

Vitamin D deficiency and age at menarche: a prospective study^{1–3}

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ABSTRACT

Background: Early menarche is a risk factor for cardiometabolic disease and cancer. Latitude, which influences sun exposure, is inversely related to age at menarche. This association might be related to vitamin D, but to our knowledge it has not been investigated in prospective epidemiologic studies.

Objective: We studied the association between vitamin D status and the occurrence of menarche in a prospective study in girls from Bogota, Colombia.

Design: We measured plasma 25-hydroxyvitamin D [25(OH)D] concentrations in a random sample of 242 girls (mean \pm SD age: 8.8 ± 1.6 y) and followed them for a median of 30 mo. Girls were asked periodically about the occurrence and date of menarche. Baseline 25(OH)D concentrations were categorized as <50 nmol/L (deficient), ≥ 50 and <75 nmol/L, or ≥ 75 nmol/L (sufficient). The incidence of menarche was compared between groups by using time-to-event analyses.

Results: A total of 57% of girls in the vitamin D-deficient group reached menarche during follow-up compared with 23% of girls in the vitamin D-sufficient group (P -trend = 0.0004). The estimated mean (\pm SE) ages at menarche in the same groups were 11.8 ± 0.2 y and 12.6 ± 0.2 y, respectively ($P = 0.0009$). After adjustment for baseline age and BMI-for-age z score in a Cox proportional hazards model, the probability of menarche was twice as high in vitamin D-deficient girls than in girls who were vitamin D-sufficient (HR: 2.05; 95% CI: 1.03, 4.07; $P = 0.04$). Similar results were obtained in girls aged ≥ 9 y at baseline (HR: 2.39; 95% CI: 1.14, 5.00; $P = 0.02$).

Conclusion: Vitamin D deficiency is associated with earlier menarche. *Am J Clin Nutr* 2011;94:1020–5.

INTRODUCTION

Early menarche is related to increased risk of adverse health outcomes during adulthood including obesity (1), type 2 diabetes (2), cardiovascular disease (3), and breast (4) and endometrial (5) cancers. In addition, early menarche has been associated with behavioral and psychosocial risk factors during adolescence, such as alcohol consumption and smoking, early sexual debut, and teenage pregnancy (6, 7). Although the timing of menarche is genetically determined to some extent (8, 9), there is also a wide variability between populations that should be explained by environmental characteristics. An ongoing secular decline in age at menarche worldwide has motivated the search for factors that affect the onset of puberty (10, 11); modification of these factors might contribute to decrease risk of adverse health outcomes related to early menarche.

A geographic north-south gradient in age at menarche was described (12); girls who live at higher latitudes appear to have an earlier initiation of menses than girls who live closer to the equator (13). Although this pattern might be explained by differences in temperature, light-darkness rhythms, and socioeconomic conditions, it also corresponds with a geographic gradient in sun exposure that, in some regions, coincided with vitamin D status (14). Vitamin D deficiency is associated with the development of adiposity in children (15), and childhood obesity could be a risk factor for early puberty (16); thus, vitamin D might play a role in the timing of puberty. However, to our knowledge, this hypothesis has not been thoroughly examined in epidemiologic investigations.

We evaluated the associations of vitamin D serostatus with age at menarche in a prospective study of school-age girls who were followed for 2.5 y in Bogota, Colombia.

SUBJECTS AND METHODS

Study population

The study was conducted in the context of the Bogota School Children Cohort, which is an ongoing longitudinal investigation of nutrition and health in school-age children. Details on the cohort design (17) and vitamin D substudy (15) have been previously reported. In brief, in February 2006, we recruited a randomly selected group of 3202 children (age range: 5–12 y) who were enrolled in public primary schools of the city. The sample was representative of low- and middle-income families who lived in Bogota because the public school system enrolls a majority of children from these strata. At the time of enrollment, we collected information on sociodemographic characteristics and health habits of participants and their families with the use of a parent self-administered questionnaire. During the next few weeks, research assistants visited children at the schools and

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obtained a fasting blood sample and anthropometric measurements. Weight was measured in light clothing to the nearest 0.1 kg on Tanita HS301 electronic scales (Tanita), and height was measured without shoes to the nearest 1 mm with wall-mounted Seca 202 stadiometers (Seca). We visited the schools again in June and November 2006 to perform additional anthropometric assessments and once yearly in 2007 and 2008. When children were absent from the school at the time of the assessment, they were visited at home. At each of these assessments, girls were asked whether or not they had already started menstruating, and if they responded affirmatively, we recorded the date of the first menstrual period.

