentration through vitamin D supplementation has a beneficial effect on aBMD in children with OI. The purpose of the present study therefore was to evaluate the efficacy of high-dose vitamin D supplementation on LS-aBMD in children with OI through a one-year randomized, double-blind study. The primary study outcome was the change in LS-aBMD z-scores; secondary outcomes were changes in cortical and trabecular volumetric BMD z-scores and lower-limb muscle power.

2. Subjects and methods

2.1. Subjects

Study participants living in North America were recruited at the Shriners Hospital for Children in Montreal between September 2012 and June 2013. Patients of both sexes were eligible if they were between 6 and 19 years of age and were diagnosed with OI of any type. Patients were ineligible if aBMD measurement of the lumbar spine could not be performed (as LS-aBMD z-score change was the primary outcome) and if they had been receiving bisphosphonate treatment for less than two years (to avoid the large fluctuation in LS-aBMD z-score that is commonly observed at the start of bisphosphonate treatment) [19]. Pregnancy at any time during the study was exclusionary. Any chronic disorder other than OI, or use of medication known to interfere with 25OHD metabolism was an exclusion factor.

The sample size was determined based on the consideration that the study should be able to detect a LS-aBMD z-score change difference of 0.2 between treatment groups, which was considered the lower threshold for a clinically relevant effect. To put this number into context, 12 months of treatment of a similar group of patients with oral alendronate was associated with a LS-aBMD z-score change of 1.0, compared to placebo [20]. Thus, the study was designed to detect changes in LS-aBMD z-score that were one fifth of the changes that are expected with oral bisphosphonate treatment. Our (unpublished) clinical observations had shown that annual changes in LS-aBMD z-scores in children with OI fluctuated with a standard deviation (SD) of 0.28. Sample size calculations (using an alpha error of 0.05 and a power of 80%) revealed that 24 patients per group were required for the analysis of the primary outcome. Assuming an attrition rate of 20%, the targeted enrolment was 30 participants per group, for a total of 60 participants.

Of the 89 patients who were assessed for inclusion, 11 did not meet inclusion criteria and 18 declined participation, resulting in the randomization of 60 patients (Fig. 1). The study was approved by the Institutional Review Board of McGill University. Signed informed consent was obtained from participants aged 18 years and from parents of participants below 18 years of age; assent was obtained from participants over 8 years of age.

2.2. Treatment protocol and follow-up

Patients were randomized in equal number to receive either 400 IU per day or 2000 IU per day of vitamin D_3 supplements. Randomization was stratified according to baseline bisphosphonate treatment status and pubertal stage. A laboratory employee not otherwise involved in the study used a computerized randomization list to determine patient allocation. Two bottles of vitamin D supplements were provided and subjects were instructed to take one pill from each bottle daily for one year. Subjects randomly assigned to the low-dose group took one placebo and one 400 IU pill of vitamin D daily. Subjects randomly assigned to the high-dose group took two pills containing 1000 IU of vitamin D daily. The supplements were of similar size, taste and appearance and were from the same manufacturer (Jamieson Inc.). Unused vitamin D pills were collected at the final study visit, and compliance was confirmed by pill count. The compliance rate was calculated as the number of pills that participants actually received, based on pill count, relative to the number of pills that participants were expected to receive according to the study protocol. Patients and investigators were blinded to the treatment assignment throughout the study.

In-person visits at the study center occurred at baseline and 12 months later. Telephone follow-up calls were conducted every 3 months to collect information on adverse events, concomitant

