# Vitamin D and attainment of peak bone mass among peripubertal Finnish girls: a 3-y prospective study[1–3]

*Marjo KM Lehtonen-Veromaa, Timo T Möttönen, Ilpo O Nuotio, Kerttu MA Irjala, Aila E Leino, and Jorma SA Viikari*

**ABSTRACT**

**Background:** Little is known about the effect of vitamin D status on bone gain in adolescents.

**Objective:** The objective was to examine whether vitamin D status is associated with accrual of bone mineral density (BMD) and bone mineral apparent density (BMAD).

**Design:** This 3-y prospective study examined the association between changes in BMD or BMAD and serum 25-hydroxyvitamin D [25(OH)D] in 171 healthy Finnish girls aged 9–15 y. Lumbar spine and femoral neck BMDs were measured by dual-energy X-ray absorptiometry.

**Results:** Baseline 25(OH)D correlated significantly with the unadjusted 3-y change in BMD at the lumbar spine ($r = 0.35$, $P < 0.001$) and femoral neck ($r = 0.32$, $P < 0.001$) in all participants. The difference from baseline in adjusted 3-y BMD accumulation between those with severe hypovitaminosis D [25(OH)D < 20 nmol/L] and those with a normal vitamin D status [25(OH)D ≥ 37.5 nmol/L] was 4% (12.7%, 13.1%, and 16.7% for the lowest, middle, and highest tertiles of 25(OH)D, respectively; $P$ for trend = 0.01) at the lumbar spine in the girls with advanced sexual maturation at baseline ($n = 129$). Moreover, the adjusted change in lumbar spine BMD was 27% greater in the highest vitamin D intake tertile than in the lowest tertile in the same girls ($P$ for trend = 0.016).

**Conclusions:** Pubertal girls with hypovitaminosis D seem to be at risk of not reaching maximum peak bone mass, particularly at the lumbar spine. Dietary enrichment or supplementation with vitamin D should be considered to ensure an adequate vitamin D status. *Am J Clin Nutr* 2002;76:1446–53.

**KEY WORDS** Adolescence, peripubertal girls, bone mineral density, bone mineral apparent density, serum 25-hydroxyvitamin D, bone mass, Finland

## INTRODUCTION

Osteoporosis has profound implications for an individual and for the economics of society. Although the mechanisms that lead to osteoporosis are not fully understood, 2 major contributing factors are known: peak bone mass during childhood and adolescence and the rate of bone loss during aging. The risk of osteoporotic fractures in an elderly population increases progressively as bone mineral density (BMD; in g/cm$^2$) declines—a reduction of 1 SD in the BMD of the femoral neck is associated with a doubling of the risk of hip fractures (1).

Vitamin D is obtained either from dietary sources or from cutaneous synthesis through a process initiated by ultraviolet radiation on the skin (2). Vitamin D stores can be assessed by measuring the serum concentration of 25-hydroxyvitamin D [25(OH)D], which is the most commonly used index of vitamin D status (3). However, there has been an intense debate as to which concentrations are optimal for the acquisition and maintenance of bone mass (4, 5). When serum 25(OH)D decreases, parathyroid hormone (PTH) concentrations increase, which increases the risk of osteoclast activity and, ultimately, of bone resorption (6, 7).

BMD and bone mineral apparent density (BMAD; in g/cm$^3$) are the result of the dynamic process of bone formation and bone resorption. Osteocalcin, a noncollagenous protein synthesized by the osteoblasts, is a valid marker of bone formation (8), whereas one of the new markers of bone resorption is the carboxyl terminal telopeptide of type I collagen (CTX), a degradation product of mature collagen that is excreted into the serum (8, 9). Bone mass accumulates throughout the growth period and in early adulthood. During this time, increased bone resorption and decreased bone formation may lead to a reduced peak bone mass (8). To prevent osteoporosis, therefore, it is important to identify all contributors to the process of skeletal development.

Vitamin D deficiency is not uncommon among children and adolescents, particularly during the dark seasons of the year. In the absence of sunshine, many children and adolescents have vitamin D deficiency or even severe hypovitaminosis D (4, 7, 10). Surprisingly, little is known about the true effect of vitamin D status on the acquisition of BMD and BMAD or on the biochemical markers of bone turnover in adolescents (11–14).

The purpose of this study was to examine the association between serum 25(OH)D and bone accumulation at the lumbar spine and femoral neck and the association between serum

[1] From the Paavo Nurmi Centre, Sport and Exercise Medicine Unit, Department of Physiology, University of Turku, Turku, Finland (MKML-V), and the Department of Medicine (TTM, ION, and JSAV) and the Central Laboratory (KMAI and AEL), Turku University Central Hospital, Kiinamyllynkatu, Turku, Finland.

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[3] Address reprint requests to TT Möttönen, Department of Medicine, Turku University Central Hospital, Paimio Hospital, 21540 Paimio, Finland. E-mail: timo.mottonen@tyks.fi.

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25(OH)D and some biochemical markers of bone metabolism in peripubertal girls.

## SUBJECTS AND METHODS

### Subjects

The study group comprised 191 healthy white girls aged 9–15 y (66 gymnasts, 65 runners, and 60 nonathletic control subjects with an average age of 12.9 y) who were participating in a long-term health study (10). The participants were enrolled as volunteers recruited from local sports clubs and schools in the city of Turku and its vicinity. Most of the control subjects were classmates of the athletes or children of hospital personnel. All participants were healthy and had no chronic diseases that could affect growth or the metabolism of calcium or vitamin D.

