Prevalence of vitamin D insufficiency in Swiss teenagers with appendicular fractures: a prospective study of 100 cases

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Abstract

Background The significance of subclinical vitamin D deficiency in the pathogenesis of fractures in children and adolescents currently remains unclear.

Objective We aimed to determine the prevalence of vitamin D insufficiency and its effect on bone mineral density (BMD) and bone mineral content (BMC) values in a collective of Swiss Caucasian children with a first episode of appendicular fracture.

Design and methods One hundred teenagers with a first episode of appendicular fracture [50 upper limb fractures (group 1) and 50 lower limb fractures (group 2)] and 50 healthy controls (group 3) were recruited into a cross-sectional study. The BMC and BMD values were measured by dual-energy X-ray absorptiometry, and serum 25

hydroxyvitamin D [25(OH)D] was assessed by electro-chemiluminescence immunoassays.

Results From the 100 injured teenagers in the study, 12 % had deficient vitamin D levels (<20 ng/mL; <50 nmol/L) and 36 % had insufficient levels (≥ 20 <30 ng/mL; ≥ 50 <78 nmol/L), whereas 6 and 34 % of healthy controls were, respectively, vitamin D deficient and insufficient. There were no significant differences for serum 25(OH)D levels, L2–L4 BMD Z-score, and L2–L4 BMC Z-score variables ($p = 0.216$) between the three groups nor for the calcaneal BMD Z-score variables ($p = 0.278$) between healthy controls and lower limb fracture victims. Investigations on the influences of serum 25(OH)D on BMD and BMC showed no correlation between serum 25(OH)D and L2–L4 BMD Z-scores ($r = -0.15$; $p = 0.135$), whereas low but significant inverse correlations were, surprisingly, detected between serum 25(OH)D and calcaneal BMD Z-scores ($r = -0.21$; $p = 0.034$) and between serum 25(OH)D and L2–L4 BMC Z-scores ($r = -0.22$; $p = 0.029$).

Conclusions A significant proportion of Swiss Caucasian teenagers were vitamin D insufficient, independent of limb fracture status, in our study. However, this study failed to show an influence of low vitamin D status on BMD and/or BMC of the lumbar spine and heel.

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Introduction

Vitamin D is essential for calcium–phosphate homeostasis and, thus, plays a key role in bone development/remodeling

[1–3]. The active vitamin D metabolite, 1,25(OH)₂D₃, is the metabolic effector of vitamin D throughout the body, and has many established direct and indirect effects, leading to a positive impact on bone mineralization [4]. Circulating levels of 25(OH)D are the best marker of vitamin status, with a long half-time of approximately 30 days [4, 5]. There is little consensus regarding the ideal 25(OH)D level [6, 7]. Nevertheless, many experts agree that 30 ng/mL represents an optimal level for 25(OH)D [6, 8], and suggest that 20 ng/mL represents the lower limit of normal [9].

A number of studies in adolescents have shown a high prevalence of subclinical vitamin D deficiency in Europe [10–15], the United States [16–20], and New Zealand [21], especially during the winter months. In elderly subjects, low vitamin D status elevates parathyroid hormone (PTH) concentrations, which, in turn, increases bone turnover and bone loss, contributes to mineralization defects, and increases the risk of hip and other fractures [22]. However, it remains unclear as to whether similar effects occur in children and adolescents. Fractures are common in the pediatric population, with an incidence of approximately 50 % in boys and 40 % in girls [23, 24]. Fracture rates peak between ages 11 and 15 years, corresponding to the period of maximum postnatal growth velocity [24–26]; the increase is often attributed to normal childhood development [27, 28]. However, many fractures are related to deficient bone health. In fact, many studies suggest that fracture risk in children may be a function of lower bone mineralization associated with genetic [29] and environmental factors, such as poor nutrition and physical inactivity [30–34]. The relationship between low vitamin D levels and fracture risk has been extensively studied above all in infants with rickets [35–40] and for osteoporotic fractures in adults [41–44], but its significance in children and adolescents has been investigated to a lesser extent. A few studies have documented vitamin D deficiency to be a common problem among otherwise healthy adolescents and correlated this deficiency with decreased bone density in youth [11, 15, 45]. More interestingly, a recent preliminary study demonstrated that a significant proportion of African American children with fractures were vitamin D insufficient [46].

