

Vitamin D₃ Dose Requirement to Raise 25-Hydroxyvitamin D to Desirable Levels in Adolescents: Results from a Randomized Controlled Trial

Laila Al-Shaar,^{1,2} Rania Mneimneh,³ Mona Nabulsi,^{1,4} Joyce Maalouf,³ and Ghada El-Hajj Fuleihan^{1,3}

¹Scholars in Health Research Program, American University of Beirut Medical Center, Beirut, Lebanon

²Vascular Medicine Program, American University of Beirut Medical Center, Beirut, Lebanon

³Calcium Metabolism and Osteoporosis Program & WHO Collaborating Center for Metabolic Bone Disorder, Division of Endocrinology, Department of Internal Medicine, American University of Beirut Medical Center, Beirut, Lebanon

⁴Department of Pediatrics and Adolescent Medicine, American University of Beirut Medical Center, Beirut, Lebanon

ABSTRACT

Several organizations issued recommendations on desirable serum 25-hydroxy vitamin D [25(OH)D] levels and doses of vitamin D needed to achieve them. Trials allowing the formulation of evidence-based recommendations in adolescents are scarce. We investigated the ability of two doses of vitamin D₃ in achieving recommended vitamin D levels in this age group. Post hoc analyses on data from a 1-year double-blind trial that randomized 336 Lebanese adolescents, aged 13 ± 2 years, to placebo, vitamin D₃ at 200 IU/day (low dose), or 2000 IU/day (high dose). Serum 25(OH)D level and proportions of children achieving levels ≥20 ng/mL and 30 ng/mL were determined. At baseline, mean 25(OH)D was 15 ± 7 ng/mL, 16.4 ± 7 ng/mL in boys, and 14 ± 8 ng/mL in girls, *p* = 0.003, with a level ≥20 ng/mL in 18% and ≥30 ng/mL in 5% of subjects. At 1 year, mean levels were 18.6 ± 6.6 ng/mL in the low-dose group, 17.1 ± 6 ng/mL in girls, and 20.2 ± 7 ng/mL in boys, *p* = 0.01, and 36.3 ± 22.3 ng/mL in the high-dose group, with no sex differences. 25(OH)D increased to ≥20 ng/mL in 34% of children in the low-dose and 96% in the high-dose group, being higher in boys in the low-dose arm only; it remained ≥30 ng/mL in 4% of children in the low-dose arm but increased to 64% in the high-dose arm. Baseline 25(OH)D level, body mass index (BMI), and vitamin D dose assigned were the most significant predictors for reaching a 25(OH)D level ≥20 ng/mL and 30 ng/mL. A daily dose of 2000 IU raised 25(OH)D level ≥20 ng/mL in 96% of adolescents (98% boys versus 93% girls). Dose-response studies are needed to determine in a definitive manner the daily allowance of vitamin D for Middle Eastern adolescents with a similar profile. © 2014 American Society for Bone and Mineral Research.

KEY WORDS: DESIRABLE LEVELS; GUIDELINES; RDA; IOM; ES; VITAMIN D DOSE; ADOLESCENTS

Introduction

Vitamin D is a steroid hormone with clear beneficial effects on musculoskeletal parameters across the life cycle.^(1,2) Vitamin D plays a central role in the skeletal health of children and adolescents,^(3,4) and despite ample sunshine throughout the year, rickets still occur in the Middle East.^(5–8) The region also registers some of the highest prevalence for hypovitaminosis D in the pediatric age group worldwide.^(9,10)

Although it is clear that daily vitamin D doses of 400 IU are sufficient to prevent rickets,⁽¹¹⁾ recommendations regarding desirable levels for bone health, and optimal doses to achieve them, in subjects with more subtle deficiencies are still debatable.^(12–15) Indeed, whereas the updated recommendations from the Institute of Medicine (IOM) issued in the same year

propose that doses of 600 IU/day are sufficient to bring more than 97.5% of the population to the designated desirable 25(OH)D level of 20 ng/mL,⁽¹²⁾ the 2011 Endocrine Society (ES) recommends dietary intakes of at least 1000 IU of vitamin D per day to consistently raise serum 25(OH)D level above 30 ng/mL.⁽¹³⁾ The revised recommendation of the American Academy of Pediatrics (AAP) increased the minimum daily intake of vitamin D supplementation from 200 IU, beginning in the first 2 months after birth and through adolescence, to 400 IU,⁽¹⁴⁾ and more recently endorsed those by the IOM (<http://pediatrics.aappublications.org/content/130/5/e1424.full.pdf+html>).

The above-described lack of consensus in part reflects the limitations incurred by the scarcity of data in general, and from nonwestern populations in particular, and the handful randomized trials conducted in the pediatric age group.⁽³⁾ In this article,

Received in original form June 4, 2013; revised form September 16, 2013; accepted September 19, 2013. Accepted manuscript online October 5, 2013.

Address correspondence to: Ghada El-Hajj Fuleihan, MD, MPH, Division of Endocrinology, American University of Beirut Medical Center, PO Box 11-0236, Riad El Solh, Beirut 1107 2020, Lebanon. E-mail: gf01@aub.edu.lb

Additional Supporting Information may be found in the online version of this article.

Journal of Bone and Mineral Research, Vol. 29, No. 4, April 2014, pp 944–951

DOI: 10.1002/jbmr.2111

© 2014 American Society for Bone and Mineral Research

we took advantage of results from a randomized vitamin D trial conducted in school children, comparing the effect of two doses of vitamin D and placebo on musculoskeletal parameters as primary outcomes, to shed light on the above controversy. The objective of this study was therefore to investigate the impact of the two doses of vitamin D₃ used in the trial on achieved serum 25(OH)D levels at 12 months. Predictors of achieved serum 25(OH)D levels were also explored.

