Vitamin-D Nutrition and Bone Mass in Adolescent Black Girls

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Objective: To examine the relationship between bone mass and serum levels of 25-hydroxyvitamin D and parathyroid hormone in African-American adolescent girls.

Study Design: A cross-sectional sample at a suburban research center.

Methods: Twenty-one adolescent black girls 12–14 years of age, were studied during winter with biochemical measurements of serum 25-hydroxyvitamin D (25-OHD) and parathyroid hormone (PTH). Bone mass assessment was done with dual energy x-ray absorbsiometry (DXA) and peripheral quantitative computed tomography of the radius (p-QCT). Anthropometric, physical activity and nutritional data were collected.

Results: All participants were vitamin-D deficient (serum 25-OHD level <50 nmol/L), of whom nine (43%) were severely vitamin-D deficient (serum 25-OHD level <20 nmol/L). Mean daily intake of dietary calcium was 540 mg/d and vitamin D was 195 IU/d. There was a positive correlation, although statistically not significant, between serum 25-OHD and various bone mass measurements. Serum PTH was inversely correlated to total body BMD (r=-0.51, p=0.02) and other bone mineral density at the lumbar spine, total femur and mid-radius.

Conclusion: Vitamin-D insufficiency is a widely prevalent problem among adolescent African-American girls. Our data implies that enhancing vitamin-D nutrition resulting in lower serum PTH levels could potentially influence their peak bone mass.

Key words: vitamins & minerals ■ bone mass ■ children/adolescents ■ African Americans

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INTRODUCTION

Bone mass in the geriatric population is determined by peak bone mass attained during young age and adult bone loss later in life. Although osteoporosis has traditionally been considered a disease of the elderly, it often has its origins during childhood due to impaired peak bone mass acquisition. It is generally accepted that those who achieve a higher peak bone mass are less at risk of having an osteoporotic fracture later in life.[1,2] Bone mineralization during childhood and adolescence is therefore of special interest since a low bone mass may result in an increased risk of fractures later in life. About 85–90% of peak adult bone mass is acquired by the pre-adolescent and adolescent years.[3,4] Although 80% of the variability in peak bone mass may be explained by genetic factors, a significant proportion is determined by environmental and nutritional factors.[5-7] Enhancing peak bone mass could have a greater influence on fracture reduction than any medication currently used to treat osteoporosis.

Although it is well established that black women have denser bones and an advantageous skeletal geometry[8] and lower risk of fractures, the hip fracture incidence is still as much as 40% that of white women.[9] In addition, with increasing longevity, the burden of osteoporotic fractures is increasing among this minority population as well.[10] Measures to prevent osteoporosis are therefore as important in African-American and other minority populations as in Caucasian women. Improvement in nutrition is a simple nonpharmacological approach to increase bone accretion in adolescents in order to enhance peak bone mass to the maximal genetic potential.

Vitamin-D supplementation improves the calcium economy and may be beneficial for skeletal mineralization during pubertal growth, especially among the most deficient, i.e., African Americans. African Americans have low serum 25-hydoxyvitamin-D (25-OHD) levels as a result of reduced production of vitamin D in the skin.[11] In the third NHANES study, up to 28% of adolescent African-American girls were reported to have vitamin-D insufficiency (25-OHD levels <62.5 nmol/L) even during summer at higher latitudes.[12] Data from a large

sample of 307 adolescents showed a high prevalence of vitamin-D deficiency in a Boston urban clinic. The highest prevalence was among African-American teenagers during winter.[13] Furthermore, obesity is a major health problem among adolescents and in particular among African-American women.[14] Obesity is associated with lower 25-OHD levels, making the population of black women even more vulnerable to hypovitaminosis D.[15]

It is now widely recognized that optimal vitamin-D nutrition is best described by levels of serum 25-OHD that are no longer accompanied by deleterious increased levels of parathyroid hormone (PTH).[16-18] Although still widely debated, most studies suggest that serum levels of 25-OHD >70–80 nmol/L must be attained to prevent secondary hyperparathyroidism.[19,20] Without sufficient sun exposure, most will not reach this level with vitamin D obtained from diet. Many investigators are now proposing to study the effect of augmenting vitamin-D nutrition at various stages of life as a strategy to improve bone health. In a study from Finland, adolescent girls followed for bone mineral density (BMD) and growth velocity over three years showed that vitamin-D status was important for optimal BMD and growth velocity.[21] We hypothesize that vitamin-D insufficiency could influence bone mass even in African-American girls even though their bone density is higher than Caucasians. We therefore chose to study a population of adolescent African-American girls to evaluate the relationship between vitamin-D indices and bone mass.

