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Original Full Length Article

Effect of vitamin D replacement on hip structural geometry in adolescents: A randomized controlled trial

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ABSTRACT

Background: We have shown in a randomized controlled trial that vitamin D increases bone mass, lean mass and bone area in adolescent girls, but not boys. These increments may translate into improvements in bone geometry and therefore bone strength. This study investigated the impact of vitamin D on hip geometric dimensions from DXA-derived hip structural analyses in adolescents who participated in the trial.
Methods: 167 girls (mean age 13.1 years) and 171 boys (mean age 12.7 years) were randomly assigned to receive weekly placebo oil or vitamin D_3, at doses of 1400 IU or 14,000 IU, in a double blind placebo-controlled 1-year trial. DXA images were obtained at baseline and one year, and hip images were analyzed using the hip structural analysis (HSA) software to derive parameters of bone geometry. These include outer diameter (OD), cross sectional area (CSA), section modulus (Z), and buckling ratio (BR) at the narrow neck (NN), intertrochanteric (IT), and shaft (S) regions. Analysis of Covariance (ANCOVA) was used to examine group differences for changes of bone structural parameters.
Results: In the overall group of girls, vitamin D supplementation increased aBMD (7.9% and 6.8% in low and high doses, versus 4.2% in placebo) and reduced the BR of NN (6.1% and 2.4% in low and high doses, versus 1.9% in placebo). It also improved aBMD (7.9% and 5.2% versus 3.6%) and CSA (7.5% and 5.1% versus 4.1%) of the IT and OD of the S (2.4% and 2.5% versus 0.8% respectively). Significant changes in the OD and BR of the NN, in the overall group of girls remained, after adjusting for lean mass, and were unaffected with further adjustments for lifestyle, pubertal status, and height measures. Conversely, boys did not exhibit any significant changes in any parameters of interest. A dose effect was not detected and subgroup analyses revealed no beneficial effect of vitamin D by pubertal stage.
Conclusions: Vitamin D supplementation improved bone mass and several DXA-derived structural bone parameters, in adolescent girls, but not boys. This occurred at a critical site, the femoral neck, and if maintained through adulthood could improve bone strength and lower the risk of hip fractures.
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Introduction

The amount of bone mass acquisition during adolescence is a determining factor for the risk of developing fractures later in life [1]. Although genetic factors play an important role in defining the individual's bone mineral density and content, environmental factors also exert an important influence on bone mass acquisition and its maintenance [2–4]. Several studies have shown that modifiable lifestyle factors such as dietary intake of calcium and vitamin D, and physical activity, with high impact weight bearing activities, can enhance bone mass accrual during growth [5–13].

Dual energy X-ray absorptiometry (DXA) technology is commonly used to assess bone mass and density in a highly precise, safe, and non-invasive way. However, the conventional analysis of the DXA data does not capture bone geometric and structural parameters that can be considered more appropriate for understanding the impact of any therapeutic intervention on bone mechanical integrity. It is also more difficult to assess the relationship between bone strength and body anthropometric measures when using BMD [14–16].

As a result, Beck et al. developed a program, known as the hip structural analysis (HSA) software, to estimate bone geometric properties and thus strength using the conventional DXA image data, based on principles that were first described by Martin and Burr [17]. Many

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investigators have used the HSA program to characterize bone geometry and strength [15,16,18–20]. This method can allow an assessment of the biomechanical basis for fracture risk reduction of various established interventions, through an understanding of their influence on bone geometry [21]. Indeed, high impact physical activities, and the age at which these activities are practiced, have been shown to have an effect not only on bone mineralization, but also on its geometric properties [19,22–25].

The effect of vitamin D supplementation on bone density has been extensively studied in adults, but much less in children and adolescents [10,13,26–32]. Indeed, these and numerous other studies demonstrate that the crucial role vitamin D plays in normal bone physiology in children is an important determinant of skeletal health in adults. It plays an important role in calcium homeostasis and skeletal mineralization through its endocrine effects on its target organs, bone, kidney, and intestine, and maintenance of normal circulating calcium and phosphate levels. The presence of the vitamin D receptor within the skeletal muscle suggests as well a role for vitamin D in muscle function [33,34].

Our randomized controlled clinical trial in school children showed that vitamin D supplementation had a positive impact on bone musculoskeletal parameters in girls in general and at pre-menarcheal stages in particular. Lean mass significantly increased in both low dose and high dose groups, and so did other bone parameters such as lumbar spine BMD in low dose, and trochanter BMC of both treatment groups [13]. However, to our knowledge, no study has assessed the effect of vitamin D supplementation on bone geometry, in any age group, including adolescence, a critical time for bone mass accrual. The purpose of our investigation was to examine the relationship between baseline vitamin D levels and HSA parameters and the impact of vitamin D supplementation in pre- and post-pubertal children on bone geometry and structure.

Methods

The analyses of the current study are post-hoc exploratory analyses using data from a randomized double blind placebo controlled trial on adolescent healthy subjects.

Full details on the study protocol, subject selection, evaluation, and data collection are available under Supplementary methods, and the CONSORT diagram as Supplementary Fig. 1.

Subjects

Subjects were those who completed the one year randomized double blind placebo controlled vitamin D trial [13]. The trial included 179 and 184 apparently healthy girls and boys respectively, recruited from 4 schools from the Greater Beirut area to ensure balanced representation geographically and socio-economically [35], between December 2001 and June 2002. Over 93% of the study participants (168 girls and 172 boys) returned for their scheduled one year assessment [13]. The age group chosen was 10–17 years, a critical age for bone mass accretion [36]. Subjects were included in the study if they were considered healthy, based on careful physical examination, and had no history of any disorders or medications known to affect bone metabolism [35]. The study was approved by the Institutional Review Board, and informed consent was obtained from all study subjects and their parents.

Intervention and data collection

Subjects were randomly assigned in a double-blind manner to receive weekly placebo oil, or a vitamin D_3 preparation, given as low dose vitamin D (1400 IU [35 μg/week]), i.e. the equivalent of 200 IU/day, or high dose vitamin D (14,000 IU [350 μg/week]), i.e. the equivalent of 2000 IU/day (Vigantol oil, Merck KGAa, Germany) for 1 year. The randomization process, dose selection, quality assurance and monitoring have been previously described in full detail [13]. The calculation of the sample size was performed based on the expected outcomes of the randomized clinical trial as detailed in the study by El-Hajj Fuleihan et al. [13]. At 12 months, the number of subjects with HSA data included in the current analyses included 111 subjects (55 girls, 56 boys) in placebo group, 113 subjects (58 girls, 55 boys) in low dose, and 114 subjects (54 girls, 60 boys) in high dose groups.