The main purposes of this study were, thus, to determine the prevalence of vitamin D insufficiency and appreciate its effect on bone mineral density (BMD)/bone mineral content (BMC) in a collective of Swiss Caucasian children with a first episode of appendicular fracture, hypothesizing that low vitamin D levels could alter BMD/BMC indices and render teenagers more susceptible to fractures.

Materials and methods

Study design and subjects

We performed an observational study of teenagers admitted to the Children's Hospital of Geneva, Switzerland, with an appendicular fracture from January 2005 to December 2008. One hundred adolescents aged between 10 and 16 years with a first episode of limb fracture (50 lower limb fractures, 50 upper limb fractures) and a control group of 50 healthy teenagers within the same age group were recruited for this study. Injured adolescents were selected if they were admitted to the ward as inpatients for orthopedic reduction or minimally invasive surgery (closed reduction and stabilization by percutaneous wires or screws) of their fracture under general anesthesia. Healthy control adolescents were recruited among patients' visitors, children of medical staff, and volunteers recruited mainly by advertising at the University Hospitals of Geneva. Exclusion criteria for both injured adolescents and healthy controls were: prior history of bone fractures; chronic disease; congenital or acquired bone disease; any condition limiting physical activity; hospitalization for more than 2 weeks in the previous 12 months; or dietary vitamin D supplementation. The study received institutional review board approval (protocol # 04-057, ped 04-002) and was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. Participants and the parents or guardians of participants provided written informed consent.

Measurements

Anthropometrics

Height was assessed to the nearest 0.1 cm in bare or stocking feet using a precision mechanical stadiometer (Holtain Ltd., Crymych, Dyfed, Wales, UK) and body weight was measured to the nearest 0.1 kg using a calibrated beam scale (Seca®, Reinach, Switzerland). The body mass index (BMI) was calculated as follows: $BMI = \text{weight (kg)} / \text{height squared (m}^2\text{)}$.

Collection and analysis of serum samples

Blood samples were collected from the basilic vein (Vacutainer, 9 or 6 mL, without additives, Greiner Bio-One Vacuette, St. Gallen, Switzerland) and centrifuged (1,580×g for 10 min at 20 °C) within 30 min of collection. The obtained seras were stored at −80 °C until further analysis. All analyses were performed simultaneously at the end of the recruitment period. Serum 25(OH)D were measured by electrochemiluminescence immunoassays

(ECLIA) on the automated Elecsys 2010 analyzer (Roche Diagnostics, Rotkreuz, Switzerland). The intra- and inter-assay variation was 4.2–6.1 %.

Data interpretation

We used previously proposed reference values to define vitamin D status [6, 8, 9]. Serum 25(OH)D concentrations were stratified as follows: vitamin D deficient (<20 ng/mL; <50 nmol/L); vitamin D insufficient (≥ 20 <30 ng/mL; ≥ 50 <78 nmol/L); and vitamin D sufficient (≥ 30 ng/mL; ≥ 78 nmol/L) [47, 48].

Bone mineral variables

Areal BMD (g/cm^2) and BMC (g) were determined at the spine level in the supine position by the same technician (G.D.C.) using dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy[®], GE Healthcare, General Electric Company, Madison, WI, USA). For injured adolescents, the first DXA scans were performed within 3 days of the fracture. The long-term stability of the instrument was assessed by measuring spine phantoms supplied by the manufacturer (Calibration Block Phantom for Prodigy, SN: 9081) and was found to have a precision of 0.2 % coefficient of variation over the duration of the study.

