JCEM THE JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM

A 16-Week Randomized Clinical Trial of 2000 International Units Daily Vitamin D 3 Supplementation in Black Youth: 25-Hydroxyvitamin D, Adiposity, and Arterial Stiffness

Yanbin Dong, Inger S. Stallmann-Jorgensen, Norman K. Pollock, Ryan A. Harris, Daniel Keeton, Ying Huang, Ke Li, Reda Bassali, De-huang Guo, Jeffrey Thomas, Gary L. Pierce, Jennifer White, Michael F. Holick and Haidong Zhu

J. Clin. Endocrinol. Metab. 2010 95:4584-4591 originally published online Jul 21, 2010; , doi: 10.1210/jc.2010-0606

A 16-Week Randomized Clinical Trial of 2000 International Units Daily Vitamin D_3 Supplementation in Black Youth: 25-Hydroxyvitamin D, Adiposity, and Arterial Stiffness

Yanbin Dong, Inger S. Stallmann-Jorgensen, Norman K. Pollock, Ryan A. Harris, Daniel Keeton, Ying Huang, Ke Li, Reda Bassali, De-huang Guo, Jeffrey Thomas, Gary L. Pierce, Jennifer White, Michael F. Holick, and Haidong Zhu

Georgia Prevention Institute, Department of Pediatrics (Y.D., I.S.S.-J., N.K.P., R.A.H., D.K., Y.H., K.L., D.G., J.T., G.L.P., H.Z.); General Pediatrics, Department of Pediatrics (R.B.), and Endocrinology, Department of Medicine (J.W.), Medical College of Georgia, Augusta, Georgia 30912; and Endocrinology, Department of Medicine (M.F.H.), Boston University Medical Center, School of Medicine, Boston, Massachusetts 02118

Context: Vitamin D insufficiency/deficiency is commonly observed in black youth.

Objