

# Low vitamin D status adversely affects bone health parameters in adolescents<sup>1–3</sup>

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## ABSTRACT

**Background:** The effects of subclinical vitamin D deficiency on bone mineral density (BMD) and bone turnover in adolescents, especially in boys, are unclear.

**Objective:** We aimed to investigate the relations of different stages of vitamin D status and BMD and bone turnover in a representative sample of adolescent boys and girls.

**Design:** BMD was measured by dual-energy X-ray absorptiometry at the nondominant forearm and dominant heel in a random sample of 12- ( $n = 260$ ) and 15-y-old ( $n = 239$ ) boys and 12- ( $n = 266$ ) and 15-y-old ( $n = 250$ ) girls. Serum 25-hydroxyvitamin D, parathyroid hormone, osteocalcin, and type I collagen cross-linked C-telopeptide were assessed by using enzyme-linked immunoassays. Relations between vitamin D status and bone health indexes were assessed by using regression modeling.

**Results:** Using multivariate regression to adjust for potential physical, lifestyle, and dietary confounding factors, we observed that 12- and 15-y-old girls with high vitamin D status ( $\geq 74.1$  nmol/L) had significantly greater forearm (but not heel) BMD ( $\beta = 0.018$ ; SE = 0.008;  $P < 0.05$  for each age group) and lower serum parathyroid hormone concentrations and bone turnover markers than did those with low vitamin D status. These associations were evident in subjects sampled throughout the year and in winter only. There was no significant relation between vitamin D status and BMD in boys.

**Conclusions:** Maintaining serum 25-hydroxyvitamin D concentrations above  $\approx 50$  nmol/L throughout the year may be a cost-effective means of improving bone health. Increased emphasis on exploring strategies for improving vitamin D status in adolescents is needed. *Am J Clin Nutr* 2008;87:1039–44.

## INTRODUCTION

It is well-established that prolonged and severe vitamin D deficiency [represented as serum 25-hydroxyvitamin D (25(OH)D) concentrations  $< 10$ – $25$  nmol/L] leads to rickets in children and osteomalacia in adults (1, 2). In contrast, the effect of subclinical vitamin D deficiency [also referred to as vitamin D insufficiency (3–5)] on skeletal health is less clear.

A number of studies in adolescents have shown a high prevalence of subclinical vitamin D deficiency in Europe (6–12), the United States (13–17), Lebanon (18), New Zealand (19, 20), and Tasmania (21), especially during the winter months. It is well-recognized that, in elderly subjects, low vitamin D status elevates parathyroid hormone (PTH) concentrations, which, in turn, increases bone turnover and bone loss, contributes to mineralization defects, and increases risk of hip and other fractures (22), but

the effects in children and adolescents are unclear. Elevated PTH concentrations may not be driven by the same mechanism in adolescents as in adults, and they may not necessarily be detrimental to bone health. For example, serum PTH concentrations are normally higher during adolescence (23, 24), when the rate of bone remodeling and consolidation is at a peak. Even though the relation between vitamin D status and PTH during adolescence remains unclear, the findings from a limited number of studies in adolescent girls provide evidence of a possible adverse effect of low vitamin D status for bone mineral acquisition and bone remodeling in adolescence (6, 9, 10). Those 3 studies were conducted in winter; therefore, the serum 25(OH)D cutoffs to define low vitamin D status were within a tight range—typically,  $< 50$  nmol/L.

There is a lack of consensus on the serum 25(OH)D concentration that reflects optimal vitamin D status, but it has been suggested that serum 25(OH)D concentrations  $> 80$  nmol/L are needed to plateau PTH concentration (25). However, Viljakainen et al (26) recently showed in Finnish girls that vitamin D supplementation significantly increased bone mineral augmentation of the femur and lumbar spine, and that a serum 25(OH)D concentration  $> 50$  nmol/L was sufficient to promote bone acquisition. Furthermore, whereas there are some data on the effect of low vitamin D status on bone health in girls, no study has been conducted in boys, despite the prevalence of low vitamin D status in this subgroup (7, 8, 14, 20, 27).

Therefore, the main objective of the present study was to investigate the relations among different stages of vitamin D status and bone mineral density (BMD) of the forearm and heel

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in a representative sample ( $n = 1015$ ) of 12- and 15 y-old adolescent boys and girls living at 54–55°N in the United Kingdom, in whom our group (27) recently showed that low vitamin D status is prevalent. In addition, we investigated the relations among vitamin D status, serum PTH, and biochemical markers of bone turnover.

