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Vitamin D Supplementation during Infancy Is Associated with Higher Bone Mineral Mass in Prepubertal Girls*

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ABSTRACT

The objective of this study was to determine whether vitamin D supplementation of breast-fed infants during the first year of life is associated with greater bone mineral content and/or areal bone mineral density (aBMD) in later childhood. The design was a retrospective cohort study. One hundred and six healthy prepubertal Caucasian girls (median age, 8 yr; range, 7–9 yr) were classified as vitamin D supplemented or unsupplemented during the first year of life on the basis of a questionnaire sent to participating families and their pediatricians. Bone area (square centimeters) and bone mineral content (grams) were determined by dual energy x-ray absorptiometry at six skeletal sites. Vitamin D receptor (VDR) 3′-gene polymorphisms (*Bsm*I) were also determined. The supplemented (n = 91) and unsupplemented (n = 15) groups were similar in terms of season of birth, growth in the first year of life, age, anthropometric parameters, and calcium intake at time of dual energy x-ray absorptiometry. The supplemented group had higher aBMD at the level of radial metaphysis (mean ± SEM, 0.301 ± 0.003 *vs.* 0.283 ± 0.008; $P = 0.03$), femoral neck (0.638 ± 0.007 *vs.* 0.584 ± 0.021; $P = 0.01$), and femoral trochanter (0.508 ± 0.006 *vs.* 0.474 ± 0.016; $P = 0.04$). At the lumbar spine level aBMD values were similar (0.626 ± 0.006 *vs.* 0.598 ± 0.019; $P = 0.1$). In a multiple regression model taking into account the effects of vitamin D supplementation, height, and VDR genotype on aBMD (dependent variable), femoral neck aBMD remained higher by 0.045 g/cm^2 in the supplemented group ($P = 0.02$).Vitamin D supplementation in infancy was found to be associated with increased aBMD at specific skeletal sites later in childhood in prepubertal Caucasian girls. (*J Clin Endocrinol Metab* **84:** 4541–4544, 1999)

THE REQUIREMENT of vitamin D for breast-fed term infants remains an area of controversy. The American Academy of Pediatrics Committee on Nutrition recommends vitamin D supplementation for breast-fed infants only when the mother's vitamin D nutrition has been inadequate, or if the infant does not benefit from adequate UV light exposure (1). Breast milk has vitamin D activity between 30–60 IU/L (2) and would theoretically provide less than the recommended daily allowance for infants (400 IU/day) (3). However, no evidence of vitamin D deficiency in unsupplemented healthy term breast-fed infants has been demonstrated. Studies comparing supplemented breast-fed infants *vs.* unsupplemented ones have documented higher levels of serum 25-hydroxyvitamin D with vitamin D supplementation but similar bone mineral content during the first year of life (4–8). These studies have measured bone mineral content (BMC) by single photon absorptiometry at the junction of the middle and distal third of the radius. This skeletal site is nonbearing and predominantly cortical bone. However, to our knowledge no studies have reported results for areal bone mineral density (aBMD) or BMC after the first year of life in supplemented *vs.* unsupplemented infants. Furthermore, no study has explored the possible effect of vitamin D supplementation at other skeletal sites than the radius, particularly at the spinal and femoral levels. Indeed, there is evidence that specific skeletal sites respond differently to environmental or genetic factors (9, 10).

The present study aimed at determining whether vitamin D supplementation during the first year of life in breast-fed infants was associated with greater aBMD/BMC in later childhood at specific skeletal sites.

Subjects and Methods

Study design and subjects

The protocol was approved by the ethics committee of the Department of Pediatrics of the University Hospital of Geneva. A retrospective cohort study was performed on 149 healthy prepubertal Caucasian girls who were enrolled in a randomized trial of calcium supplementation (9). These girls were recruited through the Public Health Youth Service. They were free of chronic disease, gastrointestinal disease capable of inducing malabsorption, and congenital or acquired bone disease and did not use regular medication. At enrolment and before calcium supplementation, they had anthropometric measurements, calcium intake assessment by a frequency questionnaire, and measurement of bone variables as previously described (9). Subsequently, the following information was obtained from birth records and from a questionnaire sent to participating families and their pediatricians: gestational age, weight and height at birth and at 1 yr of age, type of feeding during the first 6 months of life, and vitamin D supplementation. Information was obtained for 127 girls (85% response rate). From this group were excluded subjects who were exclusively formula fed during the first 6 months of life (n = 7), those born before 37 weeks gestation (n = 5), those who avoided milk products in infancy for query milk allergy (n = 3), and those for whom vitamin D supplementation could not be reliably specified (n = 6). Finally, 106 girls constituted the study population

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and were divided into supplemented (n = 91) and unsupplemented groups (n = 15).