Each subject had a baseline physical examination, including height, weight, and Tanner stages. Standing height was measured in triplicate using a wall stadiometer and average value was reported. Weight was recorded while the subject was wearing light clothes without shoes using a standard clinical balance. Pubertal status was measured by one of three physicians who were contributing to the study, according to the established criteria of Tanner [37]. Menarcheal status was determined by these physicians by inquiring with the study subjects about their menarcheal status at study entry. Because of the small number of subjects in each Tanner stage subgroup, study subjects were divided into two discrete pubertal sub-groups for each gender, pre- ($n = 33$) and post-menarche ($n = 134$) in girls, and early (Tanner I ($n = 45$) and II ($n = 47$)) vs. late (Tanner III ($n = 30$), IV ($n = 30$), V ($n = 19$)) puberty in boys, as was implemented in the original reports of the trial [13,38]. Assessment of calcium intake, exercise, sun exposure, and history of fractures was made at baseline and follow-up [35]. Exercise frequency was assessed based on a questionnaire inquiring about the average number of hours spent on sports per week. Calcium intake was evaluated through a food frequency questionnaire that stressed the consumption of dairy products by adolescents in the Lebanese population. Frequency of sun exposure was reported as the average number of hours spent in the sun for weekdays and weekends, and the prorated average was reported.

Measurement of serum 25-hydroxy vitamin D [25(OH)D] levels

Serum 25(OH)D was measured at baseline and 12 months by a competitive protein binding radio-immunoassay using the Incstar Kit (Diasorin, Incstar, Saluggia, Italy), with intra- and inter-assay CVs less than 13% at a serum concentration of 47 ng/ml. All samples were assayed together in the same run at the end of the study.

Areal bone mineral density (aBMD) measurements

BMD of the hip and total body compositions were determined at baseline and 1 year using a Hologic 4500A densitometer (Hologic, Bedford, MA; software version 11.2:3). In our center, the mean ± SD precision of the aBMD measurements, expressed as the CV, for 280 same-day duplicate scans performed during the study duration was less than 1.2 ± 0.9% for the spine, total hip, femoral neck, trochanter, and one third radius. Similar values were obtained for total body aBMD and BMC, lean mass, and fat mass.

Hip structural analysis

Proximal femur scans were analyzed at Dr Beck's laboratory, Johns Hopkins University (Baltimore, MD, USA) using the HSA program [20,39,40]. The HSA program uses conventional DXA image data to derive geometric properties of transverse bone cross-sections that are 5 mm thick in three regions. These regions, as illustrated in Fig. 1, are: the narrow neck (NN) across the narrowest segment of the femoral neck, the intertrochanteric (IT) region along the bisector of the neck–shaft angle, and the shaft at a length equivalent to 1.5 times minimum neck diameter distal to the intersection of neck and shaft axes. For each region the distribution of bone mass across the bone was extracted and the outer diameter (cm), bone cross-sectional area exclusive of soft tissue (CSA, cm^2), and cross-sectional moment of inertia (CSMI, cm^4) were directly measured from the bone mass profile. The outer diameter (OD) is a direct descriptive measure that measures the distance

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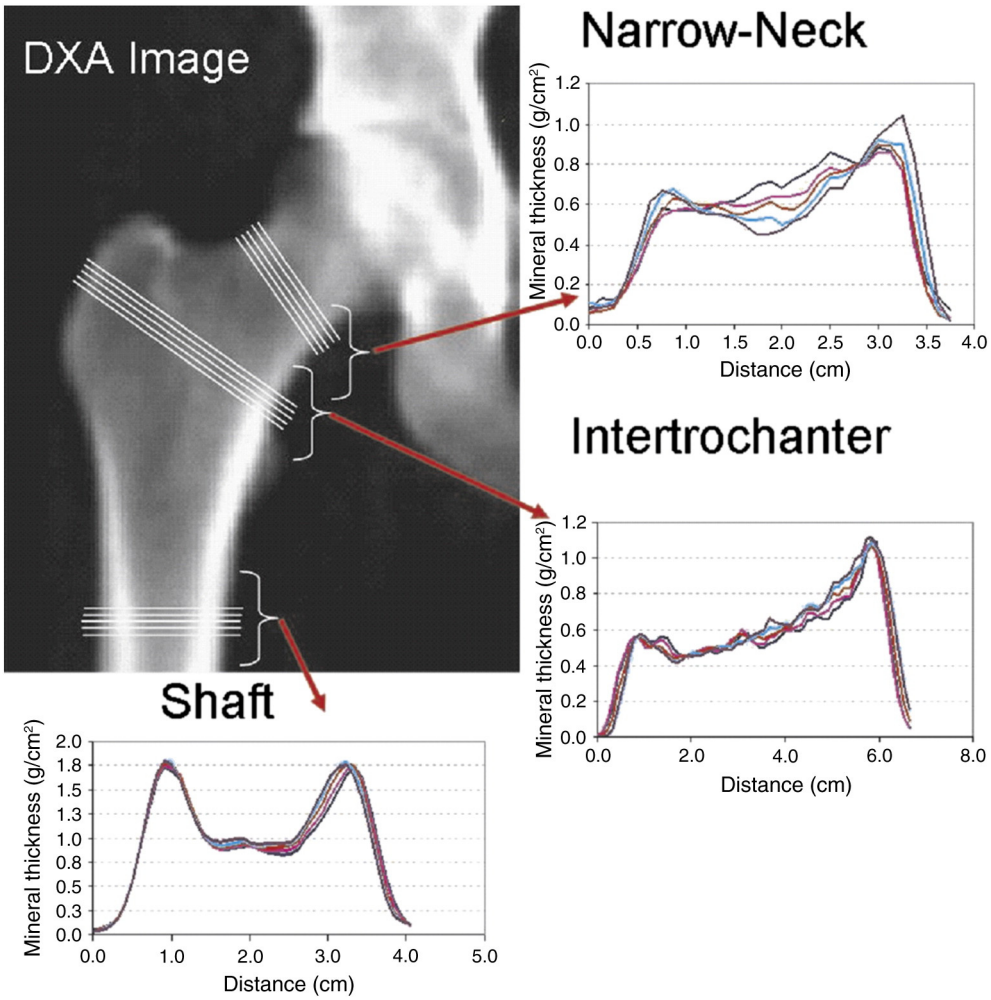

Fig. 1. A schematic of the cross sectional geometric variables calculated from the HSA program showing the different positions measured across the femur, narrow neck, intertrochanteric region, and shaft regions.

between the outer margins of the cross-section after correction for image blurring. CSA is an indicator of bone resistance to loads directed along the bone axis. Section modulus (Z), an indicator of bone bending and torsion strength, was calculated as Z = CSMI / y, where y is the distance from the centroid to the lateral cortical margin [18]. Buckling ratio (BR) is a mechanical index of wall stability in thin walled tubes, calculated as the maximum distance between the center of mass and outer cortex over the average cortical thickness (e.g. NNBR = (NN CSMI / NN Sect Mod) / NN Average cortex) [41]. High values of BR are evident in osteoporotic bone [42]. aBMD is also calculated in the conventional manner, although these regions of interest do not have their counterparts in the standard Hologic BMD analysis [18].