## SUBJECTS AND METHODS

### Design

The Young Hearts 2000 (YH2000) survey is the second in a series of cross-sectional studies examining a representative sample of adolescents from Northern Ireland. Details of subject recruitment and the inclusion and exclusion criteria were reported elsewhere (28–30). Eleven percent, 16%, 16%, 6%, 2%, 7%, 10%, 11%, 11%, and 11% of the group were sampled during January, February, March, April, May, June, September, October, November, and December, respectively. None of the subjects were sampled during July or August because of the summer vacation. Complete records were available for 1015 adolescents who had provided a blood sample and for whom data on pubertal status, anthropometry, BMD, habitual physical activity, and food intakes were also available.

Written informed consent was obtained from the subjects and from each subject's parent or guardian. Ethical approval was obtained from the Research Ethics Committee of the Queen's University of Belfast.

### Anthropometric and lifestyle data

Standing height and body weight was measured as described previously (29). Pubertal status of each subject was assessed by a pediatrician using visual signs such as nongenital secondary hair growth, vocal timbre, body habitus, general muscular development, and (in girls) overall breast development. Lifestyle and physical activity data were obtained from questionnaires, as described previously (29, 31). Dietary data were collected via a nutritionist-administered 7-d diet history method (32).

### Assessment of bone mineral density

BMD of the nondominant forearm (distal radius) and dominant heel (os calcis) was measured by dual-energy X-ray absorptiometry with a Norland Lunar peripheral instantaneous X-ray imager bone densitometer (PIXI; Lunar Corporation, Madison, WI), which has a precision of 0.5%. Before each scan, the densitometer was calibrated by using quasi-anthropomorphic phantoms according to the manufacturer's recommendations. The results of the scan were expressed as BMD (g calcium hydroxyapatite/cm<sup>2</sup>).

### Collection and preparation of samples

Nonfasting blood samples were collected by venipuncture into an evacuated tube with no additive. They were then processed to serum, which was immediately stored at –80 °C until required for analysis.

### Experimental techniques

#### *Serum 25-hydroxyvitamin D*

Concentrations of 25(OH)D were measured in serum samples by using an enzyme-linked immunosorbent assay [(ELISA)

OCTEIA 25-Hydroxy Vitamin D; Immuno Diagnostic Systems, Ltd, Boldon, United Kingdom]. The intraassay and interassay CVs for the ELISA method were 5.9% and 6.6%, respectively. The quality and accuracy of serum 25(OH)D analysis in our laboratory is ensured on an ongoing basis by participation in the Vitamin D External Quality Assessment Scheme (DEQAS) from Charing Cross Hospital (London, United Kingdom). This ELISA is used for the quantitative determination of 25(OH)D. It has 100% cross-reactivity with 25(OH)D<sub>3</sub>, and, whereas 75% cross-reactivity with 25(OH)D<sub>2</sub> was also reported, Carter et al (33) reported that the assay did not underestimate 25(OH)D<sub>2</sub> in the DEQAS samples. A comparison of the performance of our ELISA with that of a commonly used radioimmunoassay in relation to the DEQAS samples shows very good correlation (ELISA = 1.2238 × radioimmunoassay – 5.5514;  $r = 0.96$ ).

#### *Serum intact parathyroid hormone*

Intact PTH concentrations were measured in serum by using an ELISA (MD Biosciences Inc, St Paul, MN). The intraassay and interassay CVs were 3.4% and 3.8%, respectively.

#### *Serum type 1 collagen cross-linked C-telopeptides*

Type 1 collagen cross-linked C-telopeptide (CTx) was measured in the serum samples by using an ELISA (Nordic Bioscience Diagnostics A/S, Herlev, Denmark). The intraassay and interassay CVs were 6% and 5%, respectively.

#### *Serum osteocalcin*

Osteocalcin concentrations were measured in serum samples by using an ELISA (Metra Osteocalcin EIA Kit; Quidel Corporation, San Diego, CA). The intraassay and interassay CVs were 6.0% and 7.6%, respectively.