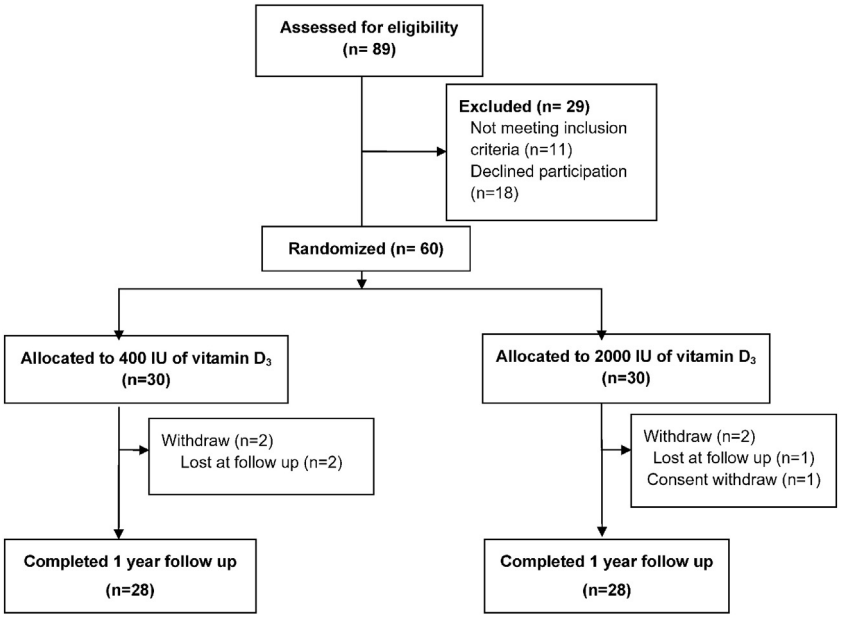

Fig. 1. CONSORT flow chart.

medication and fractures. All patients continued to receive standard medical care. Patients taking other forms of vitamin D supplements at baseline were asked to stop these supplements for the study period. Any additional intake of vitamin D through multivitamin use was accounted for in our intake assessments.

Study outcome variables were assessed at baseline and 12 months later. Based on our previous retrospective studies [17], the primary outcome variable was the change in LS-aBMD z-score. Secondary efficacy variables were changes in cortical and trabecular volumetric BMD z-scores at the radius, as well as changes in serum 25OHD, parathyroid hormone and collagen type I C-telopeptide relative to baseline. As an exploratory outcome, percent change in lower extremity muscle power per body mass was also assessed.

2.3. Anthropometric measurements

Height was measured with a Harpenden stadiometer (Holtain). Height and weight measurements were converted to age- and sex-specific z-scores based on published reference data by the Center for Disease Control and Prevention [21].

2.4. Radiological studies

LS-aBMD was measured in the antero-posterior direction at the lumbar spine (lumbar vertebra 1 to 4) by dual-energy X-ray absorptiometry (DXA) at baseline and at the final visit (QDR Discovery, Hologic Inc., Waltham, MA, USA, software version 12.3). Areal BMD results were converted to age- and sex-specific z-scores using data provided by the manufacturer. These were based on the studies of Glastre et al. [22] and Southard et al. [23] comprising a total of 353 children and adolescents. Quality control was performed daily with the use of a spine phantom provided by the manufacturer prior to conducting any measurement.

Cortical and trabecular volumetric BMD of the radius were measured using peripheral quantitative computed tomography (pQCT) of the nondominant forearm (XCT2000, Stratec Inc., Pforzheim, Germany). Quality control was conducted daily by measuring the phantom device provided by the manufacturer prior to performing any measurements in study participants. The dominant forearm was measured if the patient had intramedullary rodding on the nondominant side. Two measurement sites were assessed representing metaphyseal (4% site, for trabecular measures) and diaphyseal (65% site, for cortical measures) bone. Measurements were converted to age- and sex-specific z-scores based on pediatric reference data established by one of the coauthors [24,25]. Peripheral QCT scan images were visually inspected and excluded from analysis if movement artifacts were present. The technician rated the scans according to the following scale: 1 (no motion), 2 (minimal motion), 3 (moderate motion), 4 (severe motion) and 5 (extreme motion). Scans rated 1 or 2 were deemed acceptable whereas scans rated 3 to 5 were discarded and the measurement procedure was immediately repeated. The effective radiation dose from pQCT scans is lower than 0.01 mSv [26].