The study protocol was approved by the joint ethics committee of the Turku University and the Turku University Central Hospital. The study was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects and their parents.

### Study design

All subjects were studied at baseline over an 8-wk period in February-March 1997 and again at 6-mo intervals over 3 y. Height was measured with a fixed stadiometer (Harpenden Stadiometer; Holtain, Crymych, United Kingdom) and weight with a regularly calibrated electronic scale (EKS exclusive; EKS International, Södertälje, Sweden). Body mass index (in kg/m$^2$) was calculated and recorded. All measurements were made between 0800 and 1000 by the same observer (MKML-V). All participants received multivitamin supplementation (Optivit; Leiras, Turku, Finland) containing 10 μg vitamin $D_2$) from the beginning of October to February to ensure that they were consuming the recommended dietary allowance of vitamin D. The dosage consisted of one tablet daily during the first 2 y of the study and 2 tablets during the winter period of the third year of the study. The reason for increasing the dosage during the last year was that serum 25(OH)D did not increase by the amount provided in one tablet. Calcium supplementation (Puru-Calsor; Orion, Espoo, Finland) with 500 mg Ca as the carbonate salt was given as one tablet per day to those who consumed < 1000 mg Ca/d.

### Assessment of nutrient intake

Intakes of vitamin D and calcium were estimated at 6-mo intervals as described earlier (10). The subjects filled in semiquantitative food-frequency questionnaires that included questions about supplement use and pictures of portion sizes. The mean intake of calcium during the study period was calculated and used as a covariate for adjustment in the analysis of bone accumulation.

### Laboratory assessments

All blood samples were drawn between 0800 and 0900 after an overnight fast. Local anesthetic patches (Emla; Astra, Södertälje, Sweden) were used to reduce the discomfort of venipuncture. In menstruating subjects, samples were collected during the early follicular phase of the menstrual cycle, which was defined as the time between the fifth and the eighth day after the onset of menstrual bleeding. The blood samples were centrifuged (2100 × *g*, 10 min, room temperature) within 2 h of venipuncture, and sera were stored at −20 °C. The serum samples for 25(OH)D measurement were obtained at baseline and at 12 and 36 mo; the samples were protected from light during processing and underwent radioimmunoassay (DiaSorin Corporation, Stillwater, MN). The samples were run in duplicate; the interassay CV for serum 25(OH)D was 8.3% at a concentration of 35.3 nmol/L ($n = 50$).

Serum osteocalcin was measured at 36 mo by radioimmunoassay (CIS Bio International, Gif-sur-Yvette, Cedex, France). The intra- and interassay CVs were 1.7% and 3.1%, respectively, at a concentration of 22 ng/mL. Serum CTX was measured at 36 mo with the use of an enzyme immunologic test (Osteometer Biotech, Herlev, Denmark), and the intra- and interassay CVs were 10.3% and 4.9%, respectively, at a concentration of 7400 pmol/L.

Routine blood chemistry tests, including the measurement of serum calcium and alkaline phosphatase, were made with Hitachi 717 and 917 analyzers (Hitachi LTD, Tokyo). The serum concentrations of calcium and alkaline phosphatase (EC 3.1.3.1) in all subjects were within the reference range.

### Hypovitaminosis D

Severe hypovitaminosis D was defined as a serum 25(OH)D concentration < 20 nmol/L and moderate hypovitaminosis D as a serum 25(OH)D concentration between 20 and 37.5 nmol/L (6). The definition of hypovitaminosis D was adapted from published data, according to which the serum PTH concentration starts to increase in patients whose serum 25(OH)D concentration is < 37.5 nmol/L (6).

### Bone mineral density measurements

The BMD of the lumbar spine (L1–L4) and the nondominant hip were measured by dual-energy X-ray absorptiometry (DXA) (QDR 4500C; Hologic, Waltham, MA) at baseline and after 3 y. The data were also expressed as BMAD. By geometric scaling, the vertebral body depth was the square root of the projected area ($Ap^{0.5}$), and thus the volume was calculated as $Ap^{1.5}$. The vertebral BMAD was calculated by dividing the bone mineral content (BMC) by the vertebral body volume (BMAD = BMC/$Ap^{1.5}$) (15). For the femoral neck we used the formula developed by Katzman et al (16), who used the area squared and calculated BMAD as BMC/$Ap^2$. All measurements were performed and analyzed by the same 3 trained radiographers. To ensure quality, calibration was performed daily with a spine phantom supplied by the manufacturer. The CVs for 2 consecutive BMD measurements in 10 girls were 1.2% for the spine, 1.6% for the hip, and 0.4% for the phantom over the study period.

### Assessment of pubertal stage

One author (MKML-V) examined and recorded the pubertal Tanner stage (17). When there were discrepancies between breast stage and pubic hair stage, the breast stage was the decisive factor. To calculate the reproductive year at baseline, the age at menarche was subtracted from the current age, and the result was used as a covariate instead of age and Tanner stage. The age at menarche was determined in a poststudy interview for those subjects who did not reach menarche during the observation period; this enabled the calculation of reproductive year for data analysis.