Materials and Methods

Study population

The study group consisted of 336 healthy children and adolescents, 165 girls and 171 boys, recruited between December 2001 and June 2002, who completed a 1-year randomized double-blind placebo-controlled vitamin D trial, investigating the impact of two doses of vitamin D₃ on bone health in adolescents. The trial included apparently healthy girls and boys, recruited from four schools from the Greater Beirut area to ensure balanced representation geographically and socioeconomically.^(16,17) See the CONSORT diagram (Supplemental Fig. S1) for full details. The age group chosen was 10 to 17 years, a critical age for accretion of bone mass.⁽¹⁸⁾ Subjects were included in the study if they were considered healthy, based on careful history and physical examination, and had no history of any disorders or medications known to affect bone metabolism. Each subject had baseline height, weight, and Tanner stages determined. 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.⁽¹⁹⁾ Because of the small number of subjects in each Tanner stage subgroup, study subjects were divided into two discrete pubertal subgroups for each sex, as was reported in the original trial: premenarche ($n=33$) and postmenarche ($n=132$) in girls, and early (Tanner I-II, $n=92$) versus late (Tanner III-V, $n=79$) puberty in boys.^(16,17) Exercise frequency assessment at baseline was based on a questionnaire inquiring about the average number of hours spent on sports per week.⁽¹⁶⁾ Calcium intake was evaluated through a validated food-frequency questionnaire that stressed the consumption of dairy products by adolescents in the Lebanese population.⁽²⁰⁾ 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.⁽¹⁶⁾ The study was approved by the Institutional Review Board, and informed consent-assent was obtained from all study subjects and their parents.

Protocol and data collection

Subjects were randomly assigned in a double-blind manner to receive weekly placebo oil or a vitamin D₃ preparation, given as low-dose vitamin D₃ (1400 IU [35 µg/week]), ie, the equivalent of 200 IU/day, or high-dose vitamin D₃ (14,000 IU [350 µg/week]), ie, the equivalent of 2000 IU/day (Vigantol oil, Merck KGaA, Darmstadt, Germany) for 1 year. The randomization sequence, stratified by socioeconomic status, was generated by a computer at Merck KGaA headquarters, mailed to the study center, and administered by a senior pharmacist. All subjects received identical bottles of an oily solution containing identical volumes of diluent oil for the placebo group, diluted vigantol oil for the

low-dose group, or undiluted oil for the high-dose group. There were no differences in season-months of recruitment among the three treatment arms. Blood was collected at 0 and 12 months for routine chemistries and serum 25(OH)D levels.

Compliance monitoring

The subjects were called by study personnel every 2 weeks to prompt them to take the study drug. Subjects returned the bottles and received new bottles every 3 months. Compliance was checked by measuring the volume and, therefore, the amount of vitamin D₃ left in the returned bottles. The percentage of the dose taken was calculated as [(total volume – returned volume)/total volume] × 100. The mean percent intake of the total dose given for vitamin D₃ exceeded 95% in all three treatment arms.

Laboratory measurements and quality assurance

Serum 25(OH)D levels were measured in the Endocrine Core laboratory and all other tests in the Clinical Chemistry laboratory at the American University of Beirut Medical Center (AUB-MC). Routine chemistries were measured in the Clinical Chemistry laboratory at AUB-MC, using the auto-analyzer Hitachi 912 (Roche Diagnostics GmbH, Mannheim, Germany). 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 interassay coefficients of variation (CVs) less than 13% at a serum concentration of 47 ng/mL. Routine chemistries were run immediately, and serum was stored at -70°C for vitamin D measurements; all vitamin D measurements from an individual subject were assayed together in the same assay, at the end of the study. The Endocrine Core Laboratory is a participant in the Vitamin D External Quality Assurance Surveillance (DEQAS program, www.deqas.org) since 2002, and the Clinical Chemistry laboratory partakes in the quality assurance (QA), evaluation, and accreditation from the College of American Pathologists (CAP, www.cap.org). The endocrine core laboratory results have consistently been in compliance with the quality assurance measures for both QA programs.

Additional details regarding the randomization process, dose selection, quality assurance, and monitoring have been previously described in full detail in previous publications.^(16,21)

Normal ranges for laboratory values and desirable 25(OH)D values

The clinical ranges and cutoffs of all laboratory tests are summarized in Supplemental Table S1. For pediatric normative ranges, *The Harriet Lane Handbook*, 19th edition, was used. The cut-off points selected to determine desirable 25(OH)D levels, and thus proportion of subjects above such cut-offs, were those defined by the ES in their 2011 vitamin D guidelines, which is 25(OH)D of 30 ng/mL (75 nmol/L), and in the 2010 IOM report, which is 25(OH)D of 20 ng/mL (50 nmol/L).

Statistical analyses

Results presented in this article represent post hoc analyses performed on data collected in the completed randomized controlled trial. The calculation of the sample size for the trial was performed based on the expected primary outcomes as detailed in the study by El-Hajj Fuleihan and colleagues.^(16,17) Analysis was performed on all subjects randomized who completed the 1-year study. As detailed in the original publication, 6% of subjects dropped out of the study, but there were no differences in

dropout rates by treatment group. Continuous data are presented as mean \pm standard deviation (SD), if normally distributed, and as median and interquartile range (25th to 75th quartile) for non-normally distributed data. Numbers were rounded to integers for calcium intake and for all other variables to first decimal. The serum 25(OH)D levels were tested using independent *t* test between groups or paired *t* test or Wilcoxon signed rank test, as applicable, within the same group. Analysis of variance (ANOVA) or its nonparametric equivalence, the Kruskal-Wallis test, was used for comparison between treatment groups. Categorical variables/proportions were compared using chi-square test or Fisher's exact test as applicable or McNemar test within the same group. Correlation analysis was conducted using Pearson's correlation coefficient. Multivariate linear and logistic regression analyses were performed for exploring possible predictors of serum mean 25(OH)D at 1 year and likelihood of serum 25(OH)D levels reaching above the preset cut-off points, 20 ng/mL and 30 ng/mL. Predictors included sex, baseline 25(OH) D level, pubertal status, and vitamin D₃ dose. Other predictors were daily calcium intake, body mass index (BMI) and sun exposure entered either as values at study entry or as the mean of entry and completion values. Partial or semipartial correlations were studied to investigate the correlation between the independent variable and the predictor that has been residualized with respect to all other predictors in the model. The proportion of the independent variance associated uniquely with the predictor is the square of this semipartial correlation.

Data were analyzed using analytical computer software SPSS version 20.0 (SPSS, Chicago, IL, USA) and Sigmaplot 12. A *p* value of less than 0.05 was considered statistically significant; *p* values for comparisons in subgroups by sex and pubertal stage were corrected for multiple testing with Bonferroni adjustments.

Results

Baseline characteristics

Full details on baseline characteristics of the study group overall and by dose assignment and sex are shown in Table 1. The study

group consisted of 171 boys and 165 girls, with a mean age of 13.1 \pm 2 years. In the overall group, boys had greater BMI, calcium intake, sun exposure, and exercise activity than girls (*p* = 0.02, 0.02, <0.001, and <0.001, respectively), but there were no sex differences in these characteristics by study group (Table 1). Children had normal serum calcium, phosphate, alkaline phosphatase, and serum osteocalcin for age, and there were no differences between treatment groups (Supplemental Table S2). Boys also tended to have a higher mean 25(OH)D level than girls (16.4 \pm 7.0 versus 14.0 \pm 8.0 ng/mL, *p* = 0.003) at study entry, in addition to higher serum calcium, phosphorous, alkaline phosphatase, and osteocalcin levels (*p* < 0.001) (Supplemental Table S2).