Measurement of bone variables

BMC (grams), bone area (BA; square centimeters), and aBMD (g/cm^2) were determined by dual energy x-ray absorptiometry (DXA) using a Hologic, Inc., QDR-2000 instrument (Waltham, MA). The coefficient of variation for repeated measurements of various skeletal sites varied between 1.0–1.6% for aBMD and 0.3–3% for BMC. An estimate of volumetric mineral density [bone mineral apparent density (BMAD)] was calculated as previously described for lumbar spine, midradius, femoral diaphysis, and femoral neck (11, 12). Six skeletal sites were assessed as previously described (9): distal metaphysis of the radius, distal diaphysis of the radius, femoral neck, femoral trochanter, femoral middiaphysis, and L2–L4 vertebrae in antero-posterior view.

Vitamin D receptor (VDR) gene polymorphisms

DNA was extracted from saliva, and VDR gene allelic polymorphisms were determined by *Bsm*I endonuclease restriction after specific PCR amplification (10). Genotypic polymorphism was defined as BB (absence of restriction site on both alleles), bb (presence of restriction site on both alleles), or Bb (heterozygous).

Statistical analysis

The results are the mean ± SEM. Demographic and anthropometric characteristics and bone variables were compared between the supplemented and unsupplemented groups using a two-tailed Student's *t* test when variables were normally distributed and a Mann-Whitney U test otherwise. The Fisher exact test was used for comparison of frequencies. The possible confounding effect of VDR genotype, age, weight, and height on the relationships between vitamin D supplementation and BMC, aBMD, and BMAD (dependent variables) was taken into account using multiple linear regression. $P < 0.05$ was considered significant.

Results

Cohort and vitamin D supplementation

The demographic and anthropometric characteristics and calcium intake were similar between the supplemented and unsupplemented groups (Table 1). The supplemented group had received 400 IU/day vitamin D (cholecalciferol) for a median supplementation period of 12 months (range, 2–48 months). In the unsupplemented group reasons for nonsupplementation were lack of prescription (n = 7), poor palatability of the vitamin D supplement (n = 5), and parental choice (n = 3). The median duration of breast feeding was similar in the supplemented (4 months) and unsupplemented (5.5 months) groups. There was no association between duration of breast feeding and BMC or BMD.

TABLE 1. Demographic and anthropometric characteristics of the supplemented (VD+) and unsupplemented (VD−) groups

	VD+ (n = 91)	VD− (n = 15)
Gestational age (weeks)	39.9 ± 0.1	39.8 ± 0.4
Born in summer, no. (%)	16 (18)	5 (33)
Birth wt (g)	3235 ± 46	3189 ± 105
Birth ht (cm)	49.5 ± 0.2	50.3 ± 0.5
Wt at 1 yr (kg)	9.3 ± 0.1	9.1 ± 0.2
Ht at 1 yr (cm)	74.2 ± 0.4	74.2 ± 0.8
Age at time of DXA (yr)	7.9 ± 0.1	7.8 ± 0.1
Wt at time of DXA (kg)	26.9 ± 0.4	25.9 ± 1.1
Ht at time of DXA (cm)	128.0 ± 0.6	127.2 ± 1.2
Calcium intake at time of DXA (mg/day)	841 ± 30	837 ± 82

Values are the mean ± SEM.