2.5. Biochemical analyses

Blood samples were collected following an overnight fast between 7:30 am and 10 am at baseline and at the final visit. Serum concentrations of procollagen type I N-terminal propeptide, collagen type I C-telopeptide and serum 25OHD concentrations were analysed by an IDS-iSYS automated analyser (Immunodiagnostics Systems, Scottsdale, AZ). Serum parathyroid hormone was determined by radioimmunoassay (Diasorin, Stillwater, MN). Serum phosphorus, creatinine, alkaline phosphatase as well as ionized and total calcium values were determined by standard methods. In some cases, blood samples were of insufficient volume to perform all analysis, explaining the differences in the final number of patients analysed per biochemical outcome.

2.6. Dietary assessments

Dietary intake assessments were conducted with the parent and child helped by standardized food portions (Nasco, US) at the initial visit. Total intake of vitamin D was assessed by a registered dietitian (L.P.) completing validated food frequency questionnaires (FFQ) reflecting the past months' intake at baseline and every 6 months thereafter [27]. Additional vitamin D supplements were accounted for in dietary analyses. Also, 24-hour diet recalls were completed by telephone at 3 and 9 months according to the multiple pass method shown to be the most accurate method to estimate children's total energy intake [28]. Nutrient analyses were estimated using the Nutritionist Pro Software (Axxya Systems, Stafford, TX) based on either the Canadian Nutrient File 2010b database or the United States Department of Agriculture database according to each participant's country of residence. This distinction is essential since vitamin D fortification regulations differ within North America.

2.7. Mechanography

A ground reaction force plate (Leonardo Mechanograph Ground Reaction Force Plate, Novotec Medical Inc., Pforzheim, Germany; software version 4.2-b05.53-RES) was utilized to assess muscle power, as described [29]. A single two-legged jump and a heel rise test were conducted with all study participants who were able to perform the tests. Patients who underwent surgical procedures or suffered lower limb fractures in the previous 6 months were excluded from conducting these tests ($n = 14$ at baseline and $n = 12$ at final visit). The outcome parameter for both types of tests was peak power relative to body mass [29]. Each test was repeated three times and the trial with the highest peak power was used for analysis.

2.8. Statistical analyses

Comparisons between treatments groups were based on an intent-to-treat analysis. In case of missing data, the last observation was carried forward. In comparing groups at baseline, two-sample t-tests and χ^2 tests were used. Changes in z-scores of LS-aBMD as well as of trabecular and cortical volumetric BMD at the radius were assessed by ANOVA with baseline result as a covariate. The percent changes for biochemical values from baseline to 12 months were obtained through ANOVA, with sex, age and baseline result as covariates. A 5% significance level was maintained throughout analyses and all tests were two-sided. All data were verified prior to analysis and screened for outliers using the outlier labeling rule [30]. No data point was deemed an outlier and no data were deleted. Statistical calculations were conducted using SPSS software (version 22.0, SPSS Inc., Chicago, IL, USA). Adverse events and concomitant medications taken during study period were tabulated. To compare dietary intake methods, a Bland–Altman plot was used to assess the level of agreement between dietary data for FFQ and the 24-hour recalls for vitamin D.

3. Results

3.1. Baseline characteristics

Sixty patients were randomized, ranging from 6.0 to 18.9 years in age (Fig. 1, Table 1). Overall, more girls than boys were enrolled, and the proportion of girls tended to be higher in the 400 IU than in the 2000 IU group (Table 1). As expected in OI, mean z-scores for height and weight were low. The majority of study participants had moderate or severe OI (OI type III or IV), and 72% of patients were receiving intravenous bisphosphonate therapy.

Despite the high proportion of bisphosphonate-treated study participants, average LS-aBMD z-scores were low (Table 2). Peripheral QCT at the radius showed normal mean trabecular volumetric BMD and

Table 1
Clinical characteristics of the study population at baseline.