Measurements of the bilateral areal BMD of the os calcis (BMDOC, g/cm^2) from lower limb injured adolescents and healthy controls were performed using a peripheral DXA (Lunar Pixi[®], GE Healthcare, General Electric Company, Madison, WI, USA). The BMD of injured adolescents was assessed at fracture time under general anesthesia in the operating room. The os calcis measurements were performed using a subregion analysis program (manufacturer's program). A region of interest was localized by using anatomic features of the heel. The long-term precision and stability of the Lunar Pixi[®] instrument was performed before every use by measuring a calcaneal or a wrist phantom. The areal BMD is easily measured in children and reproducibility is comparable to that of adults, for which the standard deviation (SD) of repeated measures is roughly $0.010 \text{ g}/\text{cm}^2$ (manufacturer's literature). In our study, the mean and SD of repeated measures on children were, respectively, 0.0001 and $0.015 \text{ g}/\text{cm}^2$, and the 95 % limits of agreement were -0.029 to $+0.029 \text{ g}/\text{cm}^2$.

BMD and BMC (lumbar spine) Z-scores adjusted for age and gender were derived from the Dutch references for Caucasian children described by van der Sluis et al. [49]. Z-scores for the BMDOC were predicted using Chinn et al.'s proportional model based only on the height [50]. Z-scores were interpreted according to the three different groups (i.e., the healthy controls, the teenagers with lower

limb fractures, and the teenagers with upper limb fractures) but also to the vitamin D levels.

Statistical analysis

The difference between group characteristics in terms of sex (M/F), age (years), height (cm), weight (kg), and BMI (kg/m^2) was first verified using one-way analysis of variance (ANOVA). Subsequently, comparison between the three groups for serum 25(OH)D concentration, L2–L4 BMD Z-score, and L2–L4 BMC Z-score was carried out, also using one-way ANOVA, with group as the categorical predictive factor. If a significant difference existed between groups, Tukey's post hoc tests were conducted. Since calcaneal BMD Z-scores were only available for two of the three groups (i.e., healthy control and lower limb fractures), an unpaired Student's *t*-test was used to evaluate the calcaneal BMD Z-scores. A significant difference was defined as $p < 0.05$. Pearson correlations were conducted in order to investigate the influence of serum 25(OH)D levels on other variables.

Results

The mean ($\pm$ SD) 25(OH)D level was $32.7 (\pm 7.8) \text{ ng}/\text{mL}$ for healthy controls, $32.1 (\pm 10.3) \text{ ng}/\text{mL}$ for the upper limb fracture teenagers, and $30.5 (\pm 9.8) \text{ ng}/\text{mL}$ for those with lower limb fractures. Three (6 %) healthy controls were vitamin D deficient and 17 subjects (34 %) were insufficient, hence, 40 % of the healthy group can be considered to display abnormal 25(OH)D levels. Six adolescents with upper limb fractures (12 %) were vitamin D deficient, 16 cases were insufficient (32 %), i.e., 44 % can be considered to exhibit abnormal 25(OH)D levels. In the group of lower limb fractures, we noted that six teenagers (12 %) were vitamin D deficient and 20 subjects (40 %) were insufficient, hence, 52 % of this group exhibited abnormal 25(OH)D levels (Table 1).

The three groups were comparable as far as sex, age, height, weight, and BMI are concerned. The average and SD of the parameters extracted are presented in Table 1. No significant group effect was found for any of the serum 25(OH)D, L2–L4 BMD Z-score, or L2–L4 BMC Z-score variables ($p = 0.216$). No significant difference was found between healthy controls and the lower limb fracture group for the calcaneal BMD Z-score variables ($p = 0.278$).

Furthermore, no correlation was found between the serum 25(OH)D and L2–L4 BMD Z-scores ($r = -0.15$; $p = 0.135$) when studying the influence of serum 25(OH)D on bone mineral values. Surprisingly, low but significant inverse correlations were detected between serum 25(OH)D and calcaneal BMD Z-scores ($r = -0.21$;

Table 1 Characteristics of subjects, serum 25 hydroxyvitamin D [25(OH)D] levels, and Z-scores

