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# Hypovitaminosis D in Healthy Schoolchildren

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**ABSTRACT.** *Background.* Vitamin D is essential for skeletal growth, but there are currently no guidelines for vitamin D supplementation after infancy. This study investigates vitamin D insufficiency in healthy children.

*Methods.* Children ages 10 to 16 years from 3 private schools in Beirut, Lebanon, with differing socioeconomic status (SES) were studied: 169 in the spring of 1999 and 177 in the following fall; 83 students participated in both study phases. They had a physical examination, answered a dietary questionnaire, and blood was drawn for calcitropic hormones and indices of bone turnover.

*Results.* Overall, 52% of the students were vitamin D-insufficient; the proportion of insufficiency was 65% in the winter and 40% at the end of the summer. During both seasons, girls had lower vitamin D levels than did boys; those who followed the dress code of covered head, arms, and legs had the lowest levels. Students in the mid-SES school had lower 25-hydroxyvitamin D (25-OHD) levels than did the ones from the high-SES school. After adjusting for confounders, gender, SES, and body mass index remained the significant predictors of vitamin D levels in both seasons ( $R^2 = 0.53$ , for spring and 0.28 for fall). There was a significant inverse correlation between 25-OHD levels and parathyroid hormone levels that was best fitted by a curvilinear model ( $R^2 = 0.19$ ).

*Conclusion.* Even in a sunny country, hypovitaminosis D is common in schoolchildren, more so in the winter. Girls, especially those with a lower SES, are at particular risk. The inverse changes in parathyroid hormone suggest that insufficient vitamin D levels may deleteriously affect skeletal metabolism in healthy adolescents. Vitamin D insufficiency may be prevalent in many other countries where supplementation of milk with vitamin D is not mandatory. Our results call to a reconsideration of vitamin D supplementation in high-risk adolescents to further optimize skeletal health. *Pediatrics* 2001;107(4). URL: <http://www.pediatrics.org/cgi/content/full/107/4/e53>; *vitamin D insufficiency, bone metabolism, nutrition, gender, socioeconomic status.*

ABBREVIATIONS. 25-OHD, 25-hydroxyvitamin D; SES, socioeconomic status; PTH, parathyroid hormone; BMI, body mass index.

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Vitamin D, the precursor of the active metabolite calcitriol, 1,25(OH)<sub>2</sub> vitamin D, is a steroid hormone that is essential for bone growth and development in children and skeletal health in adults.<sup>1</sup> Although nutritional rickets caused by severe vitamin D deficiency is almost eradicated from developed countries, it is still ranked among the 5 most prevalent diseases in developing countries.<sup>2</sup> It is recognized that less severe vitamin D deficiency (otherwise called insufficiency) has a deleterious effect on skeletal health in adults and elderly<sup>3-6</sup>; however, its impact on bone metabolism in children and adolescents is less clear. The consensus has been that most adolescents should be able to synthesize sufficient vitamin D by brief exposure to sunlight and that only children living in extreme northern or southern latitudes may require supplementation with D<sup>7,8</sup>; examination of the recent evidence leads to different conclusions. Indeed, 80% of children and adolescents had insufficient vitamin D levels (25-hydroxyvitamin D [25-OHD] levels <20 ng/mL) in the winter in 4 studies conducted in Spain, France, and Finland.<sup>9-12</sup> Although it is expected that children of different genders and socioeconomic background would have different lifestyles (exercise, sunlight exposure, and nutrition), the few studies evaluating vitamin D levels in children and adolescents have mostly done so in boys and have not systematically examined the impact of socioeconomic status (SES) on vitamin D levels. An increasing body of evidence confirms a key role of calcium in bone mass accretion in children and adolescents<sup>13-15</sup>; however, the impact of vitamin D insufficiency and, therefore, repletion on skeletal integrity in this age group is yet to be determined.