SUBJECTS AND METHODS

Subjects

We recruited 12–14-year-old girls, self-declared as African American, during late winter (February to April), on Long Island, NY (latitude 40° North). Healthy volunteers were recruited from local schools, health clinics and pediatric offices through flyers and referrals. Some volunteers were recruited from among family and friends of adult research participants at our center. Approximately 25 volunteers approached our center with in a period of three months. Two were excluded because of age, one had high BMI and one refused to consent. Exclusion criterion included any chronic medical illness such as diabetes mellitus, liver or renal disease, BMI of >35 kg/m^2, use of oral contraceptives, history of anorexia nervosa or eating disorder, pregnancy, medications interfering with calcium metabolism, tobacco use, spinal disease that affects interpretation of bone densitometry. A total of 21 girls were enrolled in the study. The study was approved by the institutional review board of Winthrop-University Hospital. All subjects and their parent signed a written informed consent prior to study participation.

Study Protocol

After the informed consent, subjects underwent a detailed medical history and a physical exam. The pubertal development was assessed by Tanner staging by a single physician (SAT). The height was measured by Harpenden stadiometer, and weight was recorded. Blood was collected for biochemical measurements of serum 25-OHD, PTH and calcium. Participants completed a food frequen-

Table 1. Descriptive statistics of anthropometry, bone mass measurement, blood, urine chemistries and dietary parameters for the study group (n=21)

Measured Variable	Mean ± SD	Min–Max
Age (Years)	13.0 ± 0.95	12.0–14.0
Height (cm)	157.7 ± 4.60	149.1–165.9
Weight (kg)	57.1 ± 12.9	36.8–80.0
BMI (kg/m^2)	22.1 ± 4.7	16.2–33.3
Tanner Stage		
II	1 (5%)	
III	6 (29%)	
IV	12 (57%)	
V	2 (9%)	
Dietary Calcium (mg/d)*	540.2 ± 273.8	197.2–1,346.8
Dietary Vitamin D (IU/d)	195.2 ± 154.7	33.8–508.0
Dietary Protein (g/d)	57.7 ± 19.6	23.3-102.1
Dietary Sodium (g/d)	2.6 ± 1.4	1.2–7.1
Physical Activity (kcal/week)**	8,056 ± 5,052	1,957–22,180
Total Body BMD (g/cm^2)	1.0 ± 0.1	0.9–1.3
Serum 25-OHD (nmol/L)	25.2 ± 10.7	12.5–48.0
Serum PTH (pg/ml)	44.1 ± 18.3	19.3 ± 93.3
Serum Calcium (mg/dl)	9.4 ± 0.45	8.5–10.5

BMC: bone mineral content; BMD: bone mineral density; 25-OHD: 25-hydroxyvitamin D; PTH: parathyroid hormone; * Assessed by food frequency questionnaire; ** Compendium physical activity score from a questionnaire