Statistical analyses

Variables of interest were summarized as means (±standard deviation) or medians [interquartile range; Q1, Q3] and compared with independent t-test or the Mann–Whitney test, as appropriate depending on normality of distribution. Analysis of variance (ANOVA) or the Kruskal–Wallis test was used as applicable for evaluating differences in the outcomes of interest between the three treatment groups (placebo, low dose, high dose). Percentage change in the bone parameters was evaluated using the Analysis of Covariance (ANCOVA) technique after accounting for possible confounders, firstly after adjusting for changes in lean mass, and then after adjusting for pubertal status, height at baseline, percent height change, physical activity, calcium intake, and sun exposure. The Bonferroni post-hoc test was used to perform the different subgroup comparisons (placebo vs. low-dose vitamin D, placebo vs. high-dose vitamin D, and low-dose vitamin D vs. high-dose vitamin D). Similar analyses were performed by puberty status (pre-menarcheal girls, post-menarcheal girls, early pubertal [Tanner I and II] boys, and late pubertal [Tanner III–V] boys). Repeated Measures ANOVA (RM-ANOVA) was performed on all parameters of interest using data at baseline and 12 months. Spearman correlation coefficients were used to evaluate the relationship between vitamin D levels and different bone parameters. As the relationship between covariates and change in bone structural parameters might differ between boys and girls, each group was analyzed separately. p-Value ≤ 0.05 was used to indicate significance of tests and p-value between 0.05 and 0.1 indicated borderline significance. Analyses were performed using SPSS version 19.0 (IBM, USA) and SigmaPlot 12.0 (Systat Software Inc., San Jose, CA).

Results

Baseline characteristics and post-therapy vitamin D levels

The placebo and the two vitamin D supplemented groups had similar baseline characteristics including serum 25(OH)D levels, anthropometric and lifestyle variables, in addition to the different bone structural parameters [13] (Tables 1 & 2, Fig. 2). Baseline vitamin D level was below 10 ng/ml in 33.5% of girls and 13.5% of boys, below

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Table 1
Baseline characteristics in the overall group of girls and by treatment. Values are presented as mean (SD) or medians [interquartile range].

Variable	Overall girls (n = 167)	PBO (n = 55)	Low dose (n = 58)	High dose (n = 54)
Age (years)	13.11 [11.22–15.02]	13.60 [11.65–15.65]	12.56 [11.07–14.75]	13.08 [11.13–14.72]
Height (cm)	152.7 (9.9)	154.1 (10.0)	151.5 (9.3)	152.5 (10.5)
Weight (kg)	47.6 (11.5)	48.2 (10.7)	47.2 (11.4)	47.5 (12.7)
BMI (kg/m^2)	19.9 [17.6–21.9]	19.9 [17.2–21.8]	20.2 [17.9–23.0]	19.4 [17.5–21.9]
25(OH)D (ng/ml)	11.9 [9.1–16.6]	11.7 [8.7–11.7]	11.3 [8.7–15.6]	12.6 [10.0–18.7]
Lean mass (kg)	29.9 (5.9)	30.8 (5.9)	29.4 (5.5)	29.7 (6.5)
NN				
aBMD (g/cm^2)	0.98 (0.2)	0.99 (0.2)	0.99 (0.2)	0.97 (0.2)
Cross sectional area (cm^2)	2.36 (0.5)	2.38 (0.5)	2.38 (0.5)	2.3 (0.6)
Outer diameter (cm)	2.49 [2.38–2.65]	2.46 [2.40–2.66]	2.49 [2.40–2.64]	2.47 [2.36–2.65]
Section modulus (cm^3)	0.94 [0.75–1.12]	0.95 [0.76–1.21]	0.95 [0.78–1.13]	0.91 [0.71–1.12]
Buckling ratio	6.79 [5.80–7.99]	6.56 [5.61–8.14]	6.78 [5.74–8.06]	7.08 [6.36–7.98]
S				
aBMD (g/cm^2)	1.36 (0.24)	1.38 (0.24)	1.34 (0.24)	1.35 (0.24)
Cross sectional area (cm^2)	3.19 (0.72)	3.26 (0.70)	3.16 (0.70)	3.17 (0.78)
Outer diameter (cm)	2.46 (0.21)	2.46 (0.20)	2.46 (0.20)	2.45 (0.25)
Section modulus (cm^3)	1.51 (0.44)	1.54 (0.41)	1.49 (0.40)	1.49 (0.51)
Buckling ratio	2.41 [2.16–2.86]	2.37 [2.10–2.83]	2.47 [2.14–2.91]	2.39 [2.20–2.86]
IT				
aBMD (g/cm^2)	0.92 (0.20)	0.94 (0.20)	0.91 (0.15)	0.89 (0.16)
Cross sectional area (cm^2)	3.89 (0.83)	4.01 (0.81)	3.87 (0.80)	3.81 (0.88)
Outer diameter (cm)	4.45 (0.45)	4.46 (0.40)	4.46 (0.42)	4.44 [4.04–4.77]
Section modulus (cm^3)	2.97 (0.84)	3.03 (0.74)	2.93 (0.76)	2.95 (1.0)
Buckling ratio	6.39 [5.67–7.23]	6.18 [5.55–7.23]	6.38 [5.75–7.26]	6.62 [5.67–7.25]

25(OH)D: 25-hydroxy vitamin D; NN: narrow neck; S: shaft; IT: intertrochanteric; aBMD: areal bone mineral density.

20 ng/ml in 83.2% of girls and 80.1% of boys and below 30 ng/ml in 94.6% of girls and 94.7% of boys. There were substantial increments in serum 25(OH)D levels in response to therapy, in both genders, and in both vitamin D groups, that were significantly different from placebo. The mean increments were 3.2 ± 9.2 ng/ml (low dose), 23.8 ± 30.3 ng/ml (high dose), versus 1.5 ± 5.4 ng/ml (placebo) in girls, and 3.7 ± 5.0 ng/ml, 18.6 ± 9.4 9 ng/ml, versus 0.9 ± 5.1 ng/ml, respectively in boys (Fig. 2).

Correlations between baseline serum 25(OH)D level and HSA variables

In the overall group of girls, there was a significant correlation between baseline serum 25(OH)D level and aBMD of the NN and S ($r = 0.16$, $p = 0.04$ and $r = .17$, $p = 0.03$ respectively). In addition, baseline serum 25(OH)D levels were negatively correlated with the BR of the NN ($r = -0.22$, $p = 0.006$). Subgroup analyses revealed a higher magnitude of these correlations among post-menarcheal

Table 2
Baseline characteristics in the overall group of boys and by treatment. Values are presented as mean (SD) or medians [interquartile range].