	Upper limb fractures (<i>n</i> = 50)	Healthy controls (<i>n</i> = 50)	Lower limb fractures (<i>n</i> = 50)
Age (years)	12.9 ± 1.7	12.7 ± 2.3	13 ± 1.7
Height (cm)	158.5 ± 12.1	156.7 ± 14.6	159.1 ± 12.1
Weight (kg)	48.8 ± 11	49.2 ± 14.4	51.4 ± 12.6
BMI (kg/m ²)	19.2 ± 2.8	19.6 ± 3.3	20.1 ± 3.1
Serum 25(OH)D (ng/mL)	32.1 ± 10.3	32.7 ± 7.6	30.5 ± 9.8
Calcaneal BMD Z-score	No data	0.236 ± 0.513	0.372 ± 0.652
L2–L4 BMD Z-score	1.118 ± 0.970	1.679 ± 1.062	1.613 ± 1.360
L2–L4 BMC Z-score	0.002 ± 0.035	0.007 ± 0.037	0.004 ± 0.030

No difference between groups was found for age, height, weight, and BMI

No significant groups' effect was noted for any of serum 25(OH)D, L2–L4 BMD/BMC Z-scores, and calcaneal Z-scores

Table 2 Z-scores according to serum 25(OH)D levels

	Vitamin D deficient (<i>n</i> = 15)	Vitamin D insufficient (<i>n</i> = 53)	Vitamin D sufficient (<i>n</i> = 82)
Serum 25(OH)D (ng/mL)	17.4 ± 1.8	25.5 ± 2.8	38.4 ± 6.8
Calcaneal BMD Z-score	0.713 ± 0.544	0.387 ± 0.658	0.199 ± 0.527
L2–L4 BMD Z-score	2.125 ± 1.313	1.523 ± 1.193	1.309 ± 1.088
L2–L4 BMC Z-score	0.023 ± 0.035	0.035 ± 0.150	−0.019 ± 0.203

We could not demonstrate a positive influence of high serum 25(OH)D on bone mineral values. We even noted a significant inverse correlation between serum 25(OH)D and lumbar BMC Z-scores, and calcaneal BMD Z-scores

$p = 0.034$) and between serum 25(OH)D and L2–L4 BMC Z-scores ($r = -0.22$; $p = 0.029$) (Table 2).

Discussion

In the present study, we did not note any significant differences in the baseline serum 25(OH)D concentrations between the three groups of patients, i.e., the healthy controls, the teenagers with lower limb fractures, and those

with upper limb fractures. However, using a lower limit of 30 ng/mL to define vitamin D insufficiency, we observed a high prevalence of vitamin D insufficiency among teenagers in all groups. Our study shows that 44 % of teenagers with upper limb fractures had 25(OH)D levels below 30 ng/mL, 52 % of those with lower limb fractures demonstrated similar levels, whereas 40 % of healthy controls presented a vitamin D insufficiency.

To the best of our knowledge, there is only one preliminary study that assessed the association between serum 25(OH)D concentrations and fracture risk in older children or adolescents [46]. In this study, 59 % of African American children with fractures were vitamin D insufficient [46], and the authors noted that this prevalence was higher than the baseline levels reported in comparable populations. On the other hand, several studies have suggested that the prevalence of low vitamin D levels among pediatric orthopedic patients was frequent. McNally et al. noted, in their study, that 82 % of 730 Canadian children complaining of unexplained appendicular joint pain had “abnormally low” levels of 25(OH)D (<30 ng/mL) [51]. Szalay et al. detected an incidence of vitamin D insufficiency (<30 ng/mL) of 63 % among their orthopedic patients evaluated for aspecific musculoskeletal pain [52]. Similarly, Davies et al. demonstrated equivalent results in the UK, with 40 % of children presenting with orthopedic conditions having insufficient vitamin D levels (<50 nmol/L) [53]. However, low vitamin D status is far from being rare in Europe and has been observed during winter and spring in 17–50 % of the non-immigrant children living in northern European countries [10, 11, 54, 55]. Hence, we deduce that the prevalence of vitamin D insufficiency is not higher among teenagers with appendicular fractures, but, rather, consider that these findings reflect the situation of the general pediatric population, as previously suggested.