The parents or primary care providers of children provided written informed consent before enrollment. The study protocol was approved by the Ethics Committee of the National University of Colombia Medical School; the Institutional Review Board at the Harvard School of Public Health approved the use of data from the study.

Laboratory methods

Blood samples were collected by venipuncture and transported in ice and protected from light to the NIH (Bogota, Colombia), where plasma was separated from an EDTA-coated aliquot and cryopreserved until transportation to the United States. Plasma 25(OH)D⁴ was quantified in samples from 479 randomly selected children at the Clinical and Epidemiologic Research Laboratory of the Children's Hospital Boston (Boston, MA) by an enzyme immunoassay (Immunodiagnostic Systems Inc) that used a competitive binding technique. All samples were analyzed in duplicate. The assay had a sensitivity of 5 nmol 25(OH)D/L, intraclass CV of 5.3–6.7%, and interclass CV of 4.6–8.7%.

Data analysis

The main exposure of interest, which was vitamin D status, was classified according to 25(OH)D concentrations in 3 groups as follows: <50 (deficient), ≥50 and <75, or ≥75 (sufficient) nmol/L (18).

Analyses were restricted to the 248 girls in the random sample of 479 children in whom vitamin D was quantified. This group did not differ from the rest of girls in the cohort with regard to age, anthropometrics, sociodemographic background, or estimated age at menarche (*see* supplemental Table 1 under "Supplemental data" in the online issue). Six girls who had already experienced menarche at the time of enrollment were excluded from analyses; thus, the final sample included 242 girls. The primary endpoint considered was whether girls had menarche during follow-up and the time from enrollment when menarche occurred.

We first examined the distribution of baseline child and maternal characteristics by categories of vitamin D. The BMI-for-age *z* score was estimated according to the WHO reference (19); maternal BMI was calculated from the measured height and weight in 26% of the mothers and from self-reported data otherwise.

In bivariate analyses, we compared the proportion of girls who reached menarche by categories of vitamin D status with the use

of chi-square and Cochran-Armitage tests. Next, we conducted time-to-event analyses with the use of KM curves. In these analyses, the time to the event was the age at menarche in decimal years, which was estimated from the date of menarche and the birthdate. Girls who did not have menarche during follow-up were censored at the last time they were seen and reported that menarche had not occurred. We estimated the mean age at menarche from areas under the KM curves for each vitamin D–exposure category and compared them with the use of the log-rank test.

The association between vitamin D status and age at menarche could have been confounded by the age or adiposity level at enrollment because vitamin D could have been redistributed from blood into adipose tissue as a hydrophobic compound (20) and adiposity may have been an independent risk factor for early menarche (16). Hence, we estimated adjusted HRs with the use of Cox proportional hazards models. In these models, the outcome was the time to menarche from enrollment in decimal years, and predictors included indicator variables for vitamin D–exposure categories as well as the baseline age and BMI-for-age *z* score of the girl, which was an indicator of overall adiposity. The proportional hazards assumption was verified by introducing terms for the interaction between time and covariates into the model. Because the probability of reaching menarche during follow-up was low in girls who were the youngest at recruitment, we conducted supplemental analyses restricted to girls who were ≥9 y old at baseline. All analyses were carried out with the use of the Statistical Analysis System software (version 9.2; SAS Institute Inc).