Variable	Overall boys (n = 171)	PBO (n = 56)	Low dose (n = 55)	High dose (n = 60)
Age (years)	12.7 [11.4–14.7]	12.9 [11.8–14.8]	12.6 [11.2–14.6]	12.9 [11.4–14.8]
Height (cm)	154.9 (13.3)	156.2 (13.3)	154.4 (13.4)	154.1 (13.5)
Weight (kg)	51.5 [37.5–62.0]	54.0 [37.6–66.6]	50.3 [36.0–63.0]	49.0 [38.1–61.0]
BMI (kg/m^2)	20.4 [17.5–24.4]	20.8 [17.0–25.4]	19.8 [17.3–24.5]	20.3 [17.6–23.5]
25(OH)D (ng/ml)	15.5 [6.0–44.9]	16.0 [12.9–19.5]	15.2 [12.1–18.8]	15.5 [11.7–18.7]
Lean mass (kg)	33.4 [26.5–42.8]	35.9 [26.1–44.9]	32.4 [26.0–39.5]	31.0 [26.8–43.7]
NN				
aBMD (g/cm^2)	1.00 [0.88–1.20]	1.01 [0.89–1.22]	1.03 [0.88–1.17]	0.99 [0.87–1.19]
Cross sectional area (cm^2)	2.57 [2.17–3.32]	2.60 [2.19–3.48]	2.66 [2.12–3.23]	2.50 [2.14–3.38]
Outer diameter (cm)	2.74 (0.29)	2.79 (0.30)	2.72 (0.27)	2.71 (0.30)
Section modulus (cm^3)	1.11 [0.86–1.55]	1.15 [0.87–1.63]	1.15 [0.83–1.51]	1.05 [0.85–1.51]
Buckling ratio	7.21 (1.45)	7.22 (1.43)	7.14 (1.42)	7.25 (1.53)
S				
aBMD (g/cm^2)	1.37 [1.18–1.69]	1.38 [1.22–1.79]	1.38 [1.11–1.68]	1.36 [1.18–1.65]
Cross sectional area (cm^2)	3.23 (2.64–4.44)	3.32 [2.75–4.68]	3.38 [2.55–4.17]	3.15 [2.66–4.46]
Outer diameter (cm)	2.53 (0.26)	2.57 (0.27)	2.51(0.25)	2.49 (0.25)
Section modulus (cm^3)	1.55 [1.18–2.13]	1.67 [1.24–2.27]	1.60 [1.15–2.03]	1.48 [1.15–2.16]
Buckling ratio	2.46 (0.68)	2.41 (0.61)	2.48 (0.67)	2.49 (0.75)
IT				
aBMD (g/cm^2)	1.04 (0.20)	1.05 (0.20)	1.03 (0.20)	1.03 (0.21)
Cross sectional area (cm^2)	4.15 [3.35–5.76]	4.24 [3.40–5.87]	4.32 [3.15–5.62]	3.95 [3.42–5.69]
Outer diameter (cm)	4.49 [3.82–4.99]	4.73 [3.81–5.08]	4.47 [3.99–4.97]	4.36 [3.71–5.07]
Section modulus (cm^3)	3.06 [2.30–4.54]	3.10 [2.42–4.73]	3.40 [2.18–4.39]	2.81 [2.22–4.55]
Buckling ratio	5.61 [4.80–6.54]	5.57 [4.78–6.77]	5.59 [4.87–6.60]	5.61 [4.76–6.46]

25(OH)D: 25-hydroxy vitamin D; NN: narrow neck; S: shaft; IT: intertrochanteric; aBMD: areal bone mineral density.

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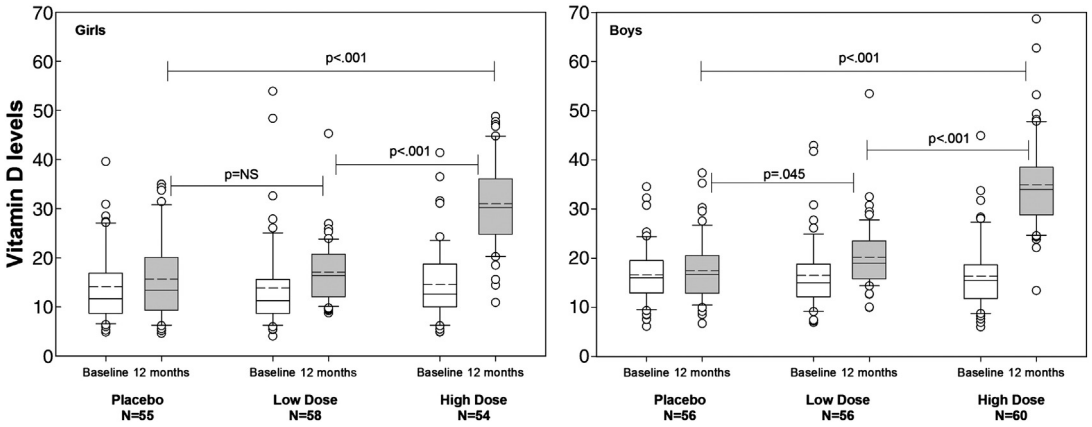

Fig. 2. Baseline and 12 months 25(OH)D levels in girls and boys across different treatment groups.

girls, a sub-group in whom additional significant correlations were noted with Z (an index of bending strength) of the NN and S regions ($r = 0.18$, $p = .046$ and $r = 0.20$, $p = 0.03$), and with CSA (an index of axial strength) and BR of the S region ($r = 0.22$, $p = 0.01$ and $r = -0.24$, $p = .007$ respectively). In pre-menarcheal girls, serum 25(OH)D levels also correlated with CSA and Z of the NN ($r = 0.36$, $p = 0.04$ and $r = 0.38$, $p = 0.03$ respectively), in addition to aBMD of the S ($r = 0.41$, $p = 0.03$) (Supplementary Table 1).

In the overall group of boys, no significant correlations were detected between baseline 25(OH)D levels and the different hip structural parameters (data not shown).

Effect of vitamin D supplementation on the geometry changes

Unadjusted analyses

In the overall group of girls, and as shown in Supplementary Table 2, vitamin D supplementation significantly increased the aBMD at the NN [+7.85% (low dose), +6.79% (high dose), vs. +4.23% in placebo, $p = .049$], and similarly at the IT region (median +7.85% and +5.20% vs. +3.63% in placebo, $p = .02$), but not at the S; thus confirming the previously reported consistent increments in aBMD in the regions of interest routinely obtained on traditional DXA images [13]. It also increased the OD of the S region (+2.43% and +2.51% in low and high dose groups respectively vs. +0.83% in placebo, $p = .04$), and the CSA of the IT region (median +7.54 and +5.13% vs. +4.08% in placebo, $p = .04$), and significantly reduced the BR of the NN (median −6.06% and −2.40% vs. −1.74% in placebo, $p = .03$). These changes were not detected in premenarcheal or post-menarcheal girls (data not shown), where only aBMD of the IT region of premenarcheal girls was significantly increased (+14% and +10% vs. +5.5% in placebo, $p = 0.02$).