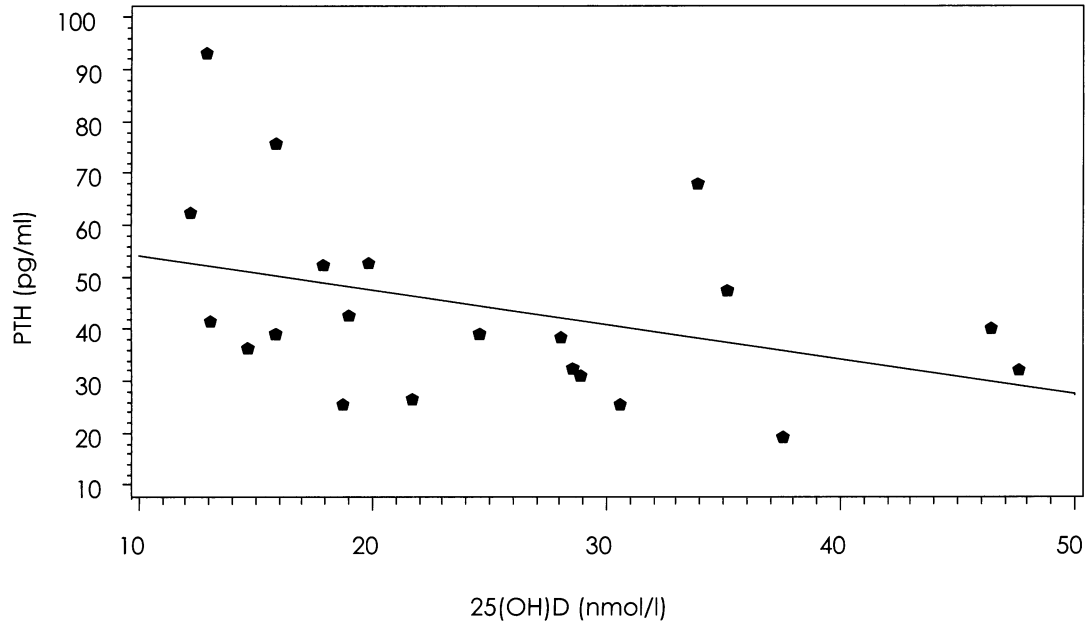
cy questionnaire at each visit for dietary calcium and vitamin-D intake with the assistance of a nurse. In addition, a three-day diet record was obtained, which was analyzed by Nutritionist Pro™ version 1.2 (First DataBank, San Bruno, CA). A recall physical activity log was completed by participants, and the compendium of physical activity was used to evaluate habitual physical activity.[22]

Bone Density Measurements

Each participant was measured by dual energy x-ray absorptiometry (DXA) on Hologic QDR 4500 (pediatric version 9.0). Whole body, lumbar spine, proximal femur and nondominant forearm were assessed by a single technician. The coefficient of variation at our center for lumbar spine (L1–4) is 0.81%, total hip is 0.62%, total body is 0.49%, and mid-radius is 0.78%.

Peripheral quantitative computed tomography (p-QCT) (Norland STRATEC XCT 960, Pforzheim, Germany) of the nondominant forearm was performed on all subjects. The ultradistal "4% site" and proximal "33% site" of the forearm were measured. For the ultradistal site, the scanner was positioned on the distal forearm and a coronal computed radiograph (scout view) was carried out. The 4% site corresponded to 4% of the forearm length from a reference line drawn through the middle of the ulnar border of the articular cartilage on scout view. Cross-sectional area (CSA) of the distal radius was determined after detecting the outer bone contour at a threshold of 267 mg/cm^3. Total vBMD (vBMD-tot) and trabecular vBMD (vBMD-trab) was determined as the mean density of the 45% central area of the bone's cross-section. The proximal radius was assessed by positioning the scanner at a site on the forearm whose distance from the ulnar styloid process corresponded to

Figure 1. Inverse relationship between serum 25-hydroxyvitamin D and serum parathyroid hormone in adolescent African-American girls (n=21)



r = -0.44, n=21, p<.05

Table 2. Spearman correlations among serum, parathyroid hormone and bone mass measurements (n=21)

Bone Mass Measurements	Mean (SD)	Correlation with PTH	
		R	p
Total-body BMC by DXA (g/cm^2)	1.01 (0.10)	-0.51	**0.02**
BMD spine L1-4 by DXA (g/cm^2)	0.90 (0.15)	-0.48	**0.03**
BMD total femur by DXA (g/cm^2)	0.92 (0.15)	-0.70	**0.0004**
BMD mid-radius by DXA (g/cm^2)	0.53 (0.07)	-0.45	**0.04**
Trabecular density at ultradistal radius by p-QCT (mg/cm^3)	213.3 (46.1)	-0.27	0.31
Total bone density at proximal 1/3 radius by p-QCT (mg/cm^3)	810.5 (72.7)	-0.24	0.31
Cortical bone density at proximal 1/3 radius by p-QCT (mg/cm^3)	1100.0 (40.8)	-0.18	0.45