RESULTS

The mean (\pm SD) age of girls at recruitment was 8.8 ± 1.6 y, and the mean BMI *z* score was 0.13 ± 0.93 . The mean (\pm SD) plasma vitamin D concentrations was 71.0 ± 18.3 nmol/L; 11.6% of girls were vitamin D–deficient, whereas 51.2% of girls had 25(OH)D concentrations ≥50 and <75 nmol/L. At baseline, vitamin D deficiency was positively associated with the age, BMI *z* score, and overweight status of girls and with maternal age, single mother status, and BMI (Table 1).

The median length of follow-up was 30.0 mo (IQR: 28.5, 31.5 mo), which did not vary according to vitamin D status. Eighty-six girls (35.5%) had menarche during follow-up. The mean (\pm SE) age at menarche, which was estimated from the KM time-to-event curve was 12.4 ± 0.1 y. Vitamin D status was inversely associated with the probability of having menarche. In vitamin D–deficient girls, 57.1% of girls had menarche during follow-up, whereas the proportion was 39.5% in girls with 25(OH)D concentrations ≥50 and <75 nmol/L and 23.3% in girls who were vitamin D–sufficient (*P*-trend = 0.0004). Consistent with this finding, the estimated mean (\pm SE) age at menarche was lowest for vitamin D–deficient girls (11.8 ± 0.2 y) compared with girls whose 25(OH)D concentrations were ≥50 and <75 nmol/L (12.5 ± 0.1 y) or girls who were vitamin D–sufficient (12.6 ± 0.2 y) (*P* = 0.0009, log-rank test) (Figure 1).

Because vitamin D status varied with baseline age and BMI *z* score, which is a measure of adiposity, we estimated the probability of reaching menarche in relation to plasma vitamin D concentrations with adjustment for these potential confounders with the use of Cox proportional hazards models (Table 2).

⁴ Abbreviations used: IGF-I, insulin-like growth factor I; KM, Kaplan-Meier; 25(OH)D, 25-hydroxyvitamin D.

TABLE 1Distribution of sociodemographic characteristics according to plasma 25(OH)D concentrations in 242 school-age girls from Bogota, Colombia¹

	25(OH)D concentrations			<i>P</i> -trend ²
	<50 nmol/L (<i>n</i> = 28)	≥50 and <75 nmol/L (<i>n</i> = 124)	≥75 nmol/L (<i>n</i> = 90)	
Girls' characteristics				
Serum 25(OH)D (nmol/L)	45.1 ± 3.8 ³	63.1 ± 6.1	90.0 ± 13.5	<0.0001
Age (y)	9.0 ± 1.7	9.0 ± 1.5	8.3 ± 1.6	0.005
Not born in Bogota (%)	11.1	14.0	9.6	0.58
BMI-for-age <i>z</i> score ⁴	0.45 ± 1.17	0.15 ± 0.91	-0.01 ± 0.85	0.03
Overweight or obese (%) ⁵	21.4	12.3	5.6	0.01
Stunted (%) ⁶	7.1	11.5	10.1	0.84
Time playing outdoors (h/wk)	5.5 ± 8.3	6.4 ± 6.6	7.6 ± 10.4	0.29
Television watching (h/wk)	22.0 ± 11.4	18.0 ± 11.1	18.3 ± 16.6	0.46
Length of follow-up (mo)	31.9 ± 5.9	31.1 ± 7.3	31.0 ± 7.0	0.58
Maternal characteristics				
Age (y)	36.5 ± 6.8	36.2 ± 6.4	34.4 ± 6.2	0.05
Parity	2.9 ± 1.2	2.9 ± 1.1	2.6 ± 1.0	0.09
Education (y)	9.0 ± 3.4	8.5 ± 3.3	8.5 ± 3.3	0.63
Single mother (%)	37.0	30.7	14.6	0.005
Height (cm)	159.1 ± 7.1	157.4 ± 5.5	158.0 ± 6.1	0.76
BMI (kg/m ²)	25.6 ± 4.8	24.5 ± 3.9	23.6 ± 3.6	0.03
Lower socioeconomic stratum (%) ⁷	51.9	42.1	44.6	0.72

¹ 25(OH)D, 25-hydroxyvitamin D.² For continuous characteristics, Wald's test was used for a variable that represented 25(OH)D categories as continuous; for dichotomous characteristics, the Cochran-Armitage test was used.³ Mean ± SD (all such values).⁴ According to the WHO reference (19).⁵ According to the International Obesity Task Force definition (40).⁶ Height-for-age *z* scores less than -2.0 according to the WHO reference (19).⁷ Stratum 1 or 2 according to the city's classification (maximum of 4 strata in the sample).