In the overall study group, 18% of children had a 25(OH)D level greater or equal to 20 ng/mL and only 5% had a level greater or equal to 30 ng/mL, with no sex differences at both cut-offs. The numbers were 16.4% for girls and 20% for boys for a cut-off of 20 ng/mL, and 4.8% for girls and 5.3% for boys for a cut-off of 30 ng/mL. There were no difference in means 25(OH) levels nor in proportions above specific cut-offs within sex and between sexes by dose assigned (Table 2).

Baseline 25(OH) D level was positively correlated with baseline sun exposure (*r* = 0.23, *p* < 0.001) in the overall group and in girls (*r* = 0.32, *p* < 0.001) but not boys, and with baseline physical activity (*r* = 0.27, *p* < 0.001) in the overall group and also by sex but not with BMI or total calcium intake at study entry.

Response to vitamin D₃ supplementation

The mean 25(OH)D levels achieved at 12 months were 19 \pm 6.6 ng/mL in the low-dose arm and 36 \pm 22.3 ng/mL in the high-dose arm, *p* < 0.001. Only 1 of 60 boys did not exceed a serum 25(OH)D of 20 ng/mL compared with 4 of 53 girls at the dose of 2000 IU/day. The achieved mean 25(OH)D level was slightly higher in boys than girls in the low-dose arm only (*p* = 0.01, Table 2, Fig. 1). For representation by median, 25th and 75th percentile, see Supplemental Fig. S2. The differences between the mean and median for 25(OH)D in girls provides evidence for the high variability in achieved levels, in response to

Table 1. Baseline Characteristics of the Study Subjects (Mean \pm SD or Median [25th to 75th Quartile])

Parameter	Overall	Placebo	Low dose (200 IU/d)	High dose (2000 IU/d)	<i>p</i> Value ^a
All subjects	<i>n</i> = 336	<i>n</i> = 111	<i>n</i> = 112	<i>n</i> = 113	
Age (years)	13.1 \pm 2.0	13.3 \pm 2.0	12.9 \pm 2.1	13.1 \pm 2.1	0.33
BMI (kg/m ²)	20.7 \pm 4.0	20.7 \pm 4.2	20.7 \pm 4.0	20.5 \pm 3.8	0.89
Calcium intake (mg/d)	728 \pm 363	736 \pm 341	721 \pm 374	726 \pm 376	0.95
Sun exposure (h/week)	7.0 (4.5–11)	7.0 (5–11.5)	7.0 (3.6–11)	7 (4.5–10.6)	0.50*
Exercise (h/week)	5.9 \pm 6.3	6.4 \pm 6.5	5.7 \pm 5.8	5.6 \pm 6.3	0.64
Boys	<i>n</i> = 171	<i>n</i> = 56	<i>n</i> = 55	<i>n</i> = 60	
Age (years)	13.0 \pm 2.0	13.1 \pm 1.9	12.9 \pm 2.0	13.1 \pm 2.0	0.80
BMI (kg/m ²)	21.1 \pm 4.2	21.6 \pm 4.6	21.0 \pm 4.3	20.9 \pm 3.9	0.64
Calcium intake (mg/day)	775 \pm 353	798 \pm 350	768 \pm 378	759 \pm 337	0.83
Sun exposure (h/week)	8.5 (5.3–11.0)	8.5(5.8–14.5)	7.0 (5.25–11.0)	8.5 (5.1–11.0)	0.67*
Exercise (h/week)	7.9 \pm 6.9	8.5 \pm 7.2	8.1 \pm 6.4	7.1 \pm 7.2	0.54
Girls	<i>n</i> = 165	<i>n</i> = 55	<i>n</i> = 57	<i>n</i> = 53	
Age (years)	13.2 \pm 2.1	13.6 \pm 2.1	13.0 \pm 2.2	13.1 \pm 2.1	0.27
BMI (kg/m ²)	20.1 \pm 3.6	20.0 \pm 3.5	20.3 \pm 3.7	20.1 \pm 3.7	0.85
Calcium intake (mg/day)	679 \pm 368	673 \pm 3 23	675 \pm 367	688 \pm 417	0.97
Sun exposure (h/week)	6.5 (3.5–10.0)	6.5 (4.5–11.0)	6.5 (2.8–10.3)	6.5 (3.9–9)	0.60*
Exercise (h/week)	3.8 \pm 4.9	4.1 \pm 4.9	3.4 \pm 4.0	3.9 \pm 5.6	0.67

^aDifference across the three treatment arms (placebo, low dose, high dose) by ANOVA, except when noted with an asterisk if done by Kruskal-Wallis Test. Numbers in bold indicate significant difference by sex.

Table 2. Mean Serum 25(OH)D and Proportion of Children Above Predefined 25(OH) Cut-offs at Baseline and 1 Year by Treatment Group and Sex

	Boys	Girls	<i>p</i> Value	Boys	Girls	<i>p</i> Value ^a
	25 (OH)D levels at baseline			25 (OH)D levels at 1 year		
Placebo	16.5 ± 5.9	14.2 ± 7.5	0.07	17.4 ± 6.5	15.7 ± 8.4	0.23
Low dose	16.5 ± 7.2	13.9 ± 9.3	0.11	20.2 ± 6.8	17.1 ± 6.2	0.01
High dose	16.3 ± 7.1	14.1 ± 6.7	0.09	34.9 ± 9.4	37.9 ± 31.2	0.48
<i>p</i> Value ^b	0.98	0.99	—	<0.001 ^{c,d}	<0.001 ^{c,d}	—
	% 25(OH)D level ≥20 ng/mL at baseline			% 25(OH)D level ≥20 ng/mL at 1 year		
Placebo	23	18	0.51	27	26	0.87
Low dose	20	12	0.25	42	26	0.08
High dose	17	20	0.61	98	93	0.13
<i>p</i> Value ^b	0.68	0.59	—	<0.001	<0.001	—
	% 25(OH)D level ≥30 ng/mL at baseline			% 25(OH)D level ≥30 ng/mL at 12 months		
Placebo	5	4	0.66	5	11	0.28
Low dose	6	5	0.95	6	2	0.29
High dose	5	7	0.59	72	55	0.06
<i>p</i> Value ^b	0.99 ^e	0.91 ^e	—	<0.001 ^e	<0.001 ^e	—