After adjusting for percentage change in lean mass, there was a difference in the change among the three treatment groups in the OD (+0.79% and 2.03% in low and high doses versus 2.73% in placebo, $p = 0.02$), and BR of NN (−6.6% and −4.2% versus −1.9%, $p = 0.04$), and borderline significant improvement in aBMD of the IT region of the hip (8.0% and 6.0% versus 5.0%, $p = 0.09$) (Table 3). These significant changes remained after additional adjustment for pubertal status, baseline height, percentage change in height, sun exposure, calcium intake, and physical activity (i.e. OD of NN: +0.80% and +1.97% in low and high doses versus +2.77% in placebo, $p = 0.02$; BR of NN: −6.53% and −4.16% versus −1.98%, $p = .049$; and IT aBMD: +8.1% and +5.84% versus +5.18% in placebo, $p = .06$) (Table 4, Fig. 3). Such findings were not observed in the sub-group analyses by menarcheal status in girls. Similarly, in boys, there was no evidence for any significant effect on change in aBMD or in any of the hip geometry variables assessed including the NN, IT, and S (Supplementary Table 3).

Discussion

Post-hoc exploratory analyses from our large vitamin D randomized controlled trial demonstrate interesting findings regarding the effect of vitamin D supplementation on site specific bone structural parameters in adolescent girls. This study also confirms the sexual dimorphism in the impact of vitamin D supplementation on bone geometry, as we had previously observed for bone density, between the two genders. However, no difference in the beneficial effects of vitamin D supplementation by vitamin D dose received was shown in this study.

The positive correlations between 25(OH)D levels at study entry and various parameters of hip geometry are quite interesting and encouraging in view of the additional positive effect of vitamin D on various indices of bone structure observed at one year in girls. These correlations, however, were not present across all femur measurement sites, nor did they reach significance in subgroup analyses by pubertal status in girls. This is possibly explained by the relatively small sample size in sub-groups by pubertal stage and more importantly by the expected timing for changes in bone mass and geometry at the hip across puberty as previously described by Jackowski et al., in

Table 3
Effect of vitamin D supplementation on % change in HSA parameters in overall group of girls after adjusting for percentage change in lean mass using Analysis of Covariance (ANCOVA). Mean (SE).

% change in HSA parameters	PBO (n = 55)	Low dose (n = 58)	High dose (n = 54)	p-Value
% change in NN				
aBMD (g/cm^2)	5.11 (0.98)	7.52 (0.95)	6.24 (1.00)	0.21
Cross sectional area (cm^2)	7.95 (0.96)	8.32 (0.93)	8.31 (0.98)	0.95
Outer diameter (cm)	**2.73 (0.50)**	**0.79 (0.49)**	**2.03 (0.52)**	**0.02***
Section modulus (cm^3)	11.33 (1.32)	11.42 (1.27)	11.72 (1.35)	0.98
Buckling ratio	**−1.85 (1.31)**	**−6.60 (1.26)**	**−4.23 (1.34)**	**0.04***
% change in S				
aBMD (g/cm^2)	8.29 (0.91)	8.90 (0.92)	8.38 (0.91)	0.88
Cross sectional area (cm^2)	9.74 (0.91)	11.31 (0.92)	10.82 (0.92)	0.47
Outer diameter (cm)	11.35 (0.44)	2.18 (0.44)	2.23 (0.44)	0.29
Section modulus (cm^3)	10.88 (1.21)	13.11 (1.22)	12.98 (1.21)	0.35
Buckling ratio	−8.94 (1.45)	−8.21 (1.47)	−7.28 (1.46)	0.72
% change in IT				
aBMD (g/cm^2)	5.09 (0.95)	8.00 (0.93)	6.04 (0.96)	0.09
Cross sectional area (cm^2)	6.97 (1.03)	9.41 (0.99)	8.74 (1.04)	0.22
Outer diameter (cm)	1.81 (0.68)	1.34 (0.66)	2.54 (0.68)	0.45
Section modulus (cm^3)	10.06 (1.96)	12.92 (1.91)	12.86 (1.98)	0.50
Buckling ratio	−3.61 (1.22)	−5.37 (1.23)	−3.17 (1.22)	0.41

NN: narrow neck; S: shaft; IT: intertrochanteric; aBMD: areal bone mineral density.
Numbers in bold indicate significant difference among the three different treatment groups; $p \leq 0.05$.
* Significant difference between low dose and placebo group.

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Table 4
Effect of vitamin D supplementation on % change in HSA parameters in overall group of girls after adjusting for baseline height, percentage change in lean mass and height, sun exposure, physical activity, calcium intake, and menarcheal status using Analysis of Covariance (ANCOVA).

% change in HSA parameters	PBO (n = 56)	Low dose (n = 56)	High dose (n = 60)	p-Value
% change in NN				
aBMD (g/cm^2)	5.25 (0.96)	7.50 (0.92)	6.12 (0.98)	0.72
Cross sectional area (cm^2)	8.16 (0.88)	8.31 (0.84)	8.12 (0.90)	0.99
Outer diameter (cm)	**2.77 (.50)**	**0.80 (0.48)**	**1.97 (0.51)**	**0.02***
Section modulus (cm^3)	11.73 (1.19)	11.31 (1.14)	11.41 (1.22)	0.97
Buckling ratio	**−1.98 (1.32)**	**−6.53 (1.26)**	**−4.16 (1.34)**	**0.049***
% change in S				
aBMD (g/cm^2)	8.32 (.84)	9.10 (0.85)	8.15 (0.84)	0.70
Cross sectional area (cm^2)	9.85 (.77)	11.62 (0.78)	10.39 (0.78)	0.26
Outer diameter (cm)	1.42 (0.42)	2.27 (0.42)	2.06 (0.42)	0.33
Section modulus (cm^3)	11.02 (1.04)	13.51 (1.05)	12.45 (1.05)	0.25
Buckling ratio	−8.86 (1.41)	−8.38 (1.42)	−7.18 (1.42)	0.70
% change in IT				
aBMD (g/cm^2)	5.18 (0.91)	8.10 (0.88)	5.84 (0.92)	0.06
Cross sectional area (cm^2)	7.13 (0.85)	9.49 (0.83)	8.48 (0.86)	0.14
Outer diameter (cm)	1.88 (0.62)	1.32 (0.61)	2.51 (0.63)	0.40
Section modulus (cm^3)	10.12 (1.63)	13.05 (1.60)	12.66 (1.65)	0.39
Buckling ratio	−3.58 (1.21)	−5.60 (1.22)	−3.00 (1.22)	0.29

NN: narrow neck; S: shaft; IT: intertrochanteric; aBMD: areal bone mineral density.
Numbers in bold indicate significant difference among the three different treatment groups; $p \leq 0.05$.
* Significant difference between low dose and placebo group.

the longitudinal Saskatchewan adolescent study [43]. Indeed, changes in various hip geometry variables occurred between 17 and 19 years in girls, and later in boys [43].