	400 IU (n = 30)	2000 IU (n = 30)	p
Male/female	9/21	16/14	0.07
Age (years)	11.7 (3.3)	11.6 (3.3)	0.86
Height (z-score)	−3.0 (2.5)**	−2.4 (2.3)**	0.39
Weight (z-score)	−1.5 (1.5)**	−0.7 (1.7)*	0.07
OI type (I/IV/III, V, and VI)	(12/11/7)	(11/14/5)	0.62
Pubertal stage (prepubertal/others)	12/18	13/17	0.79
Bisphosphonate status (treated/not treated)	22/8	21/9	0.77

Data are mean (SD). *Significantly different from zero at $p < 0.05$; **$p < 0.001$ (one-sample t-test).

slightly elevated cortical volumetric BMD (Table 2, Supplementary Table 1). Serum concentrations for calcium, phosphorus, alkaline phosphatase and parathyroid hormone were within normal limits for all participants. The average baseline serum 25OHD concentration was 65.6 nmol/L (SD: 20.4) with no difference between treatment groups; 21% of patients had a serum 25OHD concentration <50 nmol/L (Supplementary Fig. 1). The mean total vitamin D intake from food and supplemental sources in the entire population was 453 IU (SD: 262) per day, with no differences between the two randomization groups.

3.2. Changes during the intervention period

Compliance was determined for the 51 study participants who returned their unused vitamin D pills at the end of the study. Compliance rates were 63% (SD: 30) and 71% (SD: 20) in the 400 IU and 2000 IU treatment groups, respectively ($p = 0.28$). The 25OHD serum concentration increased or remained unchanged in 90% of study participants during the study period (Fig. 2). The 25OHD level decreased in three individuals who were randomized to the 2000 IU group. Of these, two individuals did not return unused pills at the end of the study, and the third had a documented low compliance (45%). In one study participant who was randomized to the 400 IU group, the serum 25OHD concentration increased by 79 nmol/L during the study interval. The cause of this larger than expected increase could not be determined, as his stated total vitamin D intake (from both diet and supplement) did not change during the study period. In the entire study cohort, the mean

Table 2
Baseline characteristics by treatment allocation.

	n	400 IU	n	2000 IU	p
Bone densitometry					
Lumbar spine aBMD (z-score)	30	−2.0 (1.1)**	30	−2.1 (1.2)**	0.64
Radius trabecular vBMD (z-score)	27	−0.6 (1.9)	26	−0.2 (1.9)	0.44
Radius cortical vBMD (z-score)	25	0.9 (1.6)*	25	0.9 (1.5)*	0.95
Serum biochemistry					
Calcium (mmol/L)	30	2.38 (0.08)	29	2.42 (0.08)	0.08
Phosphorus (mmol/L)	30	1.56 (0.19)	29	1.57 (0.2)	0.81
Alkaline phosphatase (U/L)	30	189 (97)	29	205 (102)	0.52
PTH (pmol/L)	27	2.3 (1.0)	25	2.1 (1.1)	0.70
25OHD (nmol/L)	30	66.4 (21.9)	28	64.7 (19.1)	0.77
PINP (μg/L)	29	200 (147)	29	266 (189)	0.14
CTX (ng/mL)	29	0.90 (0.53)	28	0.98 (0.52)	0.57
Total vitamin D intake from food and supplemental sources (IU/d)					
Total vitamin D intake by FFQ (IU/d)	30	464 (270)	30	443 (259)	0.76
Mechanography tests (peak power per body mass, W/kg)					
Heel rise test	16	4.44 (2.16)	18	5.31 (2.16)	0.25
Single two-legged jump	11	27.3 (7.1)	11	34.3 (10.1)	0.07

Data are mean (SD). p values represent the significance of the difference between both groups (independent sample t-test). *Significantly different from zero at $p < 0.01$; **$p < 0.001$ (one-sample t-test). BMC: bone mineral content, CTX: type I C-telopeptide, FFQ: food frequency questionnaire, PINP: procollagen type I N-terminal propeptide, PTH: parathyroid hormone, vBMD: volumetric bone mineral density.