The primary objective of the current study was to test the hypothesis that vitamin D insufficiency is prevalent in healthy school adolescents. The secondary objectives were:

1. To establish that such insufficiency is more common in girls than in boys and in schools of lower SES.
2. To determine the impact of low vitamin D levels on biochemical indices of bone remodeling during a critical period for bone mass accretion.<sup>16,17</sup>

## METHODS

### Participants and Protocol

Three private schools in Beirut, Lebanon were targeted for participant selection: 2 because their students received their medical care at our institution and 1 through personal contact. Two schools were categorized as high SES, the students' yearly fees for

the age group studied were \$5000 to \$7000, and the third one was categorized as medium SES with yearly school fees of \$1500. The age group chosen was 10 to 16 years, because this is a critical period for skeletal mass accretion.<sup>16,17</sup> Excluded were children with any medical conditions or on any medications known to affect skeletal metabolism. To evaluate the impact of seasonal variations on vitamin D levels, even at a latitude of 33.5°N, the study was implemented in 2 periods: in the spring (March to April 1999) and the second, which was originally planned for the end of summer (October), was delayed because of logistic reasons and ultimately took place in the following fall (November to December 1999).

All schoolchildren ages 10 to 16 years from the 3 schools were offered to participate: 169 agreed to the study in the spring and 177 in the following fall; 83 participants participated in both phases. In spring, all participants had their height and weight measured and answered a dietary questionnaire based on 7-day food frequency questionnaire. Height and weight percentiles were derived using growth curves published by the US National Center for Health Statistics<sup>18</sup> because national standards are not available. Blood was drawn for serum vitamin D and parathyroid hormone (PTH) levels before noon. During the fall, the 7-day food frequency questionnaire was more detailed covering intake of calcium from dairy and vitamin D intake. The student's Tanner stage was determined through careful breast and genital examination. Blood was drawn for serum 25-OHD, PTH, osteocalcin, bone-specific alkaline phosphatase, and C-telopeptide cross-links levels. The protocol was approved by the institutional review board at the American University of Beirut and written informed consent was obtained from all study participants and/or their parents/legal guardians. It was obtained twice for those who participated in both study phases.

### Hormonal Assays

Serum 25-OHD was measured by a competitive protein-binding assay using the Diasorin Incstar kit (Diasorin, Saluggia, Italy). For 25-OHD, the manufacturer's normal range is 9 to 47 ng/L, the lower limit is 5 ng/mL, the intraassay coefficient of variation is <11%, and the interassay coefficient of variation is <13% at a serum concentration of 47 ng/mL.

Serum intact PTH level were measured with ELSA-PTH immunoradiometric assay (Cis Bio International, Gif-Sur-Yvette, Cedex, France). The manufacturer's normal range is 8 to 76 pg/mL, the detection limit of the assay is 0.7 pg/mL, and the intraassay and interassay coefficients of variation are below 7% at PTH concentrations 6 to 95 pg/mL.

The serum osteocalcin levels were determined with an immunoradiometric sandwich assay (ELSA-Osteo kit, CIS Bio International, Gif-Sur-Yvette, Cedex, France). Serum C-telopeptide cross-links were measured using an enzyme-linked immunosorbent assay (Cross-Laps Immuno-Biological Laboratories, Hamburg, Germany). Serum bone-specific alkaline phosphatase levels were measured with an enzyme immunoassay (Alkphase-B, Metra Biosystems, Mountain View, CA).

### Definition of Vitamin D Deficiency and Insufficiency

Based on the suggested cutoffs for adults, vitamin D deficiency was defined as a 25-OHD <10 ng/mL, and insufficiency as a 25-OHD between 10 and 20 ng/mL.<sup>19</sup>

### Statistical Methods

Results are expressed as mean  $\pm$  standard deviation unless mentioned otherwise. Comparison of continuous variables between various subgroups of participants was performed using a 2-tailed *t* test. The relationship between 25-OHD and continuous variables (age, body mass index [BMI], sun exposure, calcium intake, vitamin D intake, and Tanner stage) was evaluated using a Pearson correlation coefficient. A linear multiple-regression model was used to determine the predictors for 25-OHD levels in the spring and fall. Curves relating serum 25-OHD and PTH levels were fitted using linear and nonlinear regression models using GraphPad Prism (GraphPad Prism Software, Version 1.02, GraphPad, San Diego, CA). The analyses were performed using SPSS Software, Version 9.0 (SPSS, Chicago, IL).

Significance was at a *P* < .05. *P* values were unadjusted for multiple testing.