### Statistical analysis

The statistical analyses were performed by using SPSS for WINDOWS software (version 12; SPSS Inc, Chicago, IL). Data that were not normally distributed were logarithmically [natural log (ln)] transformed before statistical analysis, to achieve near-normal distributions. Tests for independence were used to compare demographic variables such as age grouping, sex, season of sampling, and pubertal status and to compare dietary factors between the subjects included in the current analysis ( $n = 1015$ ) and the complete YH2000 dataset ( $n = 2017$ ). Age × sex interactions for height, weight, forearm BMD, heel BMD, serum 25(OH)D, PTH, osteocalcin, CTx, physical activity, and dietary vitamin D and calcium were examined by using 2-factor analysis of variance. Serum 25(OH)D concentrations were grouped into tertiles. Regression analyses, for each age-sex group, were then undertaken to assess the extent of the association between vitamin D status and BMD and bone turnover marker concentrations. The tertiles of serum 25(OH)D were coded by using a dummy variable-coding scheme, which allowed comparisons to be made between the high-status group and the moderate-status group and between the high-status group and the low-status group. Univariate models were constructed with nondominant forearm BMD or dominant heel BMD as the dependent variable and 3 categories of vitamin D status (ie, low, moderate, and high) as the explanatory variables. Multivariate models were then constructed to include adjustment for potential physical, dietary, and lifestyle confounders, including height, weight, pubertal status,



**TABLE 1**  
Descriptive characteristics of the study population<sup>1</sup>

	12-y-old Boys (n = 266)	12-y-old Girls (n = 260)	15-y-old Boys (n = 239)	15-y-old Girls (n = 250)
Height (cm)	152.3 (132.1–175.3) <sup>2</sup>	154.2 (131.9–169.7)	172.9 (143.9–196.0) <sup>2</sup>	162.7 (147.3–178.3)
Weight (kg)	43.1 (27.4–82.7) <sup>2</sup>	47.1 (29.2–88.0)	59.4 (31.9–113.4) <sup>2</sup>	56.4 (37.3–116.7)
Forearm BMD (g/cm <sup>2</sup> )	0.329 (0.226–0.602) <sup>2</sup>	0.320 (0.203–0.609)	0.383 (0.223–0.585) <sup>2</sup>	0.409 (0.258–0.575)
Heel BMD (g/cm <sup>2</sup> )	0.462 (0.275–0.777) <sup>2</sup>	0.443 (0.288–0.789)	0.555 (0.289–0.823) <sup>2</sup>	0.485 (0.223–0.719)
Serum 25(OH)D (nmol/L)	61.1 (22.2–174.1)	59.0 (15.8–144.6)	63.6 (5.0–165.6) <sup>2</sup>	57.6 (18.8–146.8)
Serum PTH (ng/mL)	48.6 (12.4–249.1) <sup>2</sup>	54.0 (16.0–251.7)	49.3 (8.6–220.4) <sup>2</sup>	41.8 (4.7–174.9)
Serum CTx (nmol/L)	1.17 (0.37–7.88) <sup>2</sup>	1.00 (0.30–2.82)	0.88 (0.27–2.82) <sup>2</sup>	0.43 (0.04–1.16)
Serum osteocalcin (ng/mL)	32.9 (11.1–77.2) <sup>2</sup>	31.0 (10.7–82.7)	31.6 (12.1–137.1) <sup>2</sup>	15.6 (8.4–33.9)
Physical activity score <sup>3</sup>	34.0 (0.0–77.8) <sup>2</sup>	18.9 (0.0–56.6)	27.4 (0.0–64.2) <sup>2</sup>	17.1 (0.0–58.5)
Vitamin D intake (μg/d)	1.87 (0.33–15.30) <sup>2</sup>	1.49 (0.18–13.40)	2.05 (0.44–39.52) <sup>2</sup>	1.42 (0.09–16.25)
Calcium intake (mg/d)	986 (382–3096) <sup>2</sup>	824 (236–1854)	1064 (302–2563) <sup>2</sup>	779 (199–1926)

<sup>1</sup> All values are median; range in parentheses. BMD, bone mineral density; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; CTx, type I collagen cross-linked C-telopeptide. Age-by-sex interaction was significant for all characteristics except physical activity and vitamin D intake,  $P \leq 0.005$  (2-factor ANOVA).

<sup>2</sup> Significantly different from girls of the same age,  $P \leq 0.05$  (independent *t* test).