^a*p* Value between sex.^b*p* Value within sex by dose assignment.^cSignificant difference between placebo and high-dose groups.^dSignificant difference between low- and high-dose groups.^eFisher exact test.

supplementation, in the high-dose group (Supplemental Fig. S2). The proportion of children who reached a serum 25(OH)D level equal to or greater than 20 ng/mL increased to 34% in the low-dose and to 96% in the high-dose vitamin D (Fig. 2). These proportions were significantly higher than those at baseline for both doses (Fig. 2) and sexes (Supplemental Fig. 3A). The proportion of children who reached a serum 25(OH)D level equivalent to or greater than 30 ng/mL remained low at 4% in the low-dose arm but increased to 64% in the high-dose arm (Fig. 2). These proportions were significantly higher than baseline for the high-dose group only, in the overall group (Fig. 2), and both sexes (Supplemental Fig. 3B). There was a trend for a higher proportion of boys than girls to reach the 25(OH)D cut-off of 20 ng/mL with the low dose only ($p = 0.08$), and of 30 ng/mL with the high dose only ($p = 0.06$) (Table 2). Three girls had serum 25(OH)D levels of 195, 161, and 102 ng/mL after 12 months of supplementation of high-dose vitamin D₃ (2000 IU/day). Four girls and one boy receiving 2000 IU/day had serum 25(OH)D levels less than 16 ng/mL at entry level and remained below 20 ng/mL after 12 months of supplementation. They were all healthy subjects, and none had concomitant hypercalcemia or evidence for compliance problems or malabsorption.⁽²¹⁾

Predictors for response to vitamin D₃ supplementation at 1 year

Unadjusted analyses

Serum 25(OH)D at 1 year was correlated with baseline 25(OH)D levels in the overall group ($r = 0.27$, $p < 0.001$) and by sex ($r = 0.38$, $p < 0.001$ in boys, $r = 0.23$, $p = 0.003$ in girls). It was also inversely correlated with average BMI ($r = -0.15$, $p = 0.007$) in the overall group and in boys ($r = -0.22$, $p = 0.005$) but not girls. There was no significant correlation between serum 25(OH)D at

1 year and either baseline or average calcium intake, sun exposure, or physical activity. The likelihood of achieving a 25 (OH)D level at or above 20 or 30 ng/mL at 1 year was largely dependent on the baseline vitamin D levels (odds ratio [OR] = 1.13 [1.09–1.18], $p < 0.001$, and [OR] = 1.07 [1.04–1.11], $p < 0.001$, respectively).

Subgroup analyses revealed no effect of pubertal maturation on the proportion of subjects reaching serum 25(OH)D at or above the two predefined cut-offs, by dose of vitamin D₃, within each sex (Supplemental Table S3), except for a trend for higher proportions reaching 25(OH)D at or above 30 ng/mL in early pubertal boys on the high dose (Supplemental Table S3). Furthermore, boys tended to have a higher likelihood of reaching 25(OH)D levels at or above 30 ng/mL than girls, a difference that was largely driven by a sex difference that was apparent in early but not late puberty (Supplemental Table S4).

Multivariate adjusted analyses

Multivariate linear regression analyses revealed that baseline 25 (OH)D, puberty, vitamin D₃ dose, and BMI were significant predictors of serum 25(OH)D level at 1 year, adjusting for all other covariates, including average sun exposure and sex, which were not significant, with R² of 0.39. The proportion of variance in serum 25(OH)D at 12 months associated uniquely by each predictor was as follows: 23.5% for high-dose vitamin D₃ used, 6.5% for baseline 25(OH)D, 2.0% for BMI, 1.4% for puberty, and 0.3% for low-dose vitamin D₃.

Multivariate logistic regression analyses revealed that vitamin D treatment at both doses, baseline 25(OH)D level, pubertal status, BMI, and sun exposure were all significant independent predictors for subjects reaching 25(OH)D level ≥20 ng/mL at 1 year, adjusting for all other covariates (Table 3). The highest odds ratio estimates were for the high-dose vitamin D₃ used,

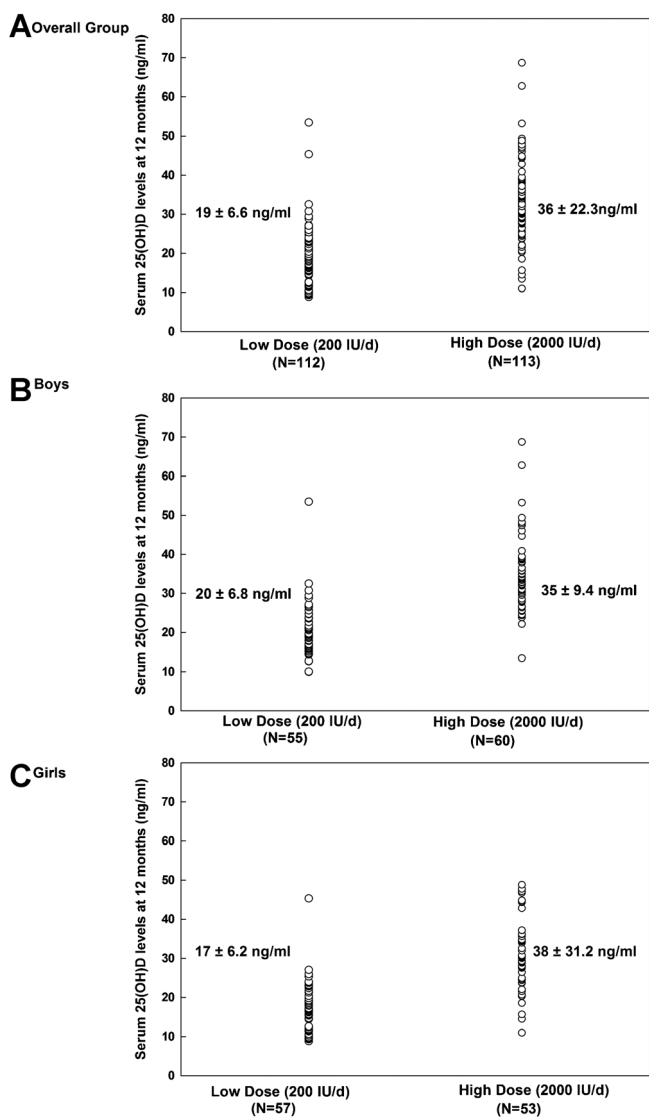


Fig. 1. Scatter plot of serum 25(OH)D at 1 year in the low- and high-dose treatment groups, in the overall group, and by sex. Values represent mean \pm standard deviation. Three subjects (not shown) had high values of serum 25(OH)D levels at 1 year: 195, 161, and 102 ng/mL.

followed by baseline level (Table 3). Treatment with vitamin D₃ at high dose only, baseline 25(OH)D level, and BMI were the only significant predictors for reaching 25(OH)D level \geq 30 ng/mL at 1 year after adjustment for all other covariates (Table 3). The highest odds ratios were again for high-dose vitamin D₃ and for baseline 25(OH)D.