## RESULTS

### Clinical Characteristics of the Study Groups

A total of 169 white students were studied in March through April 1999 (spring), with nearly equal numbers from both genders. As shown in Table 1, the mean age of the students was 13.3 years, they were at the 50th percentile for height, and slightly above that for weight (Table 1). In the subsequent fall, 177 students were studied with very similar demographics. In both phases of the study, there were no differences in age, height percentile, and weight percentile between the 2 genders; however, as expected, fractures were slightly more prevalent in boys than in girls (19.8% vs 11.4%, respectively, in spring; *P* = not significant), and these proportions were 25.3% and 12.8% in fall (*P* = .052). For the 83 participants who participated in both phases of the study, there were no differences between them and the participants studied either in spring or in fall in any of the characteristics outlined in Table 1, except for slight differences in age (<1 year; data not shown). Participants involved in both study phases account for the increase in the proportion of participants taking vitamin and/or calcium supplements in the fall (Table 1). Vitamin D intake in Table 2 represents total intake from diet (exported powdered milk contains 100 IU of vitamin D/250 mL/glass) and supplements when applicable.

### Main Predictors of Vitamin D Status

#### Gender Effect on 25-hydroxy Vitamin D Level

Girls had lower 25-OHD levels than did boys (Table 2). They also had less sun exposure and calcium intake from dairy products in both study phases. There were no gender differences in vitamin D intake in spring (Table 2). The proportion of children who were vitamin D-deficient was higher in girls than in boys during both seasons (32% vs 9% in the spring and 7.5% vs 0% in the fall in girls and boys, respectively). Similarly, the proportions of children with insufficiency were 42% versus 46% in the spring and 46% and 25.3% in the fall in girls and boys, respectively.

**TABLE 1.** Characteristics of the Study Groups During Both Phases of the Study

Variable	Spring	Fall
Time	March to April 1999	November to December 1999
<i>n</i>	169	177
Boys/girls	81/88	83/94
Age (y)	13.3 $\pm$ 1.6	13.3 $\pm$ 1.7
Height (percentile)	50 $\pm$ 28	51 $\pm$ 27
Weight (percentile)	56 $\pm$ 28	53 $\pm$ 29
SES: M/H	81/88	85/92
Fracture prevalence (%)	15.4	18.6
Proportion on vitamins	1.2%	7.9%
Proportion on calcium tablets	2.4%	10.2%
Tanner stage ( <i>n</i> )		
I-II	NA	68
III-IV	NA	59
V	NA	43
NA		7

M indicates middle; H, high; NA, not available.

**TABLE 2.** Vitamin D Levels, Sun Exposure, and Dietary Calcium and Vitamin D Intake in Both Study Phases

Variable	Spring			P Value	Fall			P Value
	All	Boys	Girls		All	Boys	Girls	
25-OHD ng/mL	17 ± 8	19 ± 7	15 ± 8	<.001 .006*	22 ± 7	24 ± 62	19 ± 7	<.001 .2*
25-OHD <10 ng/mL	21%	9%	32%		4%	0%	7.5%	
25-OHD 10–20 ng/mL	44%	46%	42%		36%	25%	46%	
Sun min/d	57 ± 48	65 ± 51	48 ± 44	.03	87 ± 62	10 ± 52	75 ± 67	.007
Calcium mg/d	608 ± 543	740 ± 644	487 ± 395	.002	710 ± 382	787 ± 393	645 ± 362	.02
Vitamin D IU/d		Not available			150 ± 159	150 ± 157	150 ± 161	NS

NS indicates not significant.

\* Comparison of proportions between genders by  $\chi^2$  test.

#### Effect of SES and Culture on Serum 25-Hydroxy Vitamin D Levels

There was a clear impact of SES on vitamin D levels during both study phases. Students attending schools of higher SES had higher vitamin D levels than did those attending the schools of middle SES (Table 3). This effect of SES on vitamin D levels was present in both genders in the spring (Table 3) and only in girls in the fall (Table 3).