Studies evaluating the impact of vitamin D on bone mass indices in adolescents are relatively scarce, with inconsistent results. In a recent systematic review and meta-analysis of six studies, that included 343 participants receiving placebo and 541 receiving vitamin D, there was no apparent effect of vitamin D supplementation on total body bone mineral content (BMC) or on bone mineral density (BMD) of the hip or forearm, but there was a trend for a small effect on lumbar spine [26]. The differences between studies might be explained by vitamin D status at entry, and possibly doses of vitamin D used. In the meta-analysis, a positive effect on total body BMC and lumbar spine BMD was revealed in analyses of studies with low serum vitamin D at entry [26]. The above review included the study we conducted in adolescents which provided the data for the current analyses.

Bone strength is determined by the mechanical properties of bone, that is, geometry and material strength. The HSA DXA based software allows a non-invasive evaluation of bone geometry. While we are unaware of any studies investigating the impact of vitamin D on hip geometry in adolescents, several have investigated other putative modulators of hip geometry including gender, anthropometric variables, body composition, puberty, exercise, hormones and disease states (anorexia nervosa, glucocorticoid use) [16,20,24,43–51]. Several lines of evidence show that bone adapts its bending strength primarily to mechanical loading, represented by lean mass and sports exercise score, and that sex steroids are associated with bone geometric structure [46,52]. In a 7 year longitudinal study of 230 adolescents, Forwood et al. demonstrated that physical activity was an independent predictor of femoral neck CSA (an index of axial strength), and of its section modulus (Z, an index of bending strength), independent of height, weight, and maturation index [52]. Findings in our study persisted after adjustment for exercise activity, but several disappeared after adjustment for changes in lean mass, confirming the profound effect of lean mass, a surrogate for muscle force, on bone geometry. Indeed, the strong association between various indices of muscle (lean mass, dynamometry, muscle cross-sectional area) and bone mass or geometry, conceptually captured by the bone–muscle unit concept, is well established during growth and adulthood [53–57]. Petit et al. also showed that overweight children and adolescents have larger indices of bone geometry in proportion to their greater lean mass in their larger body sizes, but not proportional to their fat mass [16].

The structural changes we noted in girls were present in unadjusted analyses and persisted after adjustment for lean mass first, but also after including pubertal status, height, physical activity, calcium intake and sun exposure, underscoring the additional independent positive effect of vitamin D on bone geometry, over and above that mediated through increased musculature (lean mass). In contrast, we previously noted the apparent absence of any positive effect of vitamin D on bone mass in boys [58]. And the lack of an apparent effect on bone geometry reported herein, may possibly be due to the higher 25(OH) levels at study entry in boys, and possibly the powerful effect on bone mass and structure of a larger lean mass and activity level than in girls [51]. Alternatively, other possible explanations for the observed sexual dimorphism may include the divergent effect of sex steroids on bone mass, micro-architecture, and muscle [48], the delayed timing of puberty in boys compared to girls, and the delay in changes in bone geometry in this gender compared to girls [43]. Indeed, in the latter study, the effect of exercise on bone geometry was in proportion to changes in lean mass, whereas in girls estrogen levels seemed to play an additional independent effect [43]. In a micro CT study of bone microarchitecture, Lawson et al. demonstrated a positive effect of leptin, IGF1, and androgen levels and bone microarchitecture, independent of BMI [48]. In the 10 years of longitudinal follow-up from Penn State Young Women's Health Study, predictors of adult bone

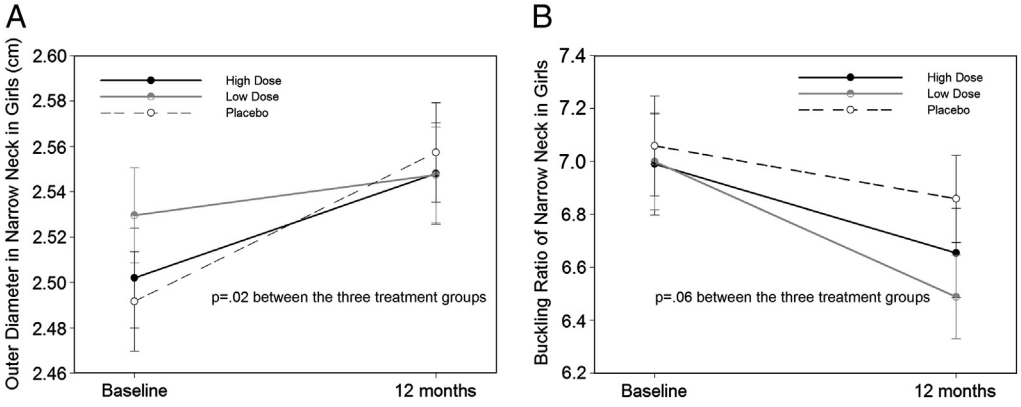

Fig. 3. Effect of vitamin D supplementation on HSA parameters in the three different treatment groups of girls after adjusting for pubertal status, baseline height, percentage change in height and lean mass, sun exposure, calcium intake, and physical activity using Repeated Measures-Analysis of Variance (RM-ANOVA). There was a difference in the change in OD (panel A) and BR (panel B) at the NN between the three groups.

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structural geometry and strength were explored, showing that bending strength depends primarily on mechanical loading (represented by lean mass and sports exercise score) and that sex steroids are associated with bone geometric structure [50].

Our study had a few limitations. These include the post-hoc nature of the analyses, the relatively small number of subjects in each group, and the large heterogeneity in a growing population which may have reduced the power to demonstrate the beneficial effects of vitamin D supplementation in the subgroup analysis by vitamin D dose received, and by pubertal status, especially in boys and early pubertal girls. Bone tissue mineral density cannot be measured from DXA data thus the HSA algorithm assumes that bones are fully mineralized at an average adult value of 1.05 g/cm^3 when deriving CSA and Z. Since mineralization is incomplete in adolescents we cannot rule out an effect of treatment on mineralization given that vitamin D deficiencies are associated with the hypomineralization of osteomalacia. Furthermore our assumption of the adult value leads to underestimation of CSA and Z and possibly on treatment effects on those parameters [44]. Moreover, the DXA data and thus the HSA program measure mass and extract dimensions from a two dimensional image on the three-dimensional hip. Therefore, a systemic error might occur in the derived geometry, if the subject's hip position is inconsistent between scans [18]. However, our QA program is quite rigorous and images were reviewed by the senior authors and it is unlikely that a systematic positioning error would be associated with treatment.