After adjustment, girls who were vitamin D-deficient were twice as likely to have menarche during follow-up than were girls who had concentrations ≥75 nmol/L (HR: 2.05; 95% CI: 1.03, 4.07; *P* = 0.04). Additional adjustments for other baseline correlates of vitamin D status, including time spent outdoors on physical activity and maternal characteristics, did not alter the results (data not shown).

Because girls who were youngest at the start of the cohort had a low probability of menarche during the 2.5-y median time of follow-up, we repeated the analyses in the group of girls who were ≥9 y of age at baseline (*n* = 122) and, thus, were more likely to contribute events during the time of the study. The mean plasma vitamin D concentration in this group was 67.8 ± 16.9 nmol/L; 13.9% of the girls were vitamin D-deficient,

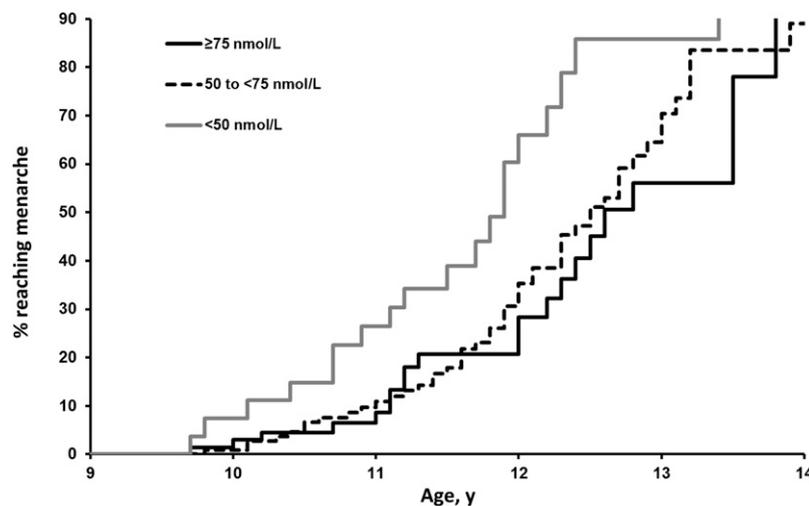


FIGURE 1. Cumulative probabilities of reaching menarche according to plasma concentrations of 25(OH)D in 242 Colombian school-age girls. The proportions of girls who reached menarche during a median of 30 mo of follow-up in groups with 25(OH)D concentrations of <50 nmol/L (deficient), ≥50 and <75 nmol/L, or ≥75 nmol/L (sufficient) were 57% (*n* = 16 of 28), 40% (*n* = 49 of 124), and 23% (*n* = 21 of 90), respectively (*P* = 0.0004, Cochran-Armitage test for trend). 25(OH)D, 25-hydroxyvitamin D.

TABLE 2

Menarche according to vitamin D concentrations at recruitment in 242 Colombian school-age girls

	<i>n</i>	Menarche during follow-up <i>n</i> (%)	Unadjusted HR (95% CI) ¹	Adjusted HR (95% CI) ¹	
				Model 1	Model 2
Vitamin D					
≥75 nmol/L	90	21 (23.3)	1.00	1.00	1.00
≥50 and <75 nmol/L	124	49 (39.5)	1.82 (1.09, 3.04)	1.12 (0.66, 1.89)	0.95 (0.56, 1.63)
<50 nmol/L	28	16 (57.1)	3.03 (1.58, 5.83)	2.54 (1.31, 4.92)	2.05 (1.03, 4.07)
<i>P</i> -trend ²		0.0004	0.0007	0.02	0.10
Age at baseline (y)	242	—	—	2.57 (2.10, 3.14)	2.78 (2.26, 3.44)
BMI-for-age <i>z</i> score	239	—	—	—	1.38 (1.09, 1.77)

¹ Derived from Cox proportional hazards models with the time to menarche as the outcome and predictors that included vitamin D categories plus baseline age (models 1 and 2) and BMI-for-age *z* score (model 2).