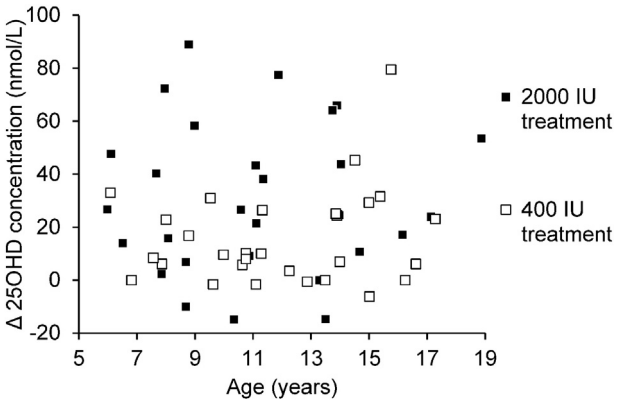

Fig. 2. Changes in serum 25OHD concentrations from baseline to final visit according to age and treatment group.

increase in serum 25OHD concentrations was twice as high in the 2000 IU group as in the 400 IU group (Table 3).

None of the other densitometric, anthropometric, biochemical and mechanographic parameters underwent significantly different changes in the two treatment groups (Table 3, Supplementary Table 2). Importantly, changes in LS-aBMD z-scores were very similar in the 400 IU and the 2000 IU treatment groups and the same was true for trabecular and cortical volumetric BMD z-scores at the radius. Similar conclusions were reached when the subgroup of 17 patients not receiving bisphosphonate treatment was analyzed separately ($p > 0.6$ for group differences in z-score change in each of the three densitometric measures).

At baseline, total vitamin D intake, including diet and supplementation, was similar between treatment groups (Table 2). The Bland–Altman plot compared dietary vitamin D intake agreement between FFQ and 24-hour recalls showing a positive mean difference, indicating an overestimation by FFQ (Fig. 3). Dietary assessments through 24-hour recalls showed similar results for vitamin D intake at 3 months and at 9 months (Supplementary Table 3). Baseline daily calcium intakes are shown in Supplementary Table 4.

Separate analyses were completed on patients with baseline serum 25OHD concentrations <50 nmol/L ($n = 6$ in the 2000 IU group, $n = 6$ in the 400 IU group). Significantly higher mean baseline parathyroid hormone levels were observed (mean: 2.9 pmol/L, SD: 1.4) compared to patients with serum 25OHD above 50 nmol/L (mean: 2.0 pmol/L, SD: 0.9) ($p = 0.01$), as is expected due to the inverse relationship between 25OHD and parathyroid serum concentrations [17]. LS-aBMD z-score change was not significantly different between treatment groups (mean: 1.1, SD: 1.1 for the 2000 IU group vs. mean: −0.2, SD: 1.1 in the 400 IU group, $p = 0.07$). Overall, participants with baseline 25OHD concentrations <50 nmol/L had significantly lower compliance (50%) during the study interval compared to participants with baseline 25OHD concentrations above 50 nmol/L (71%) ($p = 0.02$).

Another subgroup analysis was performed by separating the study population into two groups based on whether serum PTH levels were below or above the median in the present cohort. No group differences were observed in any of the outcome measures (data not shown).

3.3. Clinical adverse events

No cases of hypercalcemia or hypercalciuria were observed. No serious adverse events related to treatment were noted. The most commonly reported adverse event was headaches, but their frequency was similar in the two treatment groups ($n = 3$ in the 400 IU group; $n = 2$ in the 2000 IU group; $p = 0.64$). Some patients reported a reduction in the frequency of common colds throughout the treatment year in comparison to previous years ($n = 3$ in the 400 IU group; $n = 2$ in the 2000 IU group, $p = 0.64$).

Table 3
Changes after one year of treatment.