PTH: parathyroid hormone; DXA: dual emission x-ray absorptiometry; p-QCT: peripheral quantitative computed tomography; BMC: bone mineral content

33% of the forearm length. The entire cross-sectional area of the proximal radius was determined by detecting the outer and inner cortical bone contour at a threshold of 710 mg/cm^3. Total as well as cortical bone density (mg/cm^3) were calculated for the cross-section of the bone. The accuracy of the measurements on p-QCT was assessed using a phantom. The mean coefficient of variation for total vBMD using healthy adult volunteers at our center is 2.08%.

Biochemistry

A fasting blood sample was drawn for assessment of serum 25-OHD and PTH. Serum PTH was measured by the Allegro intact-PTH immunoassay purchased from Nichols Institute Diagnostics (San Juan Capistrano, CA).[23] The intra-assay coefficient of variation (CV) at our site is 5.2% and the inter-assay CV is 9.0%. Serum 25-OHD was measured by a radioimmunoassay (RIA) using a kit manufactured by DiaSorin Inc. (Stillwater, MN).[24] Our laboratory participated in (and is certified) by the Vitamin D External Quality Assessment Scheme (DEQAS), while the study was carried.[19,20] The intra-assay CV is 4.1% and the inter-assay CV is 7.0%. Serum calcium was measured by O-Cresolphthalein complex using automated equipment, Dimension-RXL (Dade, DE).

Statistical Analysis

The results are represented as mean ± SD. Data were analyzed using SAS v9.1 (Cary, NC). Both the Pearson product-moment and the Spearman rank-order correlation coefficients were computed. We examined the effects of other variables such as age, BMI, Tanner stage dietary calcium, sodium, protein, vitamin K and caffeine on the relationship of serum vitamin D, PTH and bone mass using partial correlations. A stepwise multiple linear regression model was used to investigate the influence of and control for combinations of variables (25-OHD, PTH, age, BMI, height and Tanner stage) on BMD. The criterion for model entry was a p value of <0.20. Because of the small number of patients in Tanner stages 2 (n=1) and 5 (n=2) and because of the non-interval nature of that measure, we dichotomized Tanner stage by grouping stages 2 and 3 together and grouping stages 4 and 5 together. We then used this variable to represent Tanner stage. A binomial "sign test" was used to detect if there was a consistent direction within sets of individually insignificant correlations.

RESULTS

The participants were 12–14 years old with a mean BMI of 22.1 (±4.7) kg/m^2 (Table 1). The study subjects were representative of a typical suburban family with working parents. The pubertal development ranged from Tanner stage 2–5. Five of the 21 participants were postmenarchal. A review of food frequency questionnaires revealed a mean dietary calcium intake of 540 (±274) mg/d and vitamin-D intake of 195 IU/d (±155 IU/d). Approximately half of the participants consumed <200 IU/d of vitamin D, and all took less than the 1,300 mg/d of calcium, recommended by the panel on dietary intake of calcium for this age.[25] The mean dietary intake of sodium and protein was 2.6 g/d and 57.7 g/d respectively. The means of serum 25-OHD level and PTH levels at baseline were 25.2 (±10.7) nmol/L and 44.1 (±18.3) pg/ml, respectively. Serum 25-OHD and PTH levels were inversely related, r=-0.44, p=0.05 (Figure 1). The individual correlation between serum 25-OHD levels and individual bone mass measurements on DXA did not reach statistical significance. However, the fact that all correlations were positive is itself statistically significant (p<0.02, sign test). The first-order (uncontrolled) Spearman correlation between serum 25-OHD levels and total-body BMD (r=0.19) lumbar spine (r=0.15) BMD, total femur BMD (r=0.33) and mid-radius BMD (0.16). Controlling for BMI, the correlation between serum 25-OHD and lumbar spine BMD, total femur BMD, mid-radius BMD and total-body BMD all increased (and in the case of total femur BMD, it rose to a statistically significant 0.55 (p=0.01). The Spearman correlations between serum 25-OHD and trabecular BMD in the distal radius, the cortical and the total BMD at the proximal radius by p-QCT were all positive, though individually not signifi-