Despite these limitations, to our knowledge this is the first to demonstrate a measurable positive effect of vitamin D supplementation on bone geometry in girls but not boys, during a critical time of growth and peak bone mass accrual. It also provides insights into pathways for such effect, which may in part be explained by the beneficial effect of vitamin D supplementation on lean mass. If confirmed and sustained into adulthood, these observed changes would be anticipated to translate into a reduced risk of hip fractures in elderly years.

Disclosures

The HSA program has been licensed by Dr. Beck's Institution to Hologic, Inc.

All authors state that they have no conflicts of interest.

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Authors' roles

Study design: GEHF, MN, RV; study conduct: GEHF, NM, JM, RE; data collection: JM, RE. Data analysis: GEHF, LA; data interpretation: All; drafting manuscript: GEHF, LA; revising manuscript content: All; approving final version of manuscript: GEHF. GEHF takes responsibility for the integrity of the data analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bone.2013.06.020.

References

[1] Bonjour JP, Rizzoli R. Bone acquisition in adolescence. In: Marcus R, Feldman D, Kelsey J, Feldman D, Kelsey Js, editors. Osteoporosis. San Diego, USA: Academic Press; 1996.
[2] Krall EA, Dawson-Hughes B. Heritable and life-style determinants of bone mineral density. J Bone Miner Res 1993;8:1–9.
[3] Runyan SM, Stadler DD, Bainbridge CN, Miller SC, Moyer-Mileur LJ. Familial resemblance of bone mineralization, calcium intake, and physical activity in early-adolescent daughters, their mothers, and maternal grandmothers. J Am Diet Assoc 2003;103:1320–5.
[4] Nabulsi M, Mahfoud Z, El-Rassi R, Al-Shaar L, Maalouf J, El-Hajj Fuleihan G. Gender differences in the heritability of musculoskeletal and body composition parameters in mother–daughter and mother–son pairs. J Clin Densitom 2013;16:223–30.
[5] Daly RM. The effect of exercise on bone mass and structural geometry during growth. Med Sport Sci 2007;51:33–49.
[6] Rizzoli R, Bianchi ML, Garabedian M, McKay HA, Moreno LA. Maximizing bone mineral mass gain during growth for the prevention of fractures in the adolescents and the elderly. Bone 2010;46:294–305.
[7] Hind K, Burrows M. Weight-bearing exercise and bone mineral accrual in children and adolescents: a review of controlled trials. Bone 2007;40:14–27.
[8] Abrams SA. Calcium and vitamin D requirements for optimal bone mass during adolescence. Curr Opin Clin Nutr Metab Care 2011;14:605–9.
[9] Stoffman N, Gordon CM. Vitamin D and adolescents: what do we know? Curr Opin Pediatr 2009;21:465–71.
[10] Abrams SA. Vitamin D, requirements of children: “all my life's a circle”. Nutr Rev 2012;70:201–6.
[11] Bueno AL, Czepielewski MA. The importance for growth of dietary intake of calcium and vitamin D. J Pediatr (Rio J) 2008;84:386–94.
[12] Ondrak KS, Morgan DW. Physical activity, calcium intake and bone health in children and adolescents. Sports Med 2007;37:587–600.
[13] El-Hajj Fuleihan G, Nabulsi M, Tamim H, Maalouf J, Salamoun M, Khalife H, et al. Effect of vitamin D replacement on musculoskeletal parameters in school children: a randomized controlled trial. J Clin Endocrinol Metab 2006;91:405–12.
[14] Fulton JP. New guidelines for the prevention and treatment of osteoporosis. National Osteoporosis Foundation. Med Health R I 1999;82:110–1.
[15] Semanick LM, Beck TJ, Cauley JA, Wheeler VW, Patrick AL, Bunker CH, et al. Association of body composition and physical activity with proximal femur geometry in middle-aged and elderly Afro-Caribbean men: the Tobago bone health study. Calcif Tissue Int 2005;77:160–6.
[16] Petit MA, Beck TJ, Shults J, Zemel BS, Foster BJ, Leonard MB. Proximal femur bone geometry is appropriately adapted to lean mass in overweight children and adolescents. Bone 2005;36:568–76.
[17] Martin RB, Burr DB. Non-invasive measurement of long bone cross-sectional moment of inertia by photon absorptiometry. J Biomech 1984;17:195–201.
[18] Petit MA, McKay HA, MacKelvie KJ, Heinonen A, Khan KM, Beck TJ. A randomized school-based jumping intervention confers site and maturity-specific benefits on bone structural properties in girls: a hip structural analysis study. J Bone Miner Res 2002;17:363–72.
[19] Ferry B, Lespessailles E, Rochcongar P, Duclos M, Courteix D. Bone health during late adolescence: effects of an 8-month training program on bone geometry in female athletes. Joint Bone Spine 2013;80:57–63.
[20] Forwood MR, Bailey DA, Beck TJ, Mirwald RL, Baxter-Jones AD, Uusi-Rasi K. Sexual dimorphism of the femoral neck during the adolescent growth spurt: a structural analysis. Bone 2004;35:973–81.
[21] Heaney RP, Abrams S, Dawson-Hughes B, Looker A, Marcus R, Matkovic V, et al. Peak bone mass. Osteoporos Int 2000;11:985–1009.
[22] Ducher G, Courteix D, Meme S, Magni C, Viala JF, Benhamou CL. Bone geometry in response to long-term tennis playing and its relationship with muscle volume: a quantitative magnetic resonance imaging study in tennis players. Bone 2005;37:457–66.
[23] Tournis S, Michopoulou E, Fatouros IG, Paspati I, Michalopoulou M, Raptou P, et al. Effect of rhythmic gymnastics on volumetric bone mineral density and bone geometry in premenarcheal female athletes and controls. J Clin Endocrinol Metab 2010;95:2755–62.
[24] Ferry B, Duclos M, Burt L, Therre P, Le Gall F, Jaffre C, et al. Bone geometry and strength adaptations to physical constraints inherent in different sports: comparison between elite female soccer players and swimmers. J Bone Miner Metab 2011;29:342–51.
[25] Ferry B, Lespessailles E, Rochcongar P, Duclos M, Courteix D. Bone health during late adolescence: effects of an 8-month training program on bone geometry in female athletes. Joint Bone Spine 2013;80:57–63.
[26] Winzenberg T, Powell S, Shaw KA, Jones G. Effects of vitamin D supplementation on bone density in healthy children: systematic review and meta-analysis. BMJ 2011;342:c7254.
[27] Bischoff-Ferrari HA. Vitamin D, and fracture prevention. Rheum Dis Clin North Am 2012;38:107–13.
[28] Bischoff-Ferrari HA, Willett WC, Orav EJ, Lips P, Meunier PJ, Lyons RA, et al. A pooled analysis of vitamin D dose requirements for fracture prevention. N Engl J Med 2012;367:40–9.
[29] Holick MF. Vitamin D, deficiency. N Engl J Med 2007;357:266–81.
[30] Viljakainen HT, Natri AM, Karkkainen M, Huttunen MM, Palssa A, Jakobsen J, et al. A positive dose–response effect of vitamin D supplementation on site-specific bone mineral augmentation in adolescent girls: a double-blinded randomized placebo-controlled 1-year intervention. J Bone Miner Res 2006;21:836–44.