	n	400 IU	n	2000 IU	p
Anthropometry					
Height (z-score)	30	0.2 (−0.1; 0.5)	30	0.0 (−0.3; 0.2)	0.33
Weight (z-score)	30	0.3 (−0.1; 0.6)	30	0.2 (−0.1; 0.6)	0.83
Bone densitometry					
Lumbar spine aBMD (z-score)[a]	30	0.0 (−0.1; 0.2)	30	0.1 (−0.1; 0.2)	0.63
Radius trabecular vBMD (z-score)[a]	27	−1.0 (−1.7; −0.3)	26	−0.4 (−1.0; 0.3)	0.22
Radius cortical vBMD (z-score)[a]	25	−0.2 (−0.6; 0.2)	25	0.0 (−0.4; 0.4)	0.53
Serum biochemistry					
Calcium (mmol/L)[b]	30	0.00 (−0.03; 0.03)	29	0.01 (−0.02; 0.04)	0.84
Phosphorus (mmol/L)[b]	30	−0.01 (−0.06; 0.03)	29	−0.02 (−0.07; 0.03)	0.83
Alkaline phosphatase (U/L)[b]	30	−4 (−15; 7)	29	−12 (−23; −1)	0.32
25OHD (nmol/L)[b]	30	15.2 (6.4; 24.1)	28	30.5 (21.3; 39.6)	0.02
PINP (μg/L)[b]	27	−4 (−38; 31)	26	−24 (−59; 11)	0.43
CTX (ng/mL)[b]	29	−0.03 (−0.14; 0.08)	28	0.02 (−0.10; 0.13)	0.58
PTH (pmol/L)[b]	27	0.0 (−0.3; 0.2)	25	−0.2 (−0.4; 0.1)	0.39
Total vitamin D intake from food and supplemental sources (IU/d)					
Total vitamin D intake by FFQ (IU/d)[b]	30	594 (385; 803)	30	1447 (1239; 1656)	<0.001
Mechanography tests (peak power per body mass, W/kg)					
Heel rise test	16	−0.1 (−0.6; 0.4)	18	−0.1 (−0.6; 0.4)	0.94
Single two-legged jump[b]	11	2.3 (−0.1; 4.7)	11	−0.9 (−3.3; 1.5)	0.08

Data are mean change with 95% CIs. p values represent the significance of the difference between both groups (ANCOVA). BMC: bone mineral content, CTX: type I C-telopeptide, FFQ: food frequency questionnaire, PINP: procollagen type I N-terminal propeptide, PTH: parathyroid hormone, vBMD: volumetric bone mineral density.
[a] Adjusted for baseline result.
[b] Adjusted for age, sex, and baseline result.

Twelve patients in the 2000 IU group and 15 patients in the 400 IU group sustained at least one fracture during the study period. The total number of long-bone fractures was 18 in the 2000 IU group and 17 in the 400 IU group. The number of long-bone fractures per patient ranged from 0 to 3 in the 2000 IU group and from 0 to 4 in the 400 IU group ($p = 0.26$).

4. Discussion

In this study on children with OI, we found that daily oral vitamin D supplementation at either 400 IU or 2000 IU per day significantly increased serum 25OHD concentrations, but that the increase in 25OHD was larger in the high-dose group. With average baseline 25OHD concentrations above 50 nmol/L, the change in LS-aBMD z-score was not different between treatment groups. We also did not observe treatment-associated differences in the changes of any other bone or muscle outcome measures.

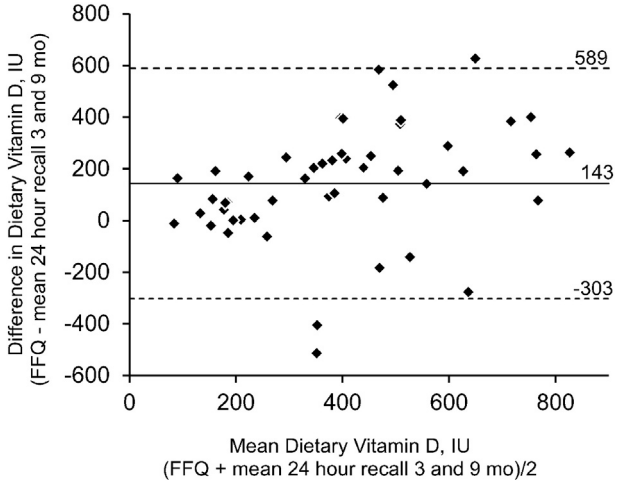

Fig. 3. Bland–Altman plot showing agreement between FFQ and mean 24-h recalls at 3 and 9 months for dietary vitamin D intake. Solid line represents the mean difference; dashed lines are plus or minus 2 SD of the mean difference. Negative mean difference indicates overall underestimation by FFQ and positive mean difference indicates overall overestimation by FFQ, n = 49.