Table 3. Results of stepwise multiple linear regression model for the association of total body bone mineral density (TBBMD) with 25-OHD, PTH, age, BMI, height and Tanner stage (TS, dichotomized)

N=21	Zero-Order Pearson r						Model Estimates	
Variable	TBBMD	Age	PTH	BMI	TS	Height	Standardized ß	Incremental R^2 (%)
Age	0.75****						0.64****	56
PTH	-0.45**	0.0					-0.32***	17
BMI	0.66***	0.48**	-0.37*				0.24	4
TS	0.61***	0.76****	-0.01	0.50**				
Height	0.27	0.29	-0.08	0.52**	0.48**			
25-OHD	0.08	-0.10	-0.39*	-0.17	-0.10	0.09		

Model estimates for included variables and inter-correlation matrix for all variables; * p<0.10; **p<0.05; ***p<0.01; **** p<0.001; Cumulative R^2=77%****

cantly different from 0.

There was some evidence of an inverse relationship between serum PTH level and total-body BMD by DXA (Figure 2 and Table 2). The Spearman correlations between serum PTH and whole-body BMD, lumbar spine, total femur and mid-radius were r=-0.51 (p=0.02), -0.48 (p=0.03), -0.70 (p=0.0004) and -0.45 (p=0.04), respectively.

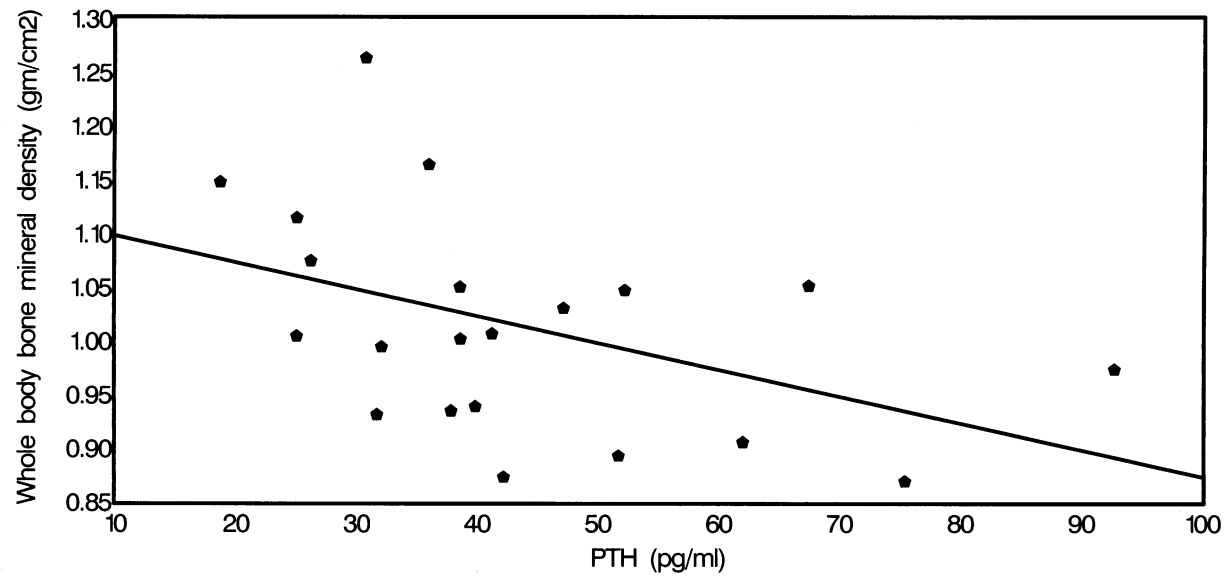
Multiple linear regression models were fitted to further examine the influence that age, BMI, height and Tanner stage have on the relationships among vitamin D, PTH and total-body BMD. Total-body BMD is chosen as a primary variable for this analysis because it reflects the entire skeleton rather than being influenced by regional factors. When both PTH and 25-OHD are included in the model while controlling for BMI and Tanner stage, PTH was consistently a better predictor of total-body BMD than 25-OHD. The regression slopes, expressed as standardized betas for the outcome variable total-body BMD are found in Table 3. The zero-order (unpartialled) Pearson correlation matrix among all the variables is included along with model estimates for the variables which jointly have significant linear relationships with total-body BMD. Age, PTH and BMI are the only variables accepted into the stepwise regression equation influencing total-body BMD. Thus, after controlling for BMI and age, serum PTH maintained its association with total-body BMD (p=0.02). The incremental R^2 for PTH was 17% in this model.