### Assessment of physical activity

The subjects completed a detailed questionnaire about their physical activity every 6 mo. Physical activity was calculated as the ratio of work metabolic rate to resting metabolic rate (in metabolic equivalents per hour per week) as described earlier

**TABLE 1**
Baseline characteristics of 171 peripubertal girls

| Characteristic | |
|---|---|
| Age (y) | 12.9 ± 1.7[1] |
| Reproductive years (y) | −0.4 ± 2.0 |
| Height (cm) | 157.8 ± 9.3 |
| Weight (kg) | 46.8 ± 9.7 |
| Dietary vitamin D intake (μg/d) | 4.3 ± 2.1 |
| Dietary calcium intake (mg/d) | 1575 ± 637 |
| Physical activity (MET h/wk)[2] | 40 (12, 75) |
| Tanner stage [*n* (%)] | |
| 1 | 31 (18.1) |
| 2 | 39 (22.8) |
| 3 | 32 (18.7) |
| 4 | 34 (19.9) |
| 5 | 35 (20.5) |

[1] $\bar{x}$ ± SD.
[2] The MET (metabolic equivalents) index was calculated by multiplying the frequency, mean duration, and mean intensity of weekly physical activity and dividing by 60. The value is the median with the interquartile range in parentheses.

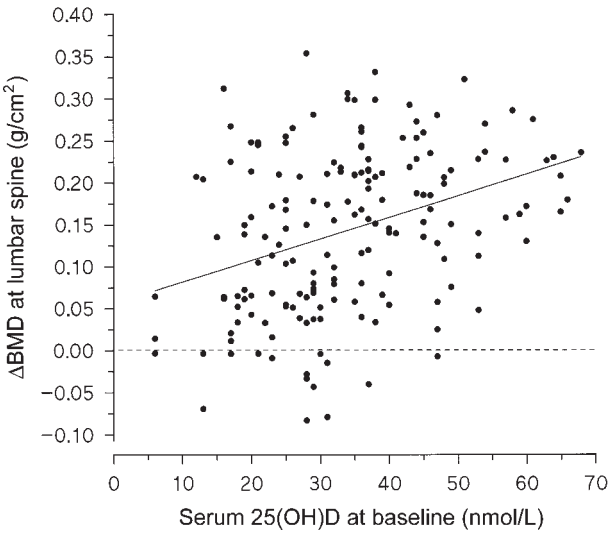


**FIGURE 1.** Relation between serum concentrations of 25-hydroxyvitamin D [25(OH)D] at baseline and the 3-y change in bone mineral density (ΔBMD) at the lumbar spine (L1–L4) among peripubertal girls ($n$ = 171). $r$ = 0.35, $P$ < 0.001.

(18). The amount of physical activity in 3 y was then mean square root transformed to correct for skewness, and the result was used as a covariate for adjustment of the incremental bone accumulation.

### Statistical analyses

All analyses were performed by using the Statistical Package for the Social Sciences for WINDOWS (release 9.0; Norusis/SPSS, Inc, Chicago). The normality of the distributions was tested by using Kolmogorov-Smirnov statistics with a Lilliefors significance or Shapiro-Wilk statistics. For variables with a normal distribution, descriptive values were expressed as means ± SDs. Statistical comparisons between measures or groups were made by analysis of variance with Tukey's honestly significant difference test. If the variables were not normally distributed, descriptive values were expressed as medians and interquartile ranges, and statistical comparisons between groups were made with the Kruskal-Wallis test with Bonferroni's adjusted Mann-Whitney $U$ test. For the most important descriptive values, 95% CIs were also given. Spearman's rank correlations were used to compare the 3-y change in BMD (ΔBMD) and of BMAD (ΔBMAD) with serum 25(OH)D and serum biochemical markers of bone metabolism with 25(OH)D concentrations. The ΔBMD and ΔBMAD of the lumbar spine and femoral neck were calculated by adjusting for the exact follow-up period. Regression analysis was performed to determine covariates for BMD and BMAD. Any covariate found to be associated with BMD or BMAD at any site was included in all subsequent analyses of covariance. Because the relation between the increases in bone measurements (dependent variables) and reproductive year were nonlinear, the study population was divided into 2 subgroups for further analysis with use of a cut-off of −2 y of reproductive year (ie, 2 y before the age of menarche at baseline). The differences in ΔBMD and ΔBMAD between vitamin D tertiles were evaluated with analysis of covariance adjusted for the following covariates: reproductive year at baseline, increase in height and weight, mean amount of physical activity during the study (square root transformed), mean intake of calcium, and baseline BMD or BMAD. Data are expressed as absolute and fractional (%) increments. In Figures 2 and 3, the method of robust locally weighted smoothing was used to fit a line through a set of points (19).

## RESULTS

The original study population consisted of 191 girls. Fifteen girls dropped out during the 3-y study, and 3 participants were excluded because of the onset of a chronic disease (celiac disease, epilepsy, and thyrotoxicosis). In addition, 2 participants were excluded because they had arrived back from Egypt and Thailand (where the conditions had been sunny) immediately before the first blood sample collection. Thus, the results of 171 subjects were included in the final analysis. The baseline characteristics of the study group are presented in **Table 1**. There were no statistically significant differences in the stage of puberty or menstruation history at the onset of the study or menstrual disturbances at the end of the study between the athletic and nonathletic groups. More than one-half of the participants had not reached menarche at the time of recruitment.