² Wald test was used for a variable representing categories of vitamin D, which was introduced into the model as a continuous predictor. For percentage of subjects who had reached menarche during follow-up, *P*-trend is from the Cochran-Armitage test.

59.8% of the girls had 25(OH)D concentrations ≥50 and <75 nmol/L, and 26.3% of the girls were vitamin D-sufficient. Seventy-one girls had menarche over a median of 30.0 mo of follow-up; the estimated mean age at menarche was 12.5 y (95% CI: 12.3, 12.7 y). Results of the association between vitamin D concentrations and age at menarche were essentially unchanged in this subset. Probabilities of menarche in groups with 25(OH)D concentrations <50, ≥50 and <75, and ≥75 nmol/L were 82.4%, 54.8%, and 53.1%, respectively (*P*-trend = 0.09), and estimated mean ± SE ages were 11.8 ± 0.3, 12.6 ± 0.1, and 12.6 ± 0.2 y (*P* = 0.002, log-rank test). After adjustment for age and BMI *z* score at baseline, vitamin D-deficient girls in this subset had 2.4 times greater probability of reaching menarche during follow-up than did girls who were vitamin D-sufficient (95% CI: 1.14, 5.00; *P* = 0.02).

DISCUSSION

In this cohort study of school-age girls, vitamin D deficiency was associated with the early onset of menses. The difference in the estimated mean age at menarche between vitamin D-sufficient and vitamin D-deficient girls was almost 1 y; this association remained strong after adjustment for key potential confounders that included age and adiposity at the time of vitamin D assessment. Confounding by physical activity outdoors was unlikely because regular exercise has not been consistently related to the timing of sexual maturation (22), and additional adjustment for indicators of physical activity did not change the estimates. Confounding by other nutrients present in food sources of vitamin D, such as fortified milk, also seems improbable. Higher intakes of milk (23) and calcium (23, 24) have each been related to earlier menarche; hence, these intakes could not explain an association of low vitamin D status with early menarche.

Inference from ecologic data indirectly suggests that a link between vitamin D status and age at menarche is possible. On the one hand, latitude is an important determinant of vitamin D status. Vitamin D synthesis is undetectable during the winter months at northern latitudes including in Edmonton (Canada, 52°N) and Boston (United States, 42°N), whereas synthesis occurs throughout the year at lower latitudes including those of Los Angeles (United States, 34°N) and San Juan (Puerto Rico, 18°N) (14). In contrast, there is an inverse correlation between

latitude and age at menarche. With the use of country-aggregated data from Grivas et al (13), the estimated correlation between age at menarche and latitude between 0° and 50° N was -0.54 (*P* = 0.003), which indicates that menarche occurs earlier in northern latitudes, where there is less vitamin D production, than near the Equator. However, direct evidence from epidemiologic investigations has been lacking. In an intervention study in Chinese girls 10 y of age, the supplementation of milk fortified with vitamin D and calcium compared with calcium only for 2 y did not have an effect on age at menarche over an extended 5-y follow-up period (25). However, the supplemental vitamin D dosage in this trial was only 3.3 μg/d, and the additional intake from diet seemed very low. By comparison, the difference in intake at the mean plasma vitamin D concentrations of vitamin D-sufficient (90.0 nmol/L) and vitamin D-deficient (45.1 nmol/L) girls in our study was 18.5 μg vitamin D/d, with the assumption that every 2.43 μg vitamin D increased blood concentrations by 1 nmol/L (26). Although in the China study, plasma vitamin D concentrations increased from 20.6 to 47.6 nmol/L in the vitamin D-fortified group, they were still within the range currently accepted as deficient (<50 nmol/L). This result suggests that a potential effect of vitamin D on menarche may be observed at doses higher than those tested in the China trial. A future clinical trial to test the effect of supplemental vitamin D on age at menarche should probably include dosages >20 μg/d. Because analyses in girls aged ≥9 y yielded similar results to those in the whole population, supplementation might not need to start at earlier ages to have an effect on age at menarche. However, the study of other potential effects of vitamin D supplementation on child health may require an earlier start of the intervention.