<sup>3</sup> Highest possible activity score = 100.

alcohol drinking, smoking habits, physical activity, supplement use, and intake of calcium and fruit [fruit intake was previously shown to influence BMD in this cohort of adolescents (29)]. The same approach was used for serum PTH, osteocalcin, and CTx concentrations. Finally, age  $\times$  sex  $\times$  vitamin D status interactions were investigated.

## RESULTS

### Baseline characteristics of adolescents

Characteristics of the adolescents included in the current analysis ( $n = 1015$ ) were compared with those of all participants in the YH2000 study ( $n = 2017$ ), which was a representative sample of adolescents in Northern Ireland. There was no significant difference in the percentage distribution of sex, age, Tanner scores, and proportion sampled during summer or winter between these 2 groups (data not shown). Similarly, there was no significant difference in height, weight, BMI, or the intake of vitamin D and calcium between these 2 groups (data not shown).

Subject characteristics, BMD measurements, biochemical markers of bone turnover, vitamin D status, and selected nutritional information for the sample by age and sex group are presented in **Table 1**. Boys 12 y old had greater forearm and heel BMD, were more physically active, had higher concentrations of serum CTx and osteocalcin, and had higher intakes of calcium and vitamin D than did their female counterparts. Girls 12 y old were taller and heavier and had higher serum PTH concentrations than did their male counterparts. There was no difference in serum 25(OH)D concentrations between 12-y-old boys and girls. Boys aged 15 y were taller and heavier; were more physically active; had greater heel BMD; had higher serum 25(OH)D, PTH, CTx, and osteocalcin; and had higher intakes of calcium and vitamin D than did their female counterparts. Girls aged 15 y had greater forearm BMD than did their male counterparts.

The significance of the age  $\times$  sex interactions ( $P < 0.05$ ) was sufficiently small to justify analyzing the age-sex groups separately. The adjusted and unadjusted associations observed between the low, medium, and high categories of vitamin D status [as serum 25(OH)D] and forearm BMD in 12- and 15-y-old boys

and girls sampled throughout the year are shown in **Table 2**. Unadjusted analyses showed that 12- and 15-y-old girls with a high vitamin D status had significantly greater forearm BMD than did subjects with a low vitamin D status. Also presented in **Table 2** are the associations between the low, medium, and high categories of vitamin D status and forearm BMD, adjusted for the influence of physical, dietary, and environmental factors. The significant positive association between high vitamin D status and forearm BMD in 12- and 15-y-old girls remained. Of the various covariates included in the model, only weight was significantly ( $P < 0.0001$ ) associated with forearm BMD in 12-y-old girls. In the 15-y-old girls, height ( $P < 0.0001$ ), weight ( $P < 0.0001$ ), pubertal status ( $P = 0.013$ ), and dietary calcium ( $P = 0.009$ ) were significantly associated with forearm BMD. However, there were no significant interactions between these factors and the tertiles of vitamin D status. Regression analysis showed no associations between tertiles of vitamin D status and forearm BMD in boys of either age group (**Table 2**) or between vitamin D status and heel BMD in any age-sex subgroup (data not shown).

The associations observed between the low, medium, and high categories of vitamin D status and serum PTH, osteocalcin, and CTx, adjusted for physical, dietary, and environmental factors, in 12- and 15-y-old boys and girls sampled throughout the year are shown in **Table 3**. Girls 12 and 15 y old with high vitamin D status had significantly lower serum PTH, osteocalcin, and CTx than did those with low vitamin D status. Girls 12 and 15 y old with high vitamin D status had significantly lower serum PTH, but not osteocalcin or CTx, than did those with moderate vitamin D status. Boys 12 and 15 y old with high vitamin D status had significantly lower serum PTH and osteocalcin, but not CTx, than did those with low vitamin D status. Boys 12 and 15 y old with high vitamin D status had significantly lower serum osteocalcin, but not PTH or CTx, than did those with moderate vitamin D status.