The mean (± SD) baseline concentration of serum 25(OH)D in winter among the whole study group was 34.0 ± 13.2 nmol/L. The baseline 25(OH)D values correlated significantly with ΔBMD at the lumbar spine ($r$ = 0.35, $P$ < 0.001) and at the femoral neck ($r$ = 0.32, $P$ < 0.001). The corresponding correlation coefficients between serum 25(OH)D and ΔBMAD at the lumbar spine and femoral neck were 0.35 ($P$ < 0.001) and 0.24 ($P$ = 0.002), respectively. The relation between serum 25(OH)D and ΔBMD at the lumbar spine is shown in **Figure 1**. The mean serum 25(OH)D concentration was 33.2 ± 11.1 nmol/L at 12 mo and 40.6 ± 15.8 nmol/L at 36 mo.

The association between 3-y ΔBMD and ΔBMAD of the lumbar spine and age, Tanner stage, and reproductive year at baseline in the peripubertal girls are shown in **Figures 2** and **3**, respectively. ΔBMD and ΔBMAD were at a maximum in the girls who, at baseline, were at Tanner stage 2 or ≈2 y before the age of menarche. The corresponding ΔBMD and ΔBMAD values at the femoral neck were not significantly different (data not shown). In the girls who experienced menarche later than ≈2 y after the

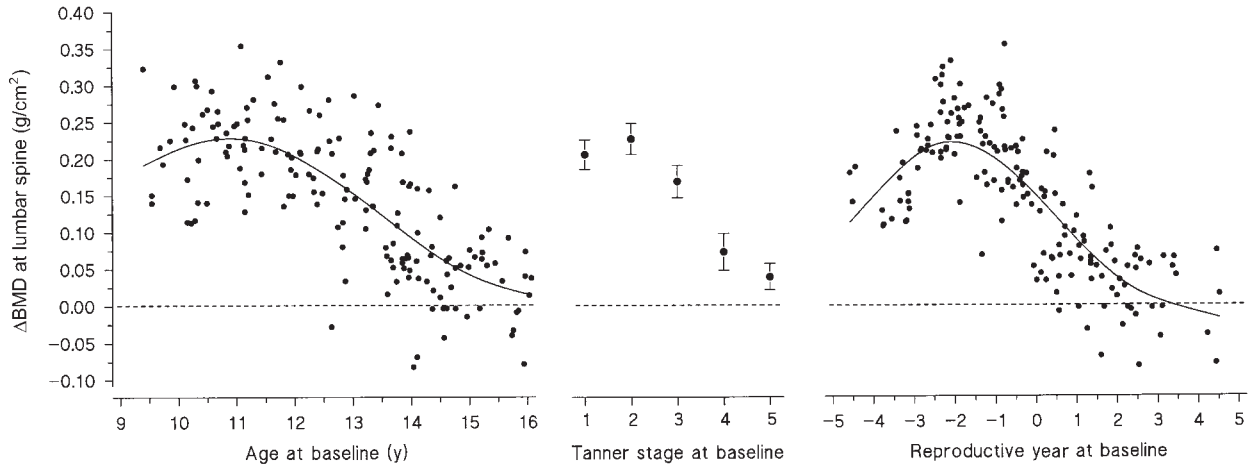


**FIGURE 2.** Association of age, Tanner stage (means; bars indicate 95% CIs), and reproductive year at baseline with the 3-y change in bone mineral density (ΔBMD) at the lumbar spine (L1–L4) among peripubertal girls ($n$ = 171).

beginning of the study, the increases in BMD and BMAD were acceleratory with age. In contrast, in the group of the girls who experienced menarche earlier than 2 y after the beginning of the study (advanced sexual maturation), the increases in BMD and BMAD were deceleratory with age. Thus, the study population was analyzed further in 2 separate groups because the increases in bone mineral measurements were not linear in the whole study population.

There were no statistically significant differences in baseline characteristics between the less mature study participants at the 3 tertiles of baseline serum 25(OH)D (**Table 2**). However, the intake of dietary vitamin D differed significantly by serum 25(OH)D tertiles over the 3-y period between the girls with advanced sexual maturation. The years of training and numbers of gymnasts, runners, and control subjects were not significantly different, by serum 25(OH)D tertile, among the less mature study participants (data not shown). The numbers of gymnasts, runners, and control subjects with advanced sexual maturation were not significantly differently by 25(OH)D tertile (lowest tertile: 14 gymnasts, 14 runners, and 18 control subjects; middle tertile: 12 gymnasts, 15 runners, and 11 control subjects; highest tertile: 16 gymnasts, 16 runners, and 13 control subjects; $P$ = 0.365). Moreover, the years of training were not significantly different by 25(OH)D tertiles (data not shown).

The true compliance with vitamin D supplementation was not significantly different by tertile during the study period (data not shown).

The unadjusted baseline BMD and BMAD values in the lumbar spine were not significantly different, by serum 25(OH)D tertile, among the less sexually maturated girls and those with advanced sexual maturation. However, the unadjusted baseline BMD and BMAD values at the femoral neck differed significantly among the girls with advanced sexual maturation by 25(OH)D tertiles (**Table 3**).