Mechanistic explanations of an effect of vitamin D deficiency on early menarche are speculative. We have previously reported that vitamin D-deficient school-age girls had a more rapid development of adiposity than did those who were vitamin D-sufficient (15), and increasing evidence from longitudinal epidemiologic studies indicated that childhood obesity could lead to accelerated sexual maturation (16, 27); thus, vitamin D status could indirectly affect the timing of menarche through its effect on obesity. Biochemical pathways might involve adipose-derived hormones. Some studies in mice (28, 29) and humans (30) indicated that increases in leptin resulted in early puberty; in contrast, 25(OH)D is inversely correlated with leptin

concentrations (31), but it is unknown whether the expression of leptin or other hormones from adipose tissue would change in response to vitamin D supplementation. There could be other biological mechanisms involved in the association of vitamin D deficiency with early menarche that are independent of obesity. IGF-I is one of the growth factors believed to modulate the onset of puberty by stimulating the gonadotropin-releasing hormone pulse (32). Zhen et al (33) showed that IGF-I increased the expression of gonadotropin-releasing hormone in vitro. Although the intracerebroventricular administration of IGF-I antibodies resulted in delayed puberty in male rats (34), intraventricular injections of IGF-I advanced puberty in female rats (35). In contrast, a recent study of 76 prepubertal girls showed an inverse correlation between IGF-I and 25(OH)D (36). Whether vitamin D concentrations directly influence IGF-I is not known. However, vitamin D receptors have been shown in different parts of the brain including the hypothalamus (37). Therefore, it is possible that vitamin D plays other unknown roles in the neuroendocrine regulation of the gonadotropic axis.

There were several strengths to our study. The longitudinal design of the study precluded a reverse causation bias, and the use of a biomarker to ascertain the exposure excluded the possibility of a recall bias. We had the possibility to adjust estimates for the potential confounding effect of key covariates including baseline age and BMI, which is a proxy for overall adiposity (38). Our estimate for age at menarche in this sample was 12.4 y and was close, albeit somewhat lower, than that previously reported for a group of public university students in the city of 12.7 y (39). The difference could be explained through a birth-cohort effect because the university students were born at least a decade before the school-age girls in the current study, and a negative secular trend in age at menarche has been described in Colombia (39). However, the similarity of the estimates supports the notion that the study sample could be representative of the population. One potential limitation of the study was the reliance on a single assessment of vitamin D at baseline to ascertain the exposure; nevertheless, 25(OH)D has high within-person correlations over time, and a single baseline measurement could provide an adequate representation of the long-term exposure (40).

In conclusion, vitamin D serostatus was positively related to age at menarche in a group of apparently healthy girls. Vitamin D deficiency was related to earlier menarche. In consideration of the health risks associated with early menarche, the benefits of delaying the onset of menses through a relatively inexpensive intervention such as vitamin D supplementation may be substantial. Therefore, it is very relevant to test the effect of vitamin D supplements on age at menarche in a randomized trial.

The authors' responsibilities were as follows—EV: designed the study, obtained funding, conducted data analyses, interpreted results, and wrote the first draft of the manuscript; CM and MM-P: contributed to the study design and data collection in the field; AB: participated in data analyses and interpretation; and all authors: contributed to the writing of the manuscript. None of the authors had any conflicts of interest in relation to this manuscript.

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