Regression models were also run by using data only from those subjects sampled during winter months (December through February). The 12- and 15-y-old girls with high vitamin D status had significantly greater forearm BMD (adjusted for the influence of physical, dietary, and environmental factors) than did those with

**TABLE 2**

Regression analysis for the relation between tertile of vitamin D status (low, moderate, or high) and forearm bone mineral density (BMD) in adolescents sampled throughout the year<sup>1</sup>

	Forearm BMD							
	12-y-old Boys (n = 266)		12-y-old Girls (n = 260)		15-y-old Boys (n = 239)		15-y-old Girls (n = 250)	
	$\beta$	SE	$\beta$	SE	$\beta$	SE	$\beta$	SE
Unadjusted								
Highest versus lowest tertile of vitamin D status	0.006	0.007	0.020 <sup>2</sup>	0.008	0.008	0.011	0.021 <sup>2</sup>	0.009
Highest versus middle tertile of vitamin D status	0.000	0.008	0.009	0.008	-0.006	0.011	0.010	0.009
Adjusted for physical, dietary, and lifestyle characteristics <sup>3</sup>								
Highest versus lowest tertile of vitamin D status	0.002	0.007	0.018 <sup>2</sup>	0.008	0.005	0.009	0.018 <sup>2</sup>	0.008
Highest versus middle tertile of vitamin D status	-0.002	0.007	0.014	0.008	-0.001	0.009	0.010	0.009

<sup>1</sup>  $\beta$  is the estimated unstandardized regression coefficient arising from multivariate regression analysis. For boys, low, moderate, or high vitamin D status was defined as serum hydroxyvitamin D concentrations  $\leq 50.1$ , 50.2–75.2, or  $\geq 75.3$  nmol/L, respectively. For girls, low, moderate, or high vitamin D status was defined as serum hydroxyvitamin D concentrations  $\leq 46.3$ , 46.4–74.0, or  $\geq 74.1$  nmol/L, respectively.  $P < 0.001$  for the interaction between age and sex;  $P = 0.488$  for the interaction between age, sex, and highest versus lowest tertile of vitamin D status; and  $P = 0.753$  for the interaction between age, sex, and highest versus middle tertile of vitamin D status.

<sup>2</sup>  $P < 0.05$ .

<sup>3</sup> Height, weight, pubertal stage, physical activity, smoking, supplement use, and intake of alcohol, calcium, and fruit.

low [ $\beta$  (SE): 0.019 (0.009);  $P < 0.05$ ] or moderate [ $\beta$  (SE): 0.016 (0.009);  $P < 0.05$ ] vitamin D status. These associations were also evident in the unadjusted model (data not shown). Girls with high vitamin D status had significantly lower serum PTH, osteocalcin, and CTx than did those with low vitamin D status [ $\beta$  (SE): -17.468 (4.951), -3.797 (1.848), and -0.112, (0.063), respectively;  $P < 0.05$ ]. There was no significant difference in serum PTH, osteocalcin, or CTx between girls with high vitamin D status and those with moderate status. Limiting analysis to those boys sampled during the winter months showed that, whereas there was no association between vitamin D status and BMD of either site or serum CTx, boys in the low and middle tertiles of serum 25(OH)D had significantly higher serum PTH [ $\beta$  (SE):

-24.250 (5.520) and -12.445 (5.476), respectively;  $P < 0.05$ ] and osteocalcin [ $\beta$  (SE): -4.804 (1.971) and -3.763 (1.948), respectively;  $P < 0.05$ ] than did boys in the highest tertile.

## DISCUSSION

The findings of the present study in a large, representative sample of healthy adolescents show that 12- and 15-y-old girls with low vitamin D status had lower BMD of the forearm and higher PTH and bone turnover marker concentrations than did those with high vitamin D status. Whereas there were significant associations in similarly aged boys between vitamin D status and

**TABLE 3**

Regression analysis for the relation between tertile of vitamin D status (low, moderate, or high) and biochemical bone measurements [serum parathyroid hormone (PTH), osteocalcin, and type I collagen cross-linked C-telopeptide (CTx)] in adolescents sampled throughout the year<sup>1</sup>