ΔBMD and ΔBMAD at the lumbar spine and femoral neck in the girls with advanced sexual maturation over the 3-y follow-up

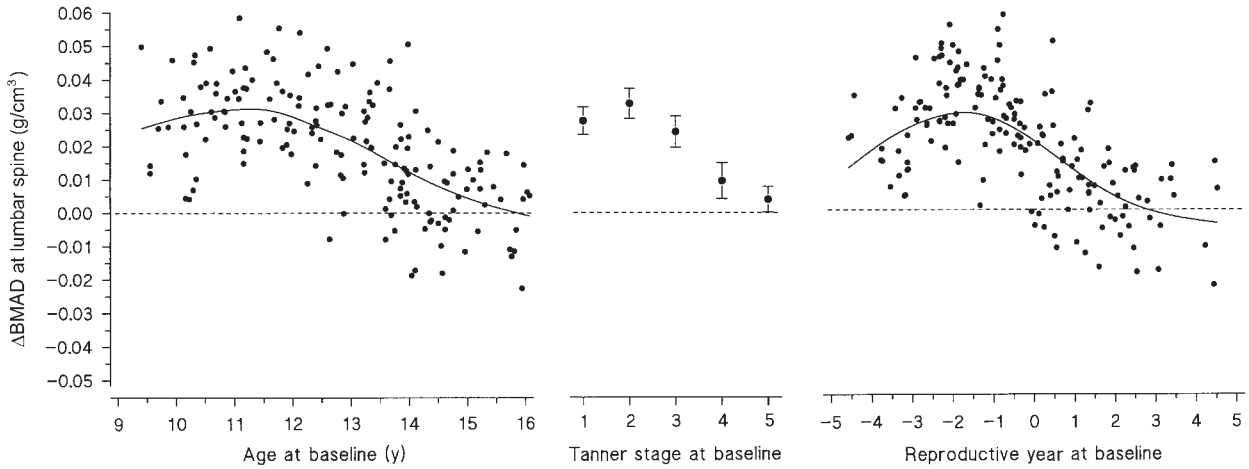


**FIGURE 3.** Association of age, Tanner stage (means; bars indicate 95% CIs), and reproductive year at baseline with the 3-y change in bone mineral apparent density (ΔBMAD) at the lumbar spine (L1–L4) among peripubertal girls ($n$ = 171).

**TABLE 2**
Characteristics of 171 peripubertal girls at baseline and over a 3-y period by tertiles of baseline serum 25-hydroxyvitamin D [25(OH)D] concentration and reproductive year at baseline

| | Reproductive year < −2 y at baseline | | | | Reproductive year ≥ −2 y at baseline | | | |
|---|---|---|---|---|---|---|---|---|
| Variable | Lowest tertile ($n$ = 16) | Middle tertile ($n$ = 12) | Highest tertile ($n$ = 14) | $P$ | Lowest tertile ($n$ = 46) | Middle tertile ($n$ = 38) | Highest tertile ($n$ = 45) | $P$ |
| Baseline serum 25(OH)D (nmol/L) | 29.6 ± 7.0[1] | 40.8 ± 3.6 | 56.4 ± 7.2 | | 19.2 ± 5.1 | 30.2 ± 2.5 | 45.1 ± 8.2 | |
| Age (y) | 11.1 ± 1.1 | 11.1 ± 1.1 | 11.2 ± 1.5 | 0.95 | 13.5 ± 1.5 | 13.5 ± 1.5 | 13.3 ± 1.5 | 0.66 |
| Reproductive year (y) | −2.7 ± 0.5 | −3.1 ± 0.8 | −2.9 ± 0.7 | 0.32 | 0.6 ± 1.6 | 0.8 ± 1.7 | 0.1 ± 1.5 | 0.09 |
| Height (cm) | 147.0 ± 7.2 | 146.8 ± 7.2 | 148.9 ± 4.5 | 0.66 | 160.6 ± 7.9 | 162.4 ± 7.4 | 160.6 ± 7.2 | 0.47 |
| Weight (kg) | 37.3 ± 6.1 | 35.5 ± 6.0 | 36.4 ± 4.6 | 0.72 | 50.5 ± 9.1 | 51.3 ± 8.2 | 48.8 ± 7.4 | 0.39 |
| Serum calcium (mmol/L) | 2.45 ± 0.06 | 2.44 ± 0.07 | 2.46 ± 0.06 | 0.86 | 2.44 ± 0.06 | 2.43 ± 0.06 | 2.45 ± 0.07 | 0.13 |
| Serum alkaline phosphatase AFOS (U/L) | 552 ± 109 | 568 ± 168 | 533 ± 115 | 0.79 | 416 ± 206 | 427 ± 222 | 508 ± 204 | 0.09 |
| 3-y change | | | | | | | | |
| Increase in height (cm) | 14.4 ± 3.6 | 13.5 ± 2.8 | 13.6 ± 3.8 | 0.77 | 4.2 ± 4.0 | 3.8 ± 3.7 | 5.8 ± 4.5 | 0.051 |
| Increase in weight (kg) | 13.2 ± 2.8 | 11.2 ± 5.1 | 10.9 ± 3.5 | 0.19 | 6.3 ± 4.0 | 6.4 ± 4.3 | 8.0 ± 5.6 | 0.18 |
| Dietary calcium intake (mg/d) | 1656 ± 343 | 1587 ± 553 | 1650 ± 433 | 0.91 | 1360 ± 469 | 1566 ± 443 | 1590 ± 498 | 0.06 |
| Dietary vitamin D intake (μg) | 4.2 ± 1.7 | 4.5 ± 2.4 | 4.1 ± 2.1 | 0.86 | 3.5 ± 1.3 | 4.0 ± 1.4 | 4.4 ± 1.7 | 0.02 |
| Physical activity (MET h/wk)[2] | 5.0 (3.7, 7.1) | 7.8 (4.3, 9.6) | 6.8 (3.9, 9.4) | 0.31 | 4.9 (3.3, 7.0) | 6.0 (3.9, 9.0) | 6.3 (3.7, 8.4) | 0.17 |