Discussion

This 1-year randomized placebo-controlled vitamin D trial demonstrates that a daily vitamin D₃ dose of 2000 IU was able to achieve a serum 25(OH)D level \geq 20 ng/mL in more than 96% of adolescents with hypovitaminosis D. Only 1 of 60 boys did not exceed a serum 25(OH)D of 20 ng/mL compared with 4 of 53 girls. In light of the low baseline 25(OH)D levels in the study group and the anticipated increments in serum level of this metabolite in response to supplementation, it is unclear that the

recommended daily vitamin D dose of 600 IU by the IOM and of 600 to 1000 IU by the ES would be sufficient to reach their respective recommended levels in Middle Eastern adolescents with an otherwise similar profile. Nevertheless, our study was not designed with a dose-response curve to assess the validity of the RDA recommendations.

Subclinical hypovitaminosis D is prevalent worldwide. Its treatment may incur beneficial effects on musculoskeletal health⁽³⁾ and thus the need to define doses to reach “desirable” levels using the evidence provided from randomized, dose-ranging, vitamin D trials. The high dose used in this study is more than threefold higher than the RDA recommended dose by the IOM of 600 IU/day. Furthermore, although closer to the upper range of 1600 IU/day recommended by the ES to reach a desirable level, the level reached in the current study with such dose is 20 ng/mL, rather than 30 ng/mL, the recommended desirable level by the ES. Our findings are in part explained by the relatively low mean 25(OH)D level of the group at study entry, but these are nevertheless reflective of circulating levels in the pediatric age groups from several nonwestern populations. Indeed, although the mean 25(OH)D at study entry was lower than the reported 25(OH)D that varied from 26 to 36 ng/mL in more than 2800 adolescents aged 12 to 19 years in the National Health and Nutrition Examination Survey (NHANES) study,⁽²²⁾ it is, however, representative of vitamin D status in adolescents in some subgroups in the United States⁽²³⁾ and in many countries in the Middle East and Asia.^(6,16,17,24–29) Indeed, a recent review, compiling information up to 2012, revealed that 30% to 75% of apparently healthy children and adolescents in the Middle East have serum 25(OH)D levels below 20 ng/mL.⁽⁶⁾ Commonly described risk factors across studies for lower values were female sex, winter season, sun exposure and conservative clothing style, pollution, and lower socioeconomic status or educational level.^(6,7,10,30,31)

The administration of doses of vitamin D₃ several folds above the RDA of 600 IU failed to raise 25(OH)D above the IOM desirable cut-off of 20 ng/mL in children and adults from Asia, India, and China.⁽⁶⁾ Indeed, in the study by Shakinba and colleagues, vitamin D administration to pubertal Iranian girls with two doses of vitamin D 3 months apart, using 50,000 IU (800 IU/day) or 100,000 IU (1600 IU/day), could not raise 25(OH)D above 20 ng/mL in all cases, whether given over a few weeks or a few months.^(32,33) Similarly, in the trial conducted by Ghazi and colleagues in adolescent schoolchildren in Tehran, mean age 16 years and baseline 25(OH)D of 13 ng/mL, randomized to 50,000 IU of vitamin D monthly, mean 25(OH)D levels achieved at 6 months were 19 ng/mL in girls and 29 ng/mL in boys.⁽³⁴⁾ Vitamin D requirements may also vary by race. Dong and colleagues randomized 49 normotensive black boys and girls, aged 16.3 ± 1.4 years, to receive 400 IU/day or 2000 IU/day over 16 weeks and demonstrated that mean 25(OH)D levels rose from 33 nmol/L to 59 nmol/L in the low-dose and to 85 nmol/L in the high-dose group.⁽³⁵⁾ Therefore, the presumption that the RDA of vitamin D needed to reach desirable 25(OH)D level is the same across population and ethnic groups, may not be correct, and is not validated from results in the above studies, nor in the current randomized controlled trial. However, none of the studies conducted in adolescents, including ours, had a detailed dose-response curve, with 600 IU/day as one of the doses used. In the elegant study of Gallagher and colleagues conducted in 163 healthy postmenopausal white women with a 25(OH)D level of 38 nmol/L, randomly assigned to receive placebo or vitamin D₃ 400, 800, 1600, 2400, 3200, 4000, or 4800 IU once daily, the