DISCUSSION

Our results indicate that African-American adolescent girls residing on Long Island (latitude 40° North) have very low serum 25-OHD levels during winter. All the participants in this study had initial serum 25-OHD levels <50 nmol/L (mean 25.2 nmol/L). In fact, the degree of vitamin-D insufficiency appears to be higher in our adolescent group as compared to the elderly. When compared to free-living, ambulatory black women aged >60 years from a prior study at our center,[26] African-American adolescent girls in the current study had 35% lower 25-OHD levels. This is probably due to increased health awareness among the elderly, where approximately 31% of the elder women took multivitamins and calcium supplements as compared to none of the young adolescent population. Similar findings have also been reported by Tangpricha et al.[27] in Boston, where they found 36% of the young adults had vitamin-D deficiency (defined as 25-OHD levels ≤50 nmol/ml) with a higher degree of insufficiency among the young as compared to the older women at the end of winter. In Maine, 48% white adolescent girls were vitamin D deficient by late winter.[28]

In recent years, much attention has been focused on increasing the calcium intake of adolescent girls. Studies with calcium supplementation to improve positive calcium balance[29,30] have suggested that 1,300 mg of calcium may not be enough and up to 1,500 mg/d is optimal calcium intake for adolescents.[31] There are, however, practical issues related to augmentation of calcium through dietary sources. As young females reach their teen years, most of them consume less dairy products because of the fear of gaining weight, which leads to a decline in calcium intake. Our participants had significantly low calcium intake. If calcium intake remains reduced, vitamin-D sufficiency becomes even more criti-

Figure 2. Serum PTH and total body BMD have an inverse relationship in African-American adolescent girls (n=21)



cal to improve the calcium economy.

Heaney and Weaver have pointed out that net absorption of calcium will not increase substantially in the absence of vitamin D-mediated active absorption.[32] At an intake of 600 mg/d (15 mmol/d) of calcium, net absorption is negative, and even 1,600 mg/d (40 mmol/d) is insufficient to offset urinary and dermal loss. In the absence of the active form of vitamin D, calcium absorption is limited to about 12.5% of the intake.[33] Attention should be focused on increasing vitamin-D intake along with calcium intake to achieve optimum bone mineralization during adolescence. In addition, optimal vitamin-D nutrition is now known to be important in extraskeletal aspects of human health.[34]

Although we failed to demonstrate a direct correlation between serum 25-OHD levels and bone mass in this small group of participants, our study suggests that elevated serum PTH levels may have an adverse influence on bone mass in African-American girls during adolescence. Similar findings have been reported by others[35] and in the large National Health and Nutrition Examination Survey (NHANES) III survey[20] among adults. Deleterious effect of hypovitaminosis D-induced secondary hyperparathyroidism in elderly people, as well as the benefits of vitamin-D supplementation are also well documented.[36] Our data are unique because unlike others it suggests that alteration in vitamin D-PTH axis can influence bone mass among African Americans, a vulnerable population, during pubertal growth, a crucial period for bone acquisition. Serum PTH, a surrogate marker for vitamin-D sufficiency, is considered a useful parameter able to reveal adequacy of calcium balance. Our data add another piece of evidence for studying the role of vitamin-D augmentation among healthy adolescents.