Overall, in both phases students in the middle SES school had less sun exposure than did those attending the higher SES school, the mean levels were  $34 \pm 34$  minutes/day and  $79 \pm 51$  minutes/day, respectively, in spring and  $63 \pm 58$  minutes/day and  $107 \pm 58$  minutes/day in fall, respectively ( $P < .001$  in both phases). The interaction among gender, SES, and season was further examined as detailed in Table 4. Boys had higher vitamin D levels than did girls, regardless of SES and season.

The effect of dress code on vitamin D levels was closely examined in the fall. A subgroup of girls from the middle SES school followed a dress code of covered head, arms, and legs. They had a 25-OHD level of  $12 \pm 5$  ng/mL compared with  $18 \pm 6$  ng/mL in girls from the same school with the usual dress code ( $P < .001$ ) and a mean level of  $22 \pm 6$  ng/mL in the students from the higher SES school ( $P = .004$ ).

#### Impact of Season on Vitamin D Levels

As shown in Table 2, overall for all study participants and within each gender the mean 25-OHD level was 4 to 5 ng/dL higher in the fall than in the spring. This seasonal effect is further demonstrated in the 83 participants who participated in both studies, both in girls and boys (Fig 1, A and B). The seasonal difference in 25-OHD level was most accen-

**TABLE 3.** Effect of SES on Vitamin D Levels by Gender

	25-OHD Levels in Spring (ng/mL)		
	H-SES	M-SES	P Value
Overall	21.4 ± 6.5	11.8 ± 6.1	<.001
Girls	19.5 ± 6.3	8.8 ± 4.6	<.001
Boys	23.6 ± 6.0	14.6 ± 6.1	<.001
	25-OHD Levels in Fall (ng/mL)		
	H-SES	M-SES	P Value
Overall	23.0 ± 5.7	19.9 ± 7.9	.003
Girls	21.9 ± 5.9	15.7 ± 6.1	<.001
Boys	24.4 ± 5.2	24.2 ± 7.1	.9

H-SES indicates high-socioeconomic status; M-SES, middle-socioeconomic status.

**TABLE 4.** Effect of Gender on Vitamin D Level by SES

	25-OHD Levels in Spring (ng/mL)		
	Boys	Girls	P Value
Overall	19.1 ± 7.5	14.7 ± 7.7	<.001
H-SES	23.6 ± 6.0	19.5 ± 6.3	.003
M-SES	14.6 ± 6.1	8.8 ± 4.6	<.001
	25-OHD Levels in Fall (ng/mL)		
	Boys	Girls	P Value
Overall	24.3 ± 6.2	19.0 ± 6.7	<.001
H-SES	24.4 ± 5.2	21.9 ± 5.8	.03
M-SES	24.2 ± 7.1	15.7 ± 6.1	<.001

H-SES indicates high-socioeconomic status; M-SES, middle-socioeconomic status.

tuated in students attending the school of middle SES but was also present in the students from the higher SES school.

#### Other Predictors of Vitamin D Levels

For each study phase the following variables were also evaluated as possible predictors of 25-OHD levels when available: age, Tanner staging, sun exposure, calcium intake, vitamin D intake, and BMI. In spring, minutes of sun exposure were positively correlated with 25-OHD levels ( $R = 0.305$ ;  $P < .001$ ); in fall, both minutes of sun exposure and vitamin D intake were positively correlated with 25-OHD levels ( $R = 0.27$  and  $R = 0.23$ , respectively;  $P < .003$ ). Tanner stage was negatively correlated with 25-OHD ( $R = -0.22$ ;  $P = .004$ ).

#### Predictors of Vitamin D Levels Adjusted for Confounders

##### Spring

Although the significant predictors for vitamin D levels on bivariate analysis were only gender, SES, and sun exposure; age, BMI, and calcium intake were also entered in a multiple linear regression model with vitamin D as the outcome variable. The significant predictors were BMI, gender, and SES, with an  $R^2 = 0.53$  ( $P = <.001$ ).