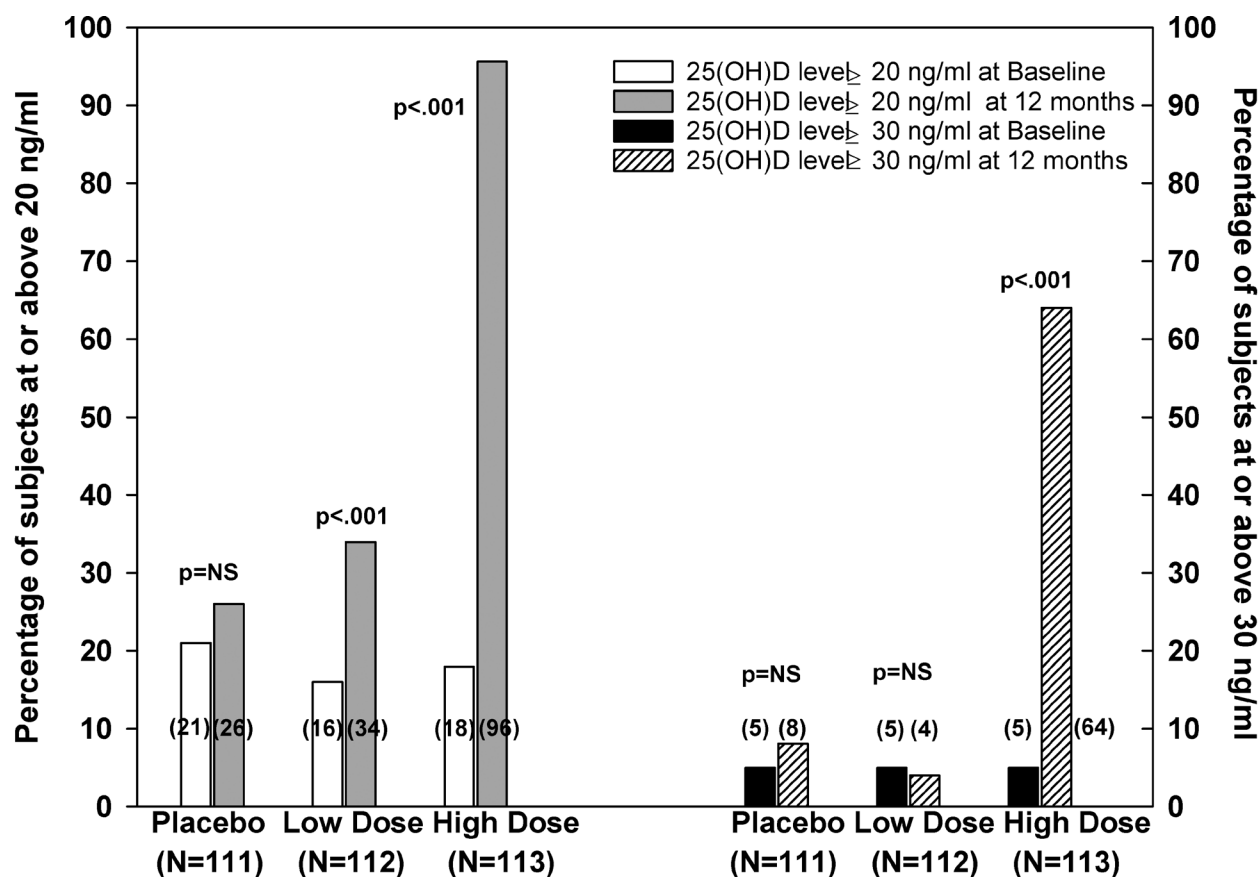


Fig. 2. Proportion of subjects with a serum 25(OH)D at or above 20 ng/mL (left panel) and 30 ng/mL (right panel) by treatment groups at baseline and after 12 months of vitamin D₃ supplementation. Numbers in parentheses represent the actual percentages in each group at baseline and 12 months of vitamin D₃ supplementation. The *p* values represent the significance level using McNemar test. *p* = NS (not significant) represents *p* values > 0.05.

relationship between vitamin D dose used and achieved serum 25 hydroxyvitamin D levels was curvilinear. In this study, the RDA of vitamin D₃ to achieve a 25(OH)D level greater than 50 nmol/L was 800 IU/day.⁽³⁶⁾ Vitamin D dose used, baseline vitamin D, pubertal status, and BMI emerged as significant predictors of serum 25(OH)D levels at 1 year in the current study, although it is important to recognize that the results derived are all exploratory. Zhao and colleagues recently demonstrated that

baseline vitamin D, season, and use of vitamin D supplementation at study entry all predicted vitamin D response variation in a study of 1179 postmenopausal non-Hispanic white women given 1100 IU/day of vitamin D.⁽³⁷⁾ Although the inverse relationship between BMI and serum 25(OH)D is well established and was confirmed in our study in the multivariate analyses, its physiologic basis is unclear.^(38,39) The most accepted explanation is deposition of 25(OH)D, a fat-soluble metabolite, in the adipose

Table 3. Predictors for Reaching 25(OH)D Levels ≥20 ng/mL or 30 ng/mL After 1 Year of Supplementation Using Multiple Logistic Regression Modeling^a

Predictors	25(OH)D levels ≥20 ng/mL		25(OH)D levels ≥30 ng/mL	
	Odds ratio (95% CI)	<i>p</i> Value	Odds ratio (95% CI)	<i>p</i> Value
Baseline 25(OH)D ≥20 ng/mL	24.2 (9.3–63.5)	<0.001	10.1 (2.5–41.6) ^b	0.001
Sex (female versus male)	0.5 (0.2–1.1)	0.07	0.5 (0.2–1.1)	0.08
Postpuberty stage (versus prepuberty)	2.3 (1.0–5.1)	0.045	1.5 (0.7–3.3)	0.34
Treatment group (versus placebo)	—	<0.001	—	<0.001
Low dose (200 IU/d)	2.4 (1.1–5.0)	0.03	0.34 (0.1–1.3)	0.12
High dose (2000 IU/d)	245.8 (71.4–846.7)	<0.001	32.7 (13–2–81.0)	<0.001
Average BMI (kg/m ²)	0.8 (0.8–0.9)	<0.001	0.8 (0.7–0.9)	<0.001
Average sun exposure (min/week)	1.002 (1.000–1.003)	0.033	1.001 (0.99–1.003)	0.21

^aOR adjusted for all covariates listed in the table.

^bPredictor is baseline 25(OH)D ≥30 ng/mL.

tissue. Subgroup analyses suggested sex differences in the effect on achieved 25(OH)D levels at 1 year, boys reaching higher level than girls, in the low-dose groups. These differences could be in part explained by other confounders such as sun exposure, BMI, and puberty, and indeed were no more significant in the adjusted analyses. Calcitriol is a significant correlate of bone mass accumulation during pubertal growth, presumably in anticipation of the high requirements for calcium, during this critical phase of skeletal development. Calcitriol was shown to be highest in a cross-sectional study of 170 adolescent girls during Tanner stages III–IV, aged 11 to 13 years,⁽⁴⁰⁾ but we are unaware of studies examining differences in 25(OH)D metabolism by pubertal stage. In the current study, the proportions of subjects reaching 25(OH)D levels ≥ 30 ng/mL, at the high dose of vitamin D, tended to differ by pubertal stages, albeit with opposing trend in boys and girls. The proportion of subjects reaching a 25(OH)D ≥ 30 ng/mL was significantly higher in boys than girls, in early puberty, at the high dose. The effect of puberty on the proportions of children reaching a 25(OH)D ≥ 20 ng/mL puberty was significant in the analyses adjusting for sex, BMI, vitamin D dose used, and sun exposure. Putman and colleagues demonstrated the lack of a significant increase in 25(OH)D at 11 weeks in a double-blind randomized clinical trial, conducted in 56 adolescents girls, aged 11 to 19 years, with baseline vitamin D sufficiency using daily doses of 200 IU or 1000 IU.⁽⁴¹⁾ We believe this may in part reflect a modulatory effect of puberty on vitamin D metabolism through IGF1 or sex steroids, although these hormones were not measured. The finding in the current study needs to be explored further because it may have important clinical implications on the possible need for sex-specific recommendations for vitamin D supplementation in adolescents with hypovitaminosis D.