	12-y-old Boys (n = 266)		12-y-old Girls (n = 260)		15-y-old Boys (n = 239)		15-y-old Girls (n = 250)	
	$\beta$	SE	$\beta$	SE	$\beta$	SE	$\beta$	SE
	PTH <sup>2</sup>							
Highest versus lowest tertile of vitamin D status	-13.283 <sup>3</sup>	5.012	-24.551 <sup>3</sup>	4.784	-20.848 <sup>3</sup>	5.421	-21.682 <sup>3</sup>	3.828
Highest versus middle tertile of vitamin D status	1.068	5.079	-9.749 <sup>3</sup>	4.478	-5.864	5.306	-7.216 <sup>4</sup>	3.942
Osteocalcin <sup>2</sup>								
Highest versus lowest tertile of vitamin D status	-5.453 <sup>3</sup>	1.755	-6.291 <sup>3</sup>	1.755	-9.989 <sup>3</sup>	3.052	-2.267 <sup>3</sup>	0.716
Highest versus middle tertile of vitamin D status	-4.826 <sup>3</sup>	1.778	-1.592	1.642	-7.248 <sup>3</sup>	2.987	-0.533	0.737
CTx <sup>2</sup>								
Highest versus lowest tertile of vitamin D status	-0.005	0.088	-0.141 <sup>3</sup>	0.063	-0.042	0.067	-0.055 <sup>5</sup>	0.031
Highest versus middle tertile of vitamin D status	0.009	0.089	-0.120 <sup>3</sup>	0.061	-0.041	0.066	0.002	0.032

<sup>1</sup>  $\beta$  is the estimated unstandardized regression coefficient arising from multivariate regression analysis. For boys, low, moderate, or high vitamin D status was defined as serum hydroxyvitamin D concentrations  $\leq 50.1$ , 50.2–75.2, or  $\geq 75.3$  nmol/L, respectively. For girls, low, moderate, or high vitamin D status was defined as serum hydroxyvitamin D concentrations  $\leq 46.3$ , 46.4–74.0, or  $\geq 74.1$  nmol/L, respectively. Age and sex interactions for PTH, osteocalcin, and CTx were significant,  $P < 0.05$ .

<sup>2</sup> Adjusted for physical, dietary, and lifestyle characteristics: height, weight, pubertal stage, physical activity, smoking, supplement use, and intake of alcohol, calcium, and fruit.

<sup>3</sup>  $P < 0.05$ .

<sup>4</sup>  $P \leq 0.068$ .

<sup>5</sup>  $P \leq 0.082$ .



serum PTH and osteocalcin, no significant association was evident between vitamin D status and BMD or serum CTx.

In the entire group of girls, sampled throughout the year, the group with low vitamin D status had serum 25(OH)D concentrations ( $<46.3$  nmol/L) that broadly encompassed the range of values between vitamin D deficiency and insufficiency [ $<25$ – $50$  nmol/L (9, 10)], whereas the group with high vitamin D status had concentrations ( $>74.1$  nmol/L) that were close to one suggested definition of optimal vitamin D status [80 nmol/L (25, 34)]. In 12- and 15-y-old girls, there was no significant difference between those with moderate (46.4–74.0 nmol/L) and high vitamin D status. These findings may suggest that serum 25(OH)D concentrations  $>46.3$  nmol/L are needed for adequate BMD accrual and rate of bone remodeling in adolescent girls. Viljakainen et al (26) recently suggested, on the basis of their findings that improving the vitamin D status of adolescent Finnish girls via vitamin D supplementation for 12 mo significantly increased bone mineral augmentation of the femur and lumbar spine, that serum 25(OH)D concentrations of  $>50$  nmol/L may be optimal. Lehtonen-Veromaa et al (6) reported that none of the girls in their study who had baseline serum 25(OH)D concentrations of  $>50$  nmol/L lost BMD at the lumbar spine. It is also notable that, unlike BMD of the femur or lumbar spine, which appears to be responsive to the serum 25(OH)D concentration in more sexually mature girls (6, 26), BMD of the forearm was influenced by the serum 25(OH)D concentration in 12- and 15-y old girls. This finding is in line with the findings of 2 studies that vitamin D status influenced forearm BMD in prepubertal (10) and peripubertal (9) girls.

The mechanism underlying the association between BMD of the forearm and vitamin D status is unclear, but that association may be related to our findings that serum PTH and bone turnover marker concentrations were higher in girls with low vitamin D status than in those with high vitamin D status. Lehtonen-Veromaa et al (6) found a significant inverse correlation between serum 25(OH)D and CTx, but not osteocalcin, in girls 9–15 y old. In general, there were no differences in bone marker concentrations between girls in the moderate and high vitamin D status groups in the present study, which, again, suggests that it is those with low vitamin D status (ie,  $<46.3$  nmol/L) who show changes in bone turnover. It is interesting that PTH was higher in girls with moderate vitamin D status than in those with high vitamin D status, which is in line with findings of some studies suggesting that, in adolescents, serum PTH either does not plateau with increasing serum 25(OH)D (9) or plateaus at serum 25(OH)D concentrations of  $>80$  nmol/L (27). The effect of low vitamin D status on bone health in boys has received very little research emphasis to date. In the present study, whereas a lack of association was found between tertiles of vitamin D status and BMD of the forearm in 12- or 15-y-old males, there were significant differences in serum osteocalcin and PTH. The reasons for the lack of BMD associations in males are unclear, and they require further investigation.