##### Fall

Similarly, although the significant predictors for vitamin D levels on bivariate analysis were only gender, SES, Tanner staging, vitamin D intake, and sun exposure, in addition we entered age, BMI, and calcium intake in a multiple linear regression model with vitamin D as the outcome variable. Again, the

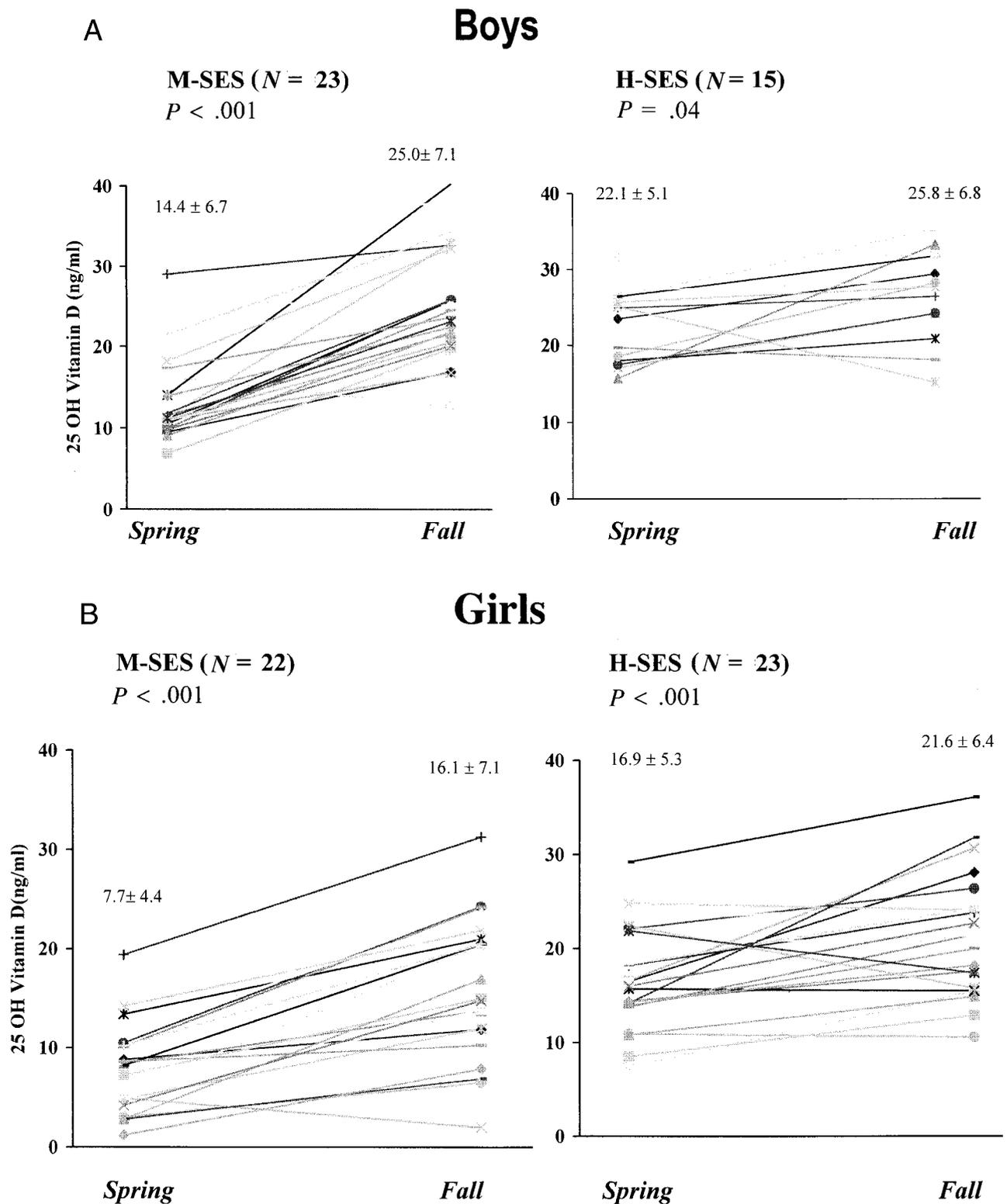


Fig 1. Seasonal variations in 25-OHD levels of those who participated in both study phases by SES in the boys (A) or girls (B).

significant predictors were BMI, gender, and school, with an  $R^2 = 0.28$  ( $P < .001$ ).

When we merged the data from both study phases and applied the same multiple-regression model, the predictors were gender, SES, and BMI and season, with an  $R^2 = 0.43$  ( $P < .001$ ).