A growing body of evidence supports a genetic basis for low 25(OH)D levels in several populations, based on polymorphisms in enzymes or protein involved in the vitamin D pathways, such as vitamin D receptor (VDR), 1- α -hydroxylase (CYP27B1), 25-hydroxylase (CYP2R1), 24-hydroxylase (CYP24A1), vitamin D binding protein (GC, DBP) and 27- and 25-hydroxylase (CYP27A1) genes.^(42,43) Two new CYP2R1 mutations were recently reported in two adolescent siblings from a Saudi family, presenting with short stature and rickets, and who required an unusually high dose of vitamin D to heal their rickets.⁽⁴⁴⁾ Although we did not assess any of these polymorphisms in subjects in this trial, our group recently demonstrated a significant association for one of the CYP2R1 single-nucleotide polymorphisms, rs10766197, with circulating 25(OH)D level in elderly Lebanese after adjusting for covariates, as has been previously reported in adults.⁽⁴⁵⁾

The study has several limitations. These include the post hoc nature of the analyses, the small sample size in the subgroup analyses needed to evaluate relevant covariates and confounders, the relatively short study duration, and the lack of an assessment of vitamin D intake. The latter would be less relevant considering the randomized nature of the study. Three subjects had serum 25(OH)D levels of 195, 161, and 102 ng/mL. Five subjects (4 girls, 1 boy) in the high-dose group had serum 25(OH)D levels less than 20 ng/mL after 12 months of supplementation. As detailed under Materials and Methods, we did not have any evidence for compliance problems in the study group, although this cannot be totally ruled out. The safety of the doses used, within the 1-year frame, has been detailed in previous publication, and although there was no evidence of vitamin D intoxication, nor of hypercalcemia resulting from vitamin D

replacement,⁽²¹⁾ the study did not assess urinary calcium excretion. The study does, however, have important strengths including the fact that it was conducted in apparently healthy adolescents with hypovitaminosis D, a condition that is prevalent worldwide, and that the analyses are based on data provided in a double-blind randomized placebo-controlled trial. It provides valuable information, for the first time to the best of our knowledge, on the dose of vitamin D needed to reach desirable levels in light of the recent vitamin D guidelines issued by IOM, ES, and AAP. Information on the vitamin D dose needed to optimize musculoskeletal health in adolescents is relatively limited and more so in the Middle East.⁽³⁾ To the best of our knowledge, the trial from which the current analyses are conducted is the only one in this region.⁽¹⁶⁾

In conclusion, results from this clinical trial clearly demonstrate that a vitamin D dose of 2000 IU/day achieved serum 25(OH)D levels at or above the recommended 25(OH)D of 20 ng/mL in 96% of the study group (98% of boys versus 93% of girls). A full dose-response curve would be best suited to assess the lowest dose needed to achieve such levels in adolescents at risk for vitamin D insufficiency, such as is the case in the Middle East and many countries in Asia.

Disclosures

All authors state that they have no conflicts of interest.

Acknowledgments

The study was supported by an educational grant from Nestle Foundation and by a grant from Merck KGaA. The authors thank the administrators, school nurses, parents, and students from the American Community School, the International College, Amlieh School, and Ashbal Al Sahel School for their support in making the study possible. The authors thank Mrs U Usta-Chanouha for her assistance in preparing the vitamin D solutions and implementing the randomization protocol, Mrs C Hajj Shahine for her tireless efforts in running the hormonal assays, and Ms Darina Bassil for her assistance in the manuscript preparation.

Authors' roles: Study design: GEHF. Study conduct: GEHF, MN, and JM. Data collection: JM. Data analysis: GEHF and LA. Data interpretation: all authors. Drafting manuscript: GEHF and RM. Revising manuscript content: all authors. Approving final version of manuscript: GEHF. GEHF takes responsibility for the integrity of the data analysis.

References

- Holick MF. Vitamin D deficiency. *N Engl J Med.* 2007;357:266–81.
- Lips P, Van Schoor NM. The effect of vitamin D on bone and osteoporosis. *Best Pract Res Clin Endocrinol Metab.* 2011;25(4):585–91.
- 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.
- Kimball S, El-Hajj Fuleihan G, Vieth R. Vitamin D: a growing perspective. *Crit Rev Clin Lab Sci.* 2008;45(4):339–415.
- El-Hajj Fuleihan G, Rahme M, Bassil D. Do desirable vitamin D levels vary globally?. In: Burckhardt P, Dawson-Hughes B, Weaver C, editors. *Nutritional influences on bone health 8th International Symposium.*: 1st ed. New York: Springer Science+Business Media; p. 273–99; 2013.
- Bassil D, Rahme M, Hoteit M, El-Hajj Fuleihan G. Hypovitaminosis D in the Middle East and North Africa: prevalence, risk factors, and impact on outcomes. *Dermato-Endocrinology.* 2013;5(2):274–98.