Bone variables

BMC was significantly higher in the supplemented group at the level of femoral neck and femoral trochanter. At the levels of the radius, femoral diaphysis, and lumbar spine, the difference was of lower magnitude (3–9%) and not statistically significant. BA at the different skeletal sites tended to be higher in the supplemented group, but did not reach statistical significance (Table 2). aBMD was significantly higher in the supplemented group at the level of the radial metaphysis (0.301 ± 0.003 *vs.* 0.283 ± 0.008; $P = 0.03$), femoral neck (0.638 ± 0.007 *vs.* 0.584 ± 0.021; $P = 0.007$), and femoral trochanter (0.508 ± 0.006 *vs.* 0.474 ± 0.016; $P = 0.04$; Fig. 1). aBMD was not statistically different at the levels of lumbar spine (0.626 ± 0.006 *vs.* 0.598 ± 0.019; $P = 0.1$), radial diaphysis (0.434 ± 0.003 *vs.* 0.427 ± 0.010; $P = 0.1$), and femoral diaphysis (1.030 ± 0.009 *vs.* 0.996 ± 0.026; $P = 0.2$). BMAD, taken as an estimate of volumetric density, was higher in the supplemented group at the level of the femoral neck only (Table 2). In multiple regression models adjusting for the effects of age, weight, and height at DXA, the differences in BMC, aBMD, and BMAD described above remained significant in the supplemented group.

VDR gene polymorphisms

As VDR gene polymorphisms have been associated with aBMD levels in prepubertal girls (10, 13), it appeared important to study the polymorphisms distribution in our population. The distribution of the VDR gene polymorphisms was slightly different in the two groups. The VDR Bb genotype was significantly more frequent in the supplemented group (47 of 86 *vs.* 3 of 15; $P = 0.02$). The prevalences of BB (8 of 86 *vs.* 4 of 15) and bb alleles (31 of 86 *vs.* 8 of 15) were not statistically different. When VDR genotype was taken into account by multiple linear regression, aBMD at the level of the femoral neck remained significantly higher in the supplemented group. After adjusting for the effects of age, weight, height at time of DXA, and VDR genotype, femoral neck aBMD remained higher by 0.045 g/cm^2 in the supplemented group ($P = 0.02$).

Discussion

In this retrospective cohort study of prepubertal girls, vitamin D supplementation in infancy was associated with higher BMC and aBMD in later childhood. The differences in BMC and aBMD at the level of the femoral neck approximated 0.5 and 0.7 SD, respectively, in favor of the supplemented group. A difference of this magnitude may have a significant impact on peak bone mass and thereby on the risk of postmenopausal fracture and could be clinically significant (14). There was a general trend for higher BA and BMC in the supplemented group at the six examined sites. This trend did not appear consistent for BMAD, which may imply that vitamin D impacts more on skeletal growth than on volumetric bone mineral density; an alternative explanation would be a lack of power to find a difference in BMAD.

Our population was homogeneous, and the supplemented and unsupplemented groups were similar in terms of calcium intake and demographic characteristics, such as race,

TABLE 2. Bone area (BA), bone mineral content (BMC), and bone mineral apparent density (BMAD) at the different skeletal sites in prepubertal girls supplemented (VD+) or unsupplemented (VD−) with vitamin D

Skeletal sites	BA (cm^2)		BMC (g)		BMAD (g/cm^3)	
	VD+ (n = 91)	VD− (n = 15)	VD+ (n = 91)	VD− (n = 15)	VD+ (n = 91)	VD− (n = 15)
Radial metaphysis	2.175 ± 0.024	2.110 ± 0.057	0.657 ± 0.012	0.601 ± 0.029		
Radial diaphysis	2.217 ± 0.019	2.183 ± 0.040	0.964 ± 0.013	0.934 ± 0.033	0.500 ± 0.004	0.500 ± 0.012
Femoral neck	2.525 ± 0.023	2.497 ± 0.049	1.614 ± 0.025	1.465 ± 0.070[a]	0.325 ± 0.004	0.299 ± 0.010[a]
Femoral trochanter	3.695 ± 0.081	3.308 ± 0.117	1.885 ± 0.050	1.576 ± 0.093[a]		
Femoral diaphysis	8.909 ± 0.132	8.517 ± 0.316	9.213 ± 0.182	8.471 ± 0.381	0.714 ± 0.006	0.726 ± 0.020
Lumbar spine L2–L4	25.303 ± 0.291	24.559 ± 0.723	15.916 ± 0.300	14.837 ± 0.896	0.239 ± 0.002	0.235 ± 0.006

Values are means ± SEM.

[a] $P < 0.05$ compared to the supplemented group.