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[31] Ward KA, Das G, Roberts SA, Berry JL, Adams JE, Rawer R, et al. A randomized, controlled trial of vitamin D supplementation upon musculoskeletal health in postmenarchal females. J Clin Endocrinol Metab 2010;95:4643–51.
[32] Sayers A, Fraser WD, Lawlor DA, Tobias JH. 25-Hydroxyvitamin-D3 levels are positively related to subsequent cortical bone development in childhood: findings from a large prospective cohort study. Osteoporos Int 2012;23:2117–28.
[33] Yoshikawa S, Nakamura T, Tanabe H, Imamura T. Osteomalacic myopathy. Endocrinol Jpn 1979;26:65–72.
[34] Ceglia L. Vitamin D, and skeletal muscle tissue and function. Mol Aspects Med 2008;29:407–14.
[35] Arabi A, Nabulsi M, Maalouf J, Choucair M, Khalife H, Vieth R, et al. Bone mineral density by age, gender, pubertal stages, and socioeconomic status in healthy Lebanese children and adolescents. Bone 2004;35:1169–79.
[36] Glastre C, Braillon P, David L, Cochat P, Meunier PJ, Delmas PD. Measurement of bone mineral content of the lumbar spine by dual energy X-ray absorptiometry in normal children: correlations with growth parameters. J Clin Endocrinol Metab 1990;70:1330–3.
[37] Tanner JM. Physical growth and development. In: Forfar JO, Ameil GC, editors. Text book of paediatrics, 1. Edinbourgh: Churchill LivingStone; 1978. p. 249–303.
[38] El-Hajj Fuleihan G, Vieth R. Vitamin D insufficiency and musculoskeletal health in children and adolescents. International Congress Series, 1297. Elsevier; 2007. p. 91–108.
[39] Beck TJ, Ruff CB, Warden KE, Scott Jr WW, Rao GU. Predicting femoral neck strength from bone mineral data. A structural approach. Invest Radiol 1990;25:6–18.
[40] Beck TJ, Ruff CB, Scott Jr WW, Plato CC, Tobin JD, Quan CA. Sex differences in geometry of the femoral neck with aging: a structural analysis of bone mineral data. Calcif Tissue Int 1992;50:24–9.
[41] Beck TJ. Hip structural analysis (HSA) program (BMD and structural geometry methodology) as used to create NHANES III dataset; 2002.
[42] El Hage R, Moussa E, Jacob C. Femoral neck geometry in overweight and normal weight adolescent girls. J Bone Miner Metab 2010;28:595–600.
[43] Jackowski SA, Kontulainen SA, Cooper DM, Lanovaz JL, Baxter-Jones AD. The timing of BMD and geometric adaptation at the proximal femur from childhood to early adulthood in males and females: a longitudinal study. J Bone Miner Res 2011;26:2753–61.
[44] Burnham JM, Shults J, Petit MA, Semeao E, Beck TJ, Zemel BS, et al. Alterations in proximal femur geometry in children treated with glucocorticoids for Crohn disease or nephrotic syndrome: impact of the underlying disease. J Bone Miner Res 2007;22:551–9.
[45] Macdonald H, Kontulainen S, Petit M, Janssen P, McKay H. Bone strength and its determinants in pre- and early pubertal boys and girls. Bone 2006;39:598–608.
[46] Jackowski SA, Faulkner RA, Farthing JP, Kontulainen SA, Beck TJ, Baxter-Jones AD. Peak lean tissue mass accrual precedes changes in bone strength indices at the proximal femur during the pubertal growth spurt. Bone 2009;44:1186–90.
[47] Maimoun L, Coste O, Mariano-Goulart D, Galtier F, Mura T, Philibert P, et al. In peripubertal girls, artistic gymnastics improves areal bone mineral density and femoral bone geometry without affecting serum OPG/RANKL levels. Osteoporos Int 2011;22:3055–66.
[48] Lawson EA, Miller KK, Bredella MA, Phan C, Misra M, Meenaghan E, et al. Hormone predictors of abnormal bone microarchitecture in women with anorexia nervosa. Bone 2010;46:458–63.
[49] DiVasta AD, Beck TJ, Petit MA, Feldman HA, LeBoff MS, Gordon CM. Bone cross-sectional geometry in adolescents and young women with anorexia nervosa: a hip structural analysis study. Osteoporos Int 2007;18:797–804.
[50] Petit MA, Beck TJ, Lin HM, Bentley C, Legro RS, Lloyd T. Femoral bone structural geometry adapts to mechanical loading and is influenced by sex steroids: the Penn State Young Women's Health Study. Bone 2004;35:750–9.
[51] Deere K, Sayers A, Rittweger J, Tobias JH. Habitual levels of high, but not moderate or low, impact activity are positively related to hip BMD and geometry: results from a population-based study of adolescents. J Bone Miner Res 2012;27:1887–95.
[52] Forwood MR, Baxter-Jones AD, Beck TJ, Mirwald RL, Howard A, Bailey DA. Physical activity and strength of the femoral neck during the adolescent growth spurt: a longitudinal analysis. Bone 2006;38:576–83.
[53] Schoenau E, Neu CM, Beck B, Manz F, Rauch F. Bone mineral content per muscle cross-sectional area as an index of the functional muscle–bone unit. J Bone Miner Res 2002;17:1095–101.
[54] Rauch F, Bailey DA, Baxter-Jones A, Mirwald R, Faulkner R. The 'muscle–bone unit' during the pubertal growth spurt. Bone 2004;34:771–5.
[55] Rittweger J, Beller G, Ehrig J, Jung C, Koch U, Ramolla J, et al. Bone–muscle strength indices for the human lower leg. Bone 2000;27:319–26.
[56] Doyle F, Brown J, Lachance C. Relation between bone mass and muscle weight. Lancet 1970;1:391–3.
[57] Crabtree NJ, Kibirige MS, Fordham JN, Banks LM, Muntoni F, Chinn D, et al. The relationship between lean body mass and bone mineral content in paediatric health and disease. Bone 2004;35:965–72.
[58] El Hajj Fuleihan G, Vieth R. Vitamin D insufficiency and musculoskeletal health in children and adolescents. In: Burckhardt P, Heaney RP, Dawson-Hughes B, editors. 6th International Symposium on Nutritional Aspects of Osteoporosis. Elsevier; 2007.