[1] $\bar{x}$ ± SD.
[2] Median; interquartile range in parentheses. Values are the square root of the MET (metabolic equivalents) index, calculated by multiplying the frequency, mean duration, and mean intensity of weekly physical activity and dividing by 60.

are shown in **Table 4**. The mean 3-y ΔBMD values from baseline were 16.7% in the highest, 13.1% in the middle, and 12.7% in the lowest 25(OH)D tertile at the lumbar spine ($P$ for trend = 0.01). The 3-y adjusted ΔBMD at the lumbar spine was 26%, or 0.029 g/cm$^2$ (95% CI: 0.003, 0.054 g/cm$^2$; $P$ = 0.022), larger in the highest than in the lowest serum 25(OH)D tertile group. The corresponding value for ΔBMAD was 50%, or 0.008 g/cm$^3$ (95% CI: 0.002, 0.013 g/cm$^3$; $P$ = 0.005). The adjusted ΔBMD at the femoral neck did not differ significantly by serum 25(OH)D tertile.

The differences in the adjusted ΔBMD and ΔBMAD values between the highest and lowest serum 25(OH)D tertiles were significant ($P$ for trend < 0.05) at the lumbar spine and when the girls with advanced sexual maturation were grouped according to mean wintertime serum 25(OH)D concentrations at baseline and at 12 mo: 0.027 g/cm$^2$ (95% CI: 0.001, 0.054 g/cm$^2$) and 0.007 g/cm$^3$ (95% CI: 0.001, 0.013 g/cm$^3$), respectively.

The mean dietary calcium and vitamin D intakes did not correlate significantly with ΔBMD or ΔBMAD at the lumbar spine or femoral neck in the overall study population. However, ΔBMD of the lumbar spine was 27%, or 0.030g/cm$^2$ (95% CI: 0.004, 0.056 g/cm$^2$), greater in the highest than in the lowest vitamin D intake tertile in the girls with advanced sexual maturation ($P$ for trend for all tertiles = 0.016). These values for the femoral neck did not differ significantly by vitamin D intake tertiles (data not shown). The 3-y adjusted ΔBMD and ΔBMAD values among the less mature girls were not significantly different by 25(OH)D tertile (data not shown).

There were no statistically significant associations between ΔBMD or ΔBMAD and serum osteocalcin or serum CTX at 36 mo among whole study population (data not shown). The serum concentration of CTX had a significant inverse correlation ($r$ = −0.27, $P$ < 0.001) with serum 25(OH)D at 36 mo in the whole study group (**Figure 4**). Many of the higher serum CTX values occurred in the girls whose 25(OH)D concentration was low. The CTX value adjusted for height, weight, and reproductive year was 13%, or 1.5 nmol/L (95% CI: 300, 2600 pmol/L), lower in the highest than in the lowest serum 25(OH)D tertile group at the end of the study ($P$ for trend = 0.003). The serum osteocalcin concentration did not correlate with the serum 25(OH)D concentration at 36 mo ($r$ = −0.08, $P$ = 0.348).

## DISCUSSION

The main finding in the present study was that there was a significant association between the baseline concentration of 25(OH)D

**TABLE 3**
Baseline unadjusted bone mineral density (BMD) and bone mineral apparent density (BMAD) at the lumbar spine (L1–L4) and femoral neck (FN) in 171 peripubertal girls by tertiles of baseline serum 25-hydroxyvitamin D [25(OH)D] concentration and reproductive year at baseline[1]

| | Reproductive year < −2 y | | | | Reproductive year ≥ −2 y | | | |
|---|---|---|---|---|---|---|---|---|
| Variable | Lowest tertile ($n$ = 16) | Middle tertile ($n$ = 12) | Highest tertile ($n$ = 14) | $P$ | Lowest tertile ($n$ = 46) | Middle tertile ($n$ = 38) | Highest tertile ($n$ = 45) | $P$ |
| Baseline serum 25(OH)D (nmol/L) | 29.6 ± 7.0 | 40.8 ± 3.6 | 56.4 ± 7.2 | | 19.2 ± 5.1 | 30.2 ± 2.5 | 45.1 ± 8.2 | |
| $BMD_{L1–L4}$ (g/cm$^2$) | 0.671 ± 0.093 | 0.668 ± 0.064 | 0.701 ± 0.087 | 0.518 | 0.875 ± 0.150 | 0.898 ± 0.158 | 0.839 ± 0.155 | 0.218 |
| $BMAD_{L1–L4}$ (g/cm$^3$) | 0.218 ± 0.023 | 0.213 ± 0.019 | 0.222 ± 0.026 | 0.657 | 0.245 ± 0.030 | 0.250 ± 0.033 | 0.239 ± 0.030 | 0.246 |
| $BMD_{FN}$ (g/cm$^2$) | 0.711 ± 0.075 | 0.704 ± 0.120 | 0.722 ± 0.106 | 0.903 | 0.836 ± 0.149 | 0.899 ± 0.156 | 0.814 ± 0.131 | 0.027 |
| $BMAD_{FN}$ (g/cm$^3$) | 0.357 ± 0.038 | 0.353 ± 0.071 | 0.360 ± 0.062 | 0.956 | 0.391 ± 0.064 | 0.427 ± 0.077 | 0.388 ± 0.073 | 0.026 |