#### Parathormone, Osteocalcin, Serum Bone-Specific Alkaline Phosphatase, and Cross-Laps

The physiologic impact of insufficient vitamin D levels was reflected in the inverse relationship between vitamin D levels and PTH levels ( $R = -0.35$  in spring,  $R = -0.39$  in fall;  $P < .001$ ;  $R^2 = 0.12-0.15$  for

the linear model). However, the data were better fitted with a curvilinear model ( $R^2 = 0.19$ ; Fig 2).

The correlation between serum 25-OHD and PTH levels remained significant even after correcting for age, gender, calcium intake, and Tanner staging.

The impact of vitamin D insufficiency on skeletal remodeling was further evaluated by measuring the following indices of bone remodeling: serum osteocalcin, serum bone alkaline phosphatase, and C-telopeptide cross-links. There was a significant, albeit weak, positive correlation between 25-OHD and C-telopeptide cross-links levels ( $R = 0.15$ ;  $P = .046$ ). No correlation was found between vitamin D and the indices of bone formation, namely osteocalcin and bone alkaline phosphatase.

## DISCUSSION

A significant proportion of children and adolescents suffered from vitamin D insufficiency. Even in a sunny country, clear seasonal variations were noted. As expected, sun exposure and vitamin D intake were significant predictors of vitamin D levels; however, additional important correlates were gender and SES in addition to BMI, regardless of the season.

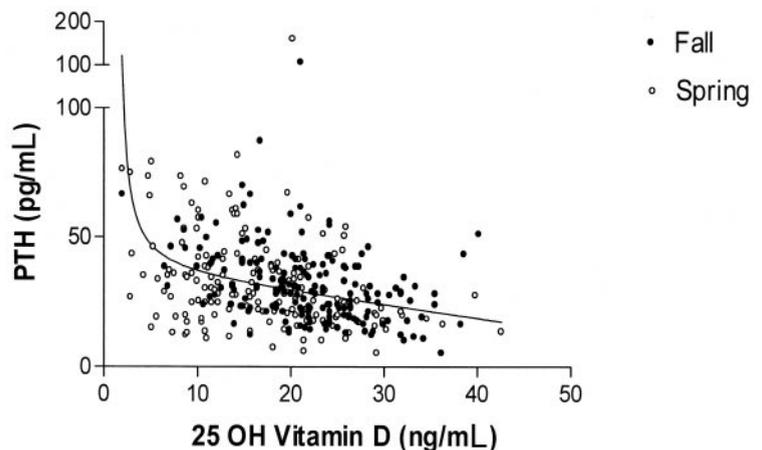
To our knowledge, this is the first study to simultaneously evaluate the impact of gender, season, and SES, as well as other known predictors of vitamin D on serum vitamin D levels. In this study, conducted at a latitude of 33.5°N, the mean 25-OHD level at the end of the winter was 17 ng/mL, a finding consistent with the gradual decrease noted in other studies conducted at more northern latitudes: it was 15.4 ng/mL in children from Northern Spain (43.5°N),<sup>9</sup> 13.5 ng/mL in adolescents Finnish girls,<sup>12</sup> and 8.2 ng/mL in male adolescents from Northern Paris (49°N).<sup>11</sup> In Lebanon, similar to other Middle Eastern and some European countries, there are no governmental regulations mandating vitamin D supplementation of milk. The main source of vitamin D is, therefore, through skin synthesis in response to sun exposure. The impact of seasonal variations on the cutaneous production of vitamin D was systematically evaluated at different latitudes.<sup>20</sup> In Los Angeles (34°N), it continued throughout the year with as little as 1 hour of sun exposure.<sup>20</sup> The question then

arises: why were the vitamin D levels low in adolescents in this study during the winter? The mean sun exposure duration was ~1 hour in the winter, in contrast to 90 minutes in the summer, with wide intragender and intergender variations. The impact of sun exposure is further underscored in the observation that girls from the middle SES school who followed the dress code of covered head, arms, and legs had the lowest vitamin D levels of all study participants.