7. Arabi A, El Rassi R, El-Hajj Fuleihan G. Hypovitaminosis D in developing countries—prevalence, risk factors and outcomes. *Nat Rev Endocrinol*. 2010;6(10):550–61.
8. El-Hajj Fuleihan G. Vitamin D deficiency in the Middle East and its health consequences. *Clin Rev Bone Miner Metab*. 2009;7:77–93.
9. Brouwer-Brolsma EM, Bischoff-Ferrari HA, Bouillon R, et al. Vitamin D: do we get enough? A discussion between vitamin D experts in order to make a step towards the harmonisation of dietary reference intakes for vitamin D across Europe. *Osteoporos Int*. 2013;24(5):1567–77.
10. Wahl DA, Cooper C, Ebeling PR, et al. A global representation of vitamin D status in healthy populations. *Arch Osteoporos*. 2012;7(1–2):155–72.
11. Lerch C, Meissner T. Interventions for the prevention of nutritional rickets in term born children. *Cochrane Database Syst Rev*. 2007;4:CD006164.
12. Ross C, Manson J, Abrams S, et al. The 2011 Report on Dietary Reference Intakes for Calcium and Vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab*. 2011;96(1):53–8.
13. Holick M, Binkley N, Bischoff-Ferrari H, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*. 2011;96(7):1911–30.
14. Wagner C, Greer F. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics*. 2008;122:1142.
15. Abrams S. Dietary guidelines for calcium and vitamin D: a new era. *Pediatrics*. 2011;127:566.
16. El-Hajj Fuleihan G, Nabulsi M, Tamim H, et al. Effect of vitamin D replacement on musculoskeletal parameters in healthy school children: a randomized controlled trial. *J Clin Endocrinol Metab*. 2006;91:405–12.
17. El-Hajj Fuleihan G, Vieth R. Vitamin D insufficiency and musculoskeletal health in children and adolescents. *Int Cong Ser*. 2007;1297:91–108.
18. 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.
19. Tanner JM. Physical growth and development. In: Forfar JO, Arneil GC, editors. *Textbook of paediatrics*. 2nd ed. Vol. 1. Edinburgh: Churchill Livingstone; p. 249–303. 1978.
20. Salamoun MM, Kizirian AS, Tannous RI, et al. Low calcium and vitamin D intake in healthy children and adolescents and their correlates. *Eur J Clin Nutr*. 2005;59(2):177–84.
21. Maalouf J, Nabulsi M, Vieth R, et al. Short term and long term safety of vitamin D₃ supplementation in school children. *J Clin Endocrinol Metab*. 2008;93(7):2693–701.
22. Looker AC, Dawson-Hughes B, Calvo MS, Gunter EW, Sahyoun NR. Serum 25-hydroxyvitamin D status of adolescents and adults in two seasonal subpopulations from NHANES III. *Bone*. 2002;30(5):771–7.
23. Gordon CM, DePeter KC, Feldman HA, Grace E, Emans SJ. Prevalence of vitamin D deficiency among healthy adolescents. *Arch Pediatr Adolesc Med*. 2004;158(6):531–7.
24. El-Hajj Fuleihan G, Nabulsi M, et al. Hypovitaminosis D in healthy schoolchildren. *Pediatrics*. 2001;107(4):E53.
25. Abdul-Razzak K, Ajlony M, Khourshed A, Obeidat B. Vitamin D deficiency among healthy infants and toddlers: a prospective study from Irbid. *Jordan. Pediatr Int*. 2011;53:839–45.
26. Bener A, Al-Ali M, Hoffmann G. Vitamin D deficiency in healthy children in a sunny country: associated factors. *Int J Food Sci Nutr*. 2009;60(Suppl 5):60–70.
27. Strand MA, Perry J, Jin M, et al. Diagnosis of rickets and reassessment of prevalence among rural children in Northern China. *Pediatr Int*. 2007;49:202–9.
28. Sachan A, Gupta R, Das V, Agarwal A, Awasthi PK, Bhatia V. High prevalence of vitamin D deficiency among pregnant women and their newborns in northern India. *Am J Clin Nutr*. 2005;81:1060–4.
29. Marwaha RK, Tandon N, Reddy DR, et al. Vitamin D and bone mineral density status of healthy schoolchildren in Northern India. *Am J Clin Nutr*. 2005;82:477–82.
30. Mithal A, Wahl DA, Bonjour JP, et al. Global vitamin D status and determinants of hypovitaminosis D. *Osteoporos Int*. 2009;20(11):1807–20.
31. Nesby-O'Dell S, Scanlon KS, Cogswell ME, et al. Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988–1994. *Am J Clin Nutr*. 2002;76(1):187–92.
32. Shakiba M, Ghadir M, Nafei Z, Akhavan Karbasi S, Lotfi MH, Shajari A. Study to evaluate two dosage regimens of vitamin D through an academic year in middle school girls: a randomized trial. *Acta Med Iran*. 2011;49(12):780–3.
33. Shakinba M, Tefagh S, Nafei Z. The optimal dose of vitamin D in growing girls during academic years: a randomized trial. *Turk J Med Sci*. 2011;41(1):33–7.
34. Ghazi A, Hosseinpanah F, M Ardakani E, Ghazi S, Hedayati M, Azizi F. Effects of different doses of oral cholecalciferol on serum 25(OH)D, PTH, calcium and bone markers during fall and winter in school-children. *Eur J Clin Nutr*. 2010;64(12):1415–22.
35. Dong Y, Stallmann-Jorgensen IS, Pollock NK, et al. A 16-week randomized clinical trial of 2000 international units daily vitamin D(3) supplementation in black youth: 25-hydroxyvitamin D, adiposity, and arterial stiffness. *J Clin Endocrinol Metab*. 2010;95(10):4584–91.
36. Gallagher JC, Sai A, Templin T 2nd, Smith L. Dose response to vitamin D supplementation in postmenopausal women: a randomized trial. *Ann Intern Med*. 2012;156(6):425–37.
37. Zhao LJ, Zhou Y, Bu F, et al. Factors predicting vitamin D response variation in non-Hispanic white postmenopausal women. *J Clin Endocrinol Metab*. 2012;97(8):2699–705.
38. Bell NH, Epstein S, Greene A, Shary J, Oexmann MJ, Shaw S. Evidence for alteration of the vitamin D-endocrine system in obese subjects. *J Clin Invest*. 1985;76(1):370–3.
39. Gordon CM. Vitamin D deficiency in adolescents: what can obesity teach us? *J Adolesc Health*. 2011;48(5):427–8.
40. Illich JZ, Badenhop NE, Jelic T, Clairmont AC, Nagode LA, Matkovic V. Calcitriol and bone mass accumulation in females during puberty. *Calcif Tissue Int*. 1997;61:104–9.
41. Putman MS, Pitts SA, Milliren CE, Feldman HA, Reinold K, Gordon CMJ. A randomized clinical trial of vitamin d supplementation in healthy adolescents. *Adolesc Health*. 2013;52(5):592–8.
42. Shea MK, Benjamin EJ, Dupuis J, et al. Genetic and non-genetic correlates of vitamins K and D. *Eur J Clin Nutr*. 2009;63:458–64.
43. Wang T, Zhang F, Richards J, Kestenbaum B, van Meurs J, Berry D. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. *Lancet*. 2010;376(9736):180–8.
44. Al Mutair A, Nasrat G, Russell D. Mutation of the CYP2R1 vitamin D 25-hydroxylase in a Saudi Arabian family with severe vitamin D deficiency. *J Clin Endocrinol Metab*. 2012;97:E2022–5.
45. Zgheib NK, Arabi A, Mahfouz R, El-hajj Fuleihan G. CYP2R1 genetic polymorphisms are associated with lower 25-hydroxy vitamin D levels in Lebanese subjects [abstract]. Presented at: 11th Annual Meeting of the European Association for Clinical Pharmacology and Therapeutics; Geneva, Switzerland; 2013 Aug 28–31.