In a complementary approach, regression analysis models were run only in those subjects sampled in winter, and, thus, they provide a more refined comparison of stages of vitamin D status within the lower range of serum 25(OH)D concentrations. Whereas there was in that analysis no association between vitamin D status and BMD in boys, the girls with low ( $<43.0$  nmol/L) and moderate (43.1–57.0 nmol/L) vitamin D status had lower BMD of the forearm than did the girls with high ( $>57.1$

nmol/L) vitamin D status. Thus, serum 25(OH)D concentrations of  $>57$  nmol/L may be required to optimize BMD of the forearm, which agrees closely with the concentrations suggested for lumbar spine and femur, ie,  $>50$  nmol/L (6, 26).

We previously showed that 36% of these adolescents had 25(OH)D concentrations  $<50$  nmol/L throughout the year (27). The prevalence of vitamin D inadequacy was much higher (50%) in subjects sampled during the winter, because of the northerly latitude ( $54$ – $55^{\circ}$ N) of Northern Ireland (27). Thus, the possible adverse effect of low vitamin D status on bone health measures during adolescence is of concern for a large proportion of that population. We also previously reported that, in this cohort during winter, low vitamin D intake ( $<1.7$   $\mu$ g/d) and being female were significant predictors of having a serum 25(OH)D concentration of  $<50$  nmol/L (27). Thus, for persons living at latitudes above  $37^{\circ}$ N in Ireland or the United Kingdom, elsewhere in Europe, and North America, low intakes of vitamin D may take on increased importance during the winter, when sunlight is of insufficient intensity to stimulate dermal vitamin D synthesis. Determination of the dietary intake of vitamin D that will support the year-round maintenance of serum 25(OH)D concentrations of  $>50$  nmol/L is important and worthy of urgent scientific research.

There was no relation between BMD of the heel and the tertile of vitamin D status in any sex or age group in the present study. To our knowledge, there has been no other report of the effect of vitamin D status on BMD of the heel. It has been suggested previously that the effect of vitamin D on the skeleton may be site-specific (6), but the reasons for this possibility are not clear. They may have to do with a modulatory effect of body weight on the influence of vitamin D on BMD, and effects were evident in the present study in the forearm but not the heel, which would experience much more load than would the forearm. In addition, it may be that differences exist in the metabolic activity in these 2 skeletal sites. It is interesting that McGartland et al (29) showed, in the same YH2000 cohort, that high fruit intake in 12- and 15-y-old girls was associated with higher BMD of the heel, but no association was observed with BMD of the forearm. Thus, the site-specific effects of nutrition on BMD may also depend on the nutritional factor in question.

The main strength of the present study is that it was conducted in a large, representative sample of adolescents, both male and female, in Northern Ireland. The limitations of the study include the lack of data on bone mineral content and fractures. Furthermore, whereas the use of a peripheral instantaneous X-ray imager to assess BMD is a convenient and acceptable method for large studies, less is known about the precision and accuracy of that method, especially among youth, as compared with the use of axial (central) dual-energy X-ray absorptiometry measurements of the hip and spine. It would be interesting to see whether the results generated in the current study would be replicated in a study that included axial measurements.

In conclusion, whereas there is no consensus about the serum 25(OH)D concentration that defines either low or optimal vitamin D status in adolescents (6, 9, 10, 25, 26, 33), we found a positive association between high vitamin D status and forearm BMD in 12- and 15-y-old girls after adjustment for potential confounders. This association likely arises because of the lower serum PTH and markers of bone remodeling seen with higher vitamin D status. With the observed associations between vitamin D status and bone health measures, a recommendation that



adolescents maintain their serum 25(OH)D concentration above an agreed cutoff (possibly  $>\approx 50$  nmol/L) may be a cost-effective means of improving bone health. Greater emphasis should be given to exploring strategies for improving vitamin D status in adolescents.

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