The increase in intracortical bone turnover during pubertal growth spurt coincides with the peak incidence of lower forearm fractures.[37] Since it has been shown that, during growth, reduced rates of remodeling are associated with increased bone density,[38] high levels of PTH could result in increased bone remodeling, increased bone fragility and a future increased risk of fracture. This phenomenon of elevation in PTH levels leading to increased bone turnover and defective mineralization of the skeleton, although well documented in the elderly,[39] may or may not be harmful during adolescence. Further studies are needed to confirm this.

In the adolescent age group, a few others have reported similar findings among Caucasians.[40-42] Outila et al.[41] reported that 14–16-year-old female participants with serum concentration of 25-OHD ≤40 nmol/L during winter with elevated PTH levels had low mean forearm BMD values at both the radius and ulna. Ilich et al.[43] report a direct correlation between vitamin D metabolites and bone acquisition in a small study of healthy Caucasian adolescent females. They found a positive association between serum calcitriol levels and the rate of increase in total-body BMD, bone mineral content (BMC) and radius BMD.[43] Lehtonen-Veromaa[21] concluded that the rate of change in BMD at the lumbar spine and femoral neck over three years among adolescent white girls was positively correlated with serum 25-OHD concentrations. A few investigators have reported contradictory findings.[44,45] Oliveri et al.[45] report a large seasonal variation in serum 25-OHD in children at the southern extremity of Argentina, without, however, a corresponding effect on bone mass. In essence, the influence of vitamin-D insufficiency on peak bone mass acquisition remains unclear, and data are limited only to the Caucasian population.

The current recommendation for adequate vitamin-D intake by the Food and Nutrition Board is 200 IU/d (5 mcg/d) during puberty.[25] Many believe that this is insufficient to achieve maximal benefits on calcium balance and bone acquisition during adolescence, especially in females who are underexposed to sunlight as a source of vitamin D.[46,47] While UV-B exposure and tanning have been shown to raise serum 25-OHD levels, they cannot be promoted clinically for the fear of skin cancer. The focus of debate has been on the total daily intake required to ensure a target serum 25-OHD concentration. In order to achieve serum 25-OHD levels >70 nmol/L, intakes much higher than the currently recommended vitamin-D daily allowance are required.[48] Furthermore, adolescent girls have a much higher demand for calcium because of a high rate of calcium accretion in the skeleton and therefore are expected to have a higher requirement for vitamin D.

Our study has some limitations, as recruiting school going children over a short period of time is challenging. Because we had a small number of participants in this study, power to detect small but clinically meaningful correlations was low. Any negative findings must be interpreted with this in mind. Nevertheless, we believe this pilot study is a useful addition to the literature as the adolescent African-American population has never been studied this way before. In addition to finding a significant linear relationship between PTH and BMD, the data presented will facilitate the design of larger cross-sectional and longitudinal studies of adolescent girls. The available techniques for biochemical measurement of 25-OHD and PTH have large coefficients of variation. To minimize these effects, we analyzed all the serum samples pooled together at the end of the study to diminish the inter-assay variability. The lack of significant correlation between serum 25-OHD and bone mass is not surprising in this small study. The subjects enrolled in the trial had low levels of 25-OHD. Therefore, the linear relationship between serum 25-OHD and bone mass may be relatively weak in a vitamin-D insufficient population. We are unable to explain the lack of significant correlation between vitamin-D indices and the DXA and p-QCT parameters in our study, except for the fact that we had a small number of participants. Absence of bio-

chemical bone turnover markers in our study limits the insight into mechanisms behind the potential ability of vitamin D to augment bone accretion.

In summary, our study suggests that improving vitamin-D nutrition may further improve skeletal mass acquisition in the African-American adolescent population, a highly vulnerable group. Prospective studies should be conducted to provide evidence for the effect of vitamin-D_3 supplementation on peak bone mass in all racial groups.

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