[1] $\bar{x}$ ± SD.

**TABLE 4**
Three-year changes in bone mineral density (ΔBMD) and bone mineral apparent density (ΔBMAD) at the lumbar spine (L1–L4) and femoral neck (FN) in the 129 peripubertal girls with advanced sexual maturation by tertiles of baseline serum 25-hydroxyvitamin D [25(OH)D][1]

| | Serum 25(OH)D tertile[1] | | | | |
|---|---|---|---|---|---|
| | Lowest ($n$ = 46) | Middle ($n$ = 38) | Highest ($n$ = 45) | $P$ for trend | Mean difference between highest and lowest tertiles (95% CI) |
| Baseline serum 25(OH)D (nmol/L) | 19.2 ± 5.1 | 30.2 ± 2.5 | 45.1 ± 8.2 | | |
| $\Delta BMD_{L1–L4}$ (g/cm$^2$) | 0.111 ± 0.007 | 0.118 ± 0.008 | 0.140 ± 0.007 | 0.010 | 0.029 (0.003, 0.054) |
| $\Delta BMAD_{L1–L4}$ (g/cm$^3$) | 0.014 ± 0.002 | 0.016 ± 0.002 | 0.021 ± 0.002 | 0.002 | 0.008 (0.002, 0.013) |
| $\Delta BMD_{FN}$ (g/cm$^2$) | 0.078 ± 0.009 | 0.083 ± 0.010) | 0.097 ± 0.009 | 0.153 | 0.019 (−0.013, 0.052) |
| $\Delta BMAD_{FN}$ (g/cm$^3$) | 0.027 ± 0.005 | 0.030 ± 0.006 | 0.031 ± 0.005 | 0.592 | 0.004 (−0.014, 0.022) |

[1] $\bar{x}$ ± SE in relation to baseline. All variables were adjusted for baseline reproductive year, baseline bone mineral values, increases in height and weight, mean intake of calcium, and mean amount of physical activity.

and 3-y ΔBMD and ΔBMAD at the lumbar spine and femoral neck among peripubertal girls. The BMD and BMAD at the lumbar spine of the girls with advanced sexual maturation increased over 3 y by 26% and 50% more, respectively, in the highest than in the lowest baseline serum 25(OH)D tertile. CTX, the serum marker of bone resorption, correlated significantly inversely with 25(OH)D concentrations at the end of the study. None of the subjects with a baseline serum 25(OH)D concentration > 50 nmol/L lost BMD at the lumbar spine. These findings indicate the importance of an adequate vitamin D status during the phase of bone modeling and skeletal consolidation of the human skeleton.

Calcium absorption and retention both peak during the period of rapid skeletal modeling in infancy and adolescence (20). Our results confirm that the dietary vitamin D intake is insufficient to maintain an optimal vitamin D status during the winter months of darkness. Webb et al (2) conducted a study in Edmonton, Canada (located at 52 °N), and found that this winter period—in which the vitamin D status is not optimal—extends from October to March. Finland is located even further north (at 60–70 °N), which clearly poses a risk of insufficient vitamin D synthesis for at least one-half of the year. In the summer, the participants in the present study had an average 25(OH)D concentration of 63 nmol/L (10), which may also be suboptimal for skeletal health because a concentration of 80 nmol/L is needed to plateau PTH concentrations (7, 21).

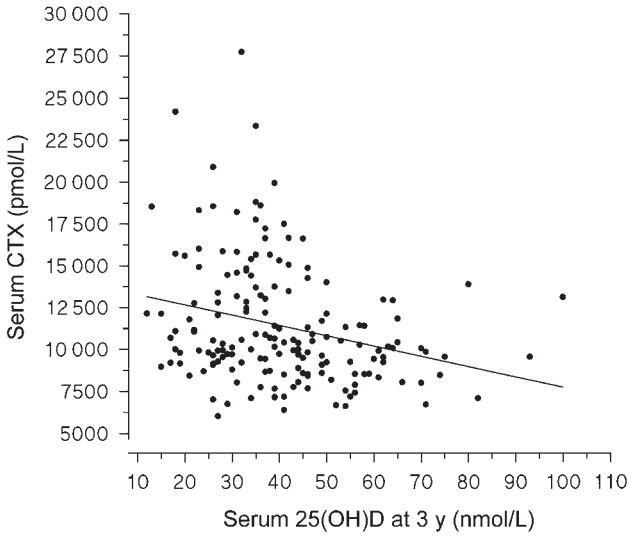


**FIGURE 4.** Relation between serum concentrations of 25-hydroxyvitamin D [25(OH)D] at 3 y and carboxyl terminal telopeptide of type I collagen (CTX) at 36 mo among peripubertal girls ($n$ = 171). $r$ = −0.27, $P$ < 0.001.