Prevalent low vitamin D levels have also been described in adults in Lebanon and Saudi Arabia by our group and others, findings that can be partially explained by low sun exposure, lack of food supplementation with vitamin D, and, in some instances, the cultural habits of the groups studied.<sup>21-23</sup>

Despite the well-recognized devastating consequences of vitamin D deficiency on the skeleton, namely rickets in children and osteomalacia in adults, the impact of more subtle deficiencies has only been recently recognized, mostly in adults and the elderly. Indeed, whereas vitamin D insufficiency leads to secondary hyperparathyroidism, bone loss, and a higher risk for fractures,<sup>3-5</sup> vitamin D repletion along with calcium decreases fractures.<sup>24,25</sup> The data in children are much more scarce. However, an increasing body of evidence suggests that insufficient vitamin D levels may be equally deleterious to skeletal health in children and adolescents. Two consecutive studies have demonstrated inverse changes between 25-OHD levels and PTH levels in French male adolescents.<sup>10,11</sup> During childhood and adolescence, reduced remodeling is associated with increments in bone mineral density.<sup>26</sup> High levels of bone remodeling, such as may result from high PTH levels, may be, therefore, deleterious to bone mass accretion. Recently a positive correlation between bone mass and sun exposure was reported in prepubertal children, further supporting an important role of replete vitamin D levels in skeletal health.<sup>27</sup> To our knowledge, the only study demonstrating a positive correlation between vitamin D levels and skeletal mass accretion measured calcitriol levels in adolescent white females.<sup>28</sup> The inverse significant negative correlation between vitamin D levels and PTH suggests a physiologic role of low 25-OHD levels and is similar to

**Fig 2.** Inverse relationship between serum 25-OHD and intact PTH levels during spring (○) and fall (●) of the study in 346 study participants.  $PTH\text{ pg/mL} = 55.98X e^{-0.03(25\text{-OHD ng/mL})}$ ;  $R^2 = 0.19$  for the curvilinear model.



what has been reported not only in adults, but also in adolescents.<sup>6,10,19</sup> In our study and that of French male adolescents, examination of the vitamin D-PTH curve reveals that at 25-OHD levels above 20 and even better 30 ng/dL, very few PTH levels above the upper limit of normal are noted. The lack of a consistent correlation between serum 25-OH D levels and indices of bone remodeling is unexplained. It may be because of the strong confounding effect of puberty on skeletal remodeling in adolescents<sup>29,30</sup> and would be best evaluated in a longitudinal study.

Recent nutritional guidelines targeted to children and adolescents to improve bone health have mainly stressed the importance of calcium and exercise. They have either omitted vitamin D or proposed that vitamin D supplementation is usually not necessary.<sup>7,31,32</sup> Evidence from our study and others<sup>9,10,12</sup> calls for a reconsideration of such strategy. The optimal vitamin D levels in children and adolescent are unclear; however, a paradigm similar to one used in adults is suggested. A vitamin D-replete state can be considered as one that does not produce secondary hyperparathyroidism. Examination of the vitamin D-PTH curve in our study and that of Guillemant et al<sup>10</sup> suggests that similar to adults, 25-OHD levels between the mid-20s and even better 30 ng/mL may be desirable to account for the seasonal decrements in vitamin D levels. A significant proportion of healthy schoolchildren were below these levels. Indeed, 65% in the winter and 40% in the summer had serum 25-OHD levels below 20 ng/mL.

Our participants were not randomly selected because there was bias for higher SES schools. It is to be expected that the findings in a more representative sample with a greater proportion of children from a lower SES may be even more worrisome.

In our study, during the winter a significant proportion of children had vitamin D insufficiency. Concomitant changes in markers of skeletal remodeling suggest that this may have an overall negative impact on peak bone mass accretion. Although the latter conclusion remains speculative at this point, our understanding of bone physiology implies that it is quite plausible. At particular risk were students from middle SES, and girls, especially those from a particular cultural background. The magnitude of the problem may be even larger in participants with a lower SES. We propose that until the advantages of a preventive strategy on bone mass are demonstrated, public health efforts to maintain not only optimal calcium, but also vitamin D intake, at least in high-risk subgroups for the latter may be warranted.

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## Hypovitaminosis D in Healthy Schoolchildren

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