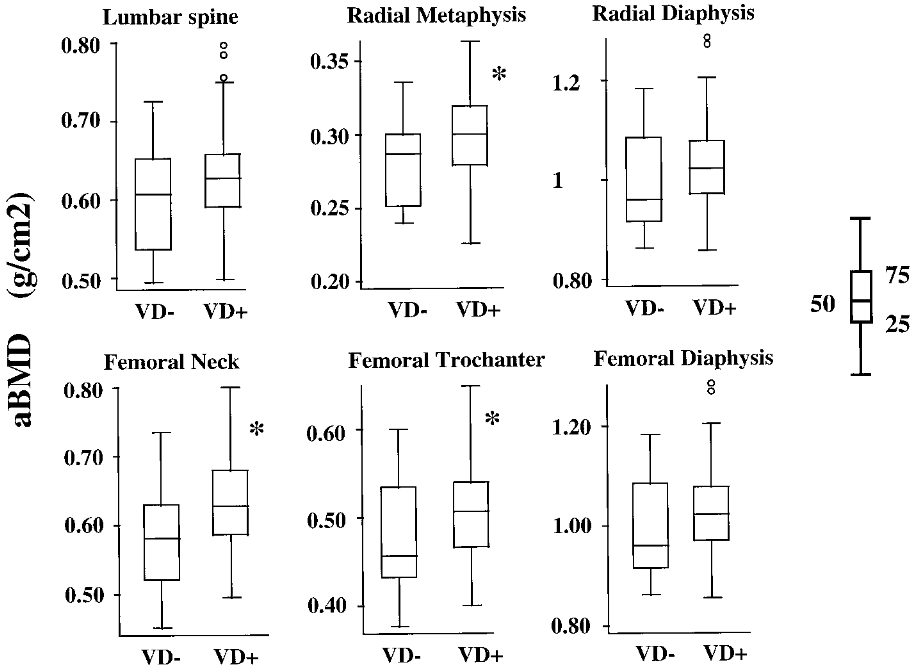

FIG. 1. Box plots illustrating the 25th, 50th, and 75th percentiles of BMD (aBMD) at six skeletal sites in the unsupplemented (VD−) and vitamin D-supplemented (VD+) subjects. aBMD was higher in the supplemented group (*) at the levels of the radial metaphysis ($P = 0.03$), femoral neck ($P = 0.007$), and femoral trochanter ($P = 0.04$).

age, and anthropometric parameters. Measurements of bone variables were made with the same instrument using a standardized protocol, and observers were unaware of the vitamin D supplementation history of participants.

Our findings are in agreement with previous studies comparing supplemented and unsupplemented healthy term breast-fed infants (4, 6, 8). These studies measured BMC at the junction of the middle and distal radius using single photon absorptiometry after a relatively short follow-up period (16 weeks to 12 months) and failed to show an increase in BMC at the distal radius level. In our study, the difference in BMC of 3% at the radial diaphysis was not statistically significant. Skeletal sites differ in structure (cortical or trabecular bone) and react differently to environmental and nutritional factors (9, 10). For instance, calcium supplementation in prepubertal girls appeared to increase bone mass accrual preferentially in the appendicular skeleton (9). In the present study BMC was significantly higher at the levels of femoral neck and trochanter in the vitamin D-supplemented group. In a way analogous to the sensitivity to calcium supplements, vitamin D supplementation may have a different impact on specific skeletal sites. Our findings might be particularly clinically relevant considering the risk of fracture at the femoral neck level in later life.

Several limitations to our study need to be stressed. An association between vitamin D supplementation and increased bone mineral mass does not imply causality. The population studied was homogenous, and no difference was found in the demographic characteristics. However several potential confounding factors, such as calcium consumption and sunlight exposure in infancy, were impossible to appraise and may contribute to the observed difference between the two groups. The information on vitamin D was evaluated several years after the supplementation, and misclassification of this variable may have occurred. The VDR BB polymorphism has been associated with lower BMD in prepubertal girls (10, 13), and this genotype tended to be more prevalent in the unsupplemented group. However, the small number of subjects precluded any reliable relationship being established. Furthermore, adjusting for VDR genotype in a multiple regression model did not attenuate the difference in femoral neck aBMD; the latter remained significantly higher in the supplemented group.

In conclusion, vitamin D supplementation in infancy could be associated with increased BMC and aBMD at specific skeletal sites in 8-yr-old prepubertal girls. If this positive influence was maintained later in life, primary prevention in the pediatric population would need serious consideration. Thus, a possible beneficial effect of early vitamin D supplementation on bone mineral mass and density at specific skeletal sites deserves further investigation in prospective controlled studies.

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