This randomized trial thus did not corroborate our previous retrospective analyses that showed a positive relationship between 25OHD concentrations and LS-aBMD z-scores [17]. It must be noted, however, that only 21% of our study participants had serum 25OHD concentrations <50 nmol/L at baseline, the lower limit of the 25OHD range recommended by the Institute of Medicine in support of bone health [7]. The present study was thus conducted mainly in children who had a vitamin D status in the recommended range.

Studies in healthy children have shown that those with serum 25OHD concentrations <50 nmol/L reap the most benefit from vitamin D supplementation on aBMD outcomes [6,31]. Similarly, when we analyzed the subgroup of our patients with baseline serum 25OHD concentrations <50 nmol/L we found that the 2000 IU group had an increase in LS-aBMD z-score that was almost significantly higher than that of the group receiving 400 IU. Even though the small sample size of this subgroup does not allow for definitive conclusions, it seems logical to focus future studies on patients screened for serum 25OHD concentrations <50 nmol/L. This is the subgroup that is most likely to benefit from vitamin D supplementation, but at the same time had the lowest compliance with vitamin D supplementation in the present study.

The relatively small percentage of study participants with 25OHD concentrations <50 nmol/L (21%) indicates that vitamin D status has improved over time in our population of children with OI. In our first retrospective study on vitamin D status in OI (which included 25OHD results that were mainly collected between 1995 and 2002), the prevalence of serum 25OHD concentrations <50 nmol/L was 52% [16]. Our subsequent analysis, mostly based on 25OHD concentrations that were determined between 2000 and 2009, found that 27% of children with OI had serum 25OHD concentrations <50 nmol/L [17]. It is likely that greater awareness of the importance of vitamin D and consequently greater vitamin D intake are reflected in this trend. It is also possible that methodological differences in the quantification of serum 25OHD (radioimmunoassay in our previous studies, chemiluminescence technique in the present study) may have contributed to differences between studies.

In fact, we observed in the present study that vitamin D consumption, as assessed through 24-hour recalls, averaged 325 IU per day.

This is more than has been reported for healthy Canadian (252 IU per day) and American (192 IU per day) youth [32,33], whereas the percentage of patients taking vitamin D supplementation was similar to Canadian and American averages [34,35]. The greater dietary vitamin D intake in individuals with OI did not translate into higher serum 25OHD concentrations (65.6 nmol/L in the present study, 71.6 nmol/L in Canadian youth, 67.0 nmol/L in American youth [36,37]). Possibly, this discrepancy is at least in part attributable to reduced sun exposure in our patients, secondary to restricted mobility. This randomized controlled trial was the first of its kind to assess the impact of high-dose vitamin D supplementation in children with OI. Very few adverse events were reported and supplementation of 2000 IU of daily vitamin D proved to be safe with no cases of hypercalcemia reported.

Among the limitations of this study was that we did not collect data reflecting endogenous vitamin D synthesis, such as skin pigmentation or sun exposure. Another study limitation was that a high proportion of participants were receiving simultaneous treatment with intravenous bisphosphonates, whereas the participants of our previous retrospective studies on the relationship between 25OHD and aBMD had not received bisphosphonates. Bisphosphonates increase aBMD, possibly making it more difficult to identify any vitamin D related effects on aBMD. As bisphosphonate treatment is the current standard of care in moderate to severe OI, concomitant use of such drugs could not be avoided. Nevertheless, we attempted to minimize the influence of bisphosphonate treatment on study outcomes by only including patients who were on stable long-term bisphosphonate treatment and by using bisphosphonate treatment status as a stratification factor in the randomization. In any case, results in the subgroup of patients not receiving bisphosphonates were very similar to those of the whole study population.

In conclusion, this one-year randomized controlled trial in children with OI found that supplementation of either 400 IU or 2000 IU of vitamin D translated into significant increases in serum 25OHD concentrations. However, increases in serum 25OHD concentrations that were already largely within the recommended range at baseline did not have a detectable effect on LS-aBMD z-scores.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bone.2016.02.013.

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