As far as we know, the present study is the first to provide results on the association between serum vitamin D and bone mass/density in teenagers at the moment of a first episode of appendicular fracture. First of all, our results suggest that there are no significant differences in L2–L4 BMD/BMC Z-scores between the three groups of patients, i.e., the healthy controls, the teenagers with lower limb fractures, and those with upper limb fractures. Furthermore, we have demonstrated that there are no significant differences in calcaneal BMD Z-scores between teenagers with lower limb fractures and healthy controls. Finally, our results show that levels of serum 25(OH)D do not predict bone mineral values. No positive correlation was found between serum 25(OH)D and L2–L4 BMD Z-scores; however, we noted a low, but significant, negative correlation between serum 25(OH)D and both calcaneal BMD and L2–L4 BMC Z-scores. Only two patients (both with lower limb fractures) had abnormal Z-scores suggesting

osteopenia (one for calcaneal BMD; Z-score = -1.42 , one for L2–L4 BMC; Z-score = -1.79), but both were considered to be vitamin D sufficient.

Several studies have examined the relationship between baseline 25(OH)D levels and BMC/BMD indices in healthy teenagers, but their results are contradictory. A positive association between baseline 25(OH)D status and BMC/BMD has been shown by El-Hajj Fuleihan et al. in adolescent girls from Beirut, for which a significant association between baseline serum 25(OH)D levels and baseline BMD of the lumbar spine, femoral neck, and radius were reported. There was also a significant association between baseline serum 25(OH)D levels and radius BMC [56]. A study conducted on adolescent girls from Finland by Lehtonen-Veromaa et al. demonstrated a relation between baseline 25(OH)D levels and the evolution of lumbar spine and femoral neck BMD during a 3-year period [14]. The difference in the percentage increase of lumbar spine BMD between the subjects with low 25(OH)D levels (<20 nmol/L) and those with higher 25(OH)D levels over the 3 years was 4 %. In another study from Finland, adolescents with 25(OH)D levels below or equal to 40 nmol/L showed significantly lower mean forearm BMD values [15]. Similarly, low 25(OH)D levels (<25 nmol/L) were found to be associated with lower forearm and/or tibial BMD in two studies performed on 10–16-year-old Irish and Finnish girls [11, 45]. However, a number of alternative studies have failed to show evidence of correlations between vitamin D levels and BMD/BMC values. Marwaha et al. found no significant correlation between BMD (measured at the distal forearm and calcaneum) and serum 25(OH)D in patients from two different socio-economic groups [57]. Ala-Houhala et al. suggested that vitamin D supplementation did not have a beneficial effect on distal radius BMC [58], whereas Khadilkar et al. demonstrated that supplementation could have a positive effect on lumbar spine BMC and BMD, but only in girls who were ≤ 2 years following menarche. Two other studies failed to show an influence of low vitamin D status on total body, hip, upper femur, and lumbar bone mineral density and/or content in 10–12-year-old Finnish girls [11] and in 16–22-year-old Californian girls [59]. There is currently no expert consensus on this issue, and the report of Cranney et al. (Agency for Healthcare Research and Quality) on the efficacy of vitamin D in relation to bone health concluded that there was only poor evidence of an association between serum 25(OH)D levels and baseline BMD or changes in BMD/BMC indices in teenagers, and that vitamin D supplementation has not confirmed a consistent benefit on BMD/BMC across sites and age groups [60].

In summary, we have shown with the present study that a substantial number of 10–16-year-old Swiss teenagers with or without limb fractures can be considered as vitamin

D insufficient on the basis of their serum 25(OH)D concentration. However, we failed to demonstrate an influence of low vitamin D status on lumbar spine or heel BMD and/or BMC. In addition, there was no significant differences in the serum 25(OH)D levels between healthy controls and adolescents with limb fractures. Our results, thus, reinforce the concept that there are probably many confounders (sexual maturity, race, genetics, diet, season, etc.) in determining the real role of vitamin D status in bone accretion during growth. Further research is, therefore, needed in order to determine the real relationship of vitamin D status on bone accretion.

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Conflict of interest None.

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