Biochemical markers of bone turnover are helpful in the study of the pathophysiology of skeletal metabolism and growth. In recent prospective studies, the serum concentrations of markers of bone resorption and formation increased rapidly during early puberty when growth velocity was highest but then started to decline before menarche, ie, during the period of the most rapid bone accumulation (18, 22–24). Little is known about the effect of the vitamin D status on bone turnover in children and adolescents without rickets. The results of 2 studies suggest that increased concentrations of bone turnover markers are associated with accelerated bone loss in adults and the elderly (25, 26). Several studies in adults and the elderly suggest that the effects of low 25(OH)D concentrations on bone metabolism may be mediated through PTH (5, 27–29). Our results indicate that serum CTX values increase when 25(OH)D values decrease. This observation agrees with what is known about the relation between PTH concentrations and vitamin D deficiency in adolescents (7).

Peak bone mass of the lumbar spine and hip is achieved around the age of 20 y (30). In the present study, bone resorption (serum CTX) was accelerated in wintertime, whereas bone formation (serum osteocalcin) did not increase in the group with the lowest serum 25(OH)D tertile, ie, at the mean serum 25(OH)D value corresponding to severe hypovitaminosis D. Reduced rates of both bone formation and resorption may have an important role in increasing bone accumulation during puberty (31). The repeated seasonal acceleration of bone resorption over the years may therefore contribute to the development of low peak bone mass in adolescent girls with hypovitaminosis D.

Because the maximum increment in the rate of BMD occurs during pubertal stages 3–4 (22, 32, 33), hypovitaminosis D during that period is expected to be more harmful to the development of BMD than is hypovitaminosis D at an earlier age. According to our results, the increases in BMD and BMAD of the lumbar spine and femoral neck were maximal in subjects who were at Tanner stage 2 of sexual development. at baseline. Our findings corroborate the observation that the last premenarcheal years are crucial for the prevention of osteoporosis (18, 22, 32).

In this 3-y follow-up study, the difference between the subjects who had severe hypovitaminosis D and those who had a normal vitamin D status regarding BMD accumulation from baseline was 4% at the lumbar spine. Previous studies reported that the spine, which is predominantly trabecular bone, is metabolically more active than is the femoral neck (34, 35). Our results agree with these reports: vitamin D status particularly affects the lumbar spine. The amount of exposure to sunlight in the prepubertal girls in Australia is associated with BMD, and this effect is more

pronounced at the spine than at the hip (36). In an earlier study, there was no effect of hypovitaminosis D on BMD of the radius among prepubertal children (37), and this finding agrees with our results in the sense that we identified no effect of vitamin D status on the BMD of the lumbar spine or hip among the less mature participants. According to a recent cross-sectional study, there is an association between BMD of the forearm and hypovitaminosis D in adolescents (14). Taken together, these studies imply that the effect of vitamin D may be site specific.

The subjects in this study were healthy and willing to commit to 3 y of participation; therefore, they probably represented the healthiest segment of the young population. To minimize the confounding effect of changing bone dimensions associated with growth, we interpreted bone acquisition also in terms of BMAD, which reduces the dependence of bone accrual on bone size and changing dimension (15, 16). The size correction is particularly important when evaluating longitudinal BMD changes in rapidly growing children and adolescents (38). Because children of the same chronologic age may differ by skeletal age and pubertal stage (39), matching for these variables is important. Moreover, matching for growth velocity according to whether growth is in the acceleratory or deceleratory phase is essential in follow-up studies, because interventions may have different effects depending on the phase of growth (8). For these reasons, we also used the reproductive year as a covariate, taking into account both age and pubertal stage.

The study participants had, in general, low dietary vitamin D intakes and low serum 25(OH)D values at the onset of this long-term health study. To guard the participants' health, all subjects were supplemented with vitamin D in the wintertime over the 3-y period, and this procedure may have reduced any differences in ΔBMD and ΔBMAD between baseline serum 25(OH)D tertiles. However, vitamin D supplementation had a weak effect on 25(OH)D concentration (40).

Prospective studies on bone mineral accumulation have shown that physical activity has a strong osteogenic effect on the growing skeleton, particularly at the femoral neck (18, 41, 42). Fortunately, the participants in the various physical activity groups were distributed equally in each serum 25(OH)D tertile. However, there were significant differences in unadjusted baseline BMD and BMAD of the femoral neck between serum 25(OH)D tertiles. Furthermore, to avoid these confounding effects, we adjusted the ΔBMD and ΔBMAD values for the baseline bone density values and the amount of physical activity over 3 y.

The findings of this study contribute to a better understanding of the disturbances in bone metabolism related to an inadequate vitamin D status. Hypovitaminosis D seems to induce deleterious effects on bone mineral accrual, particularly at the lumbar spine, precisely in a period of human growth and development when peak bone mass should be achieved. Dietary enrichment or supplementation with vitamin D should be seriously considered to ensure an adequate vitamin D status during the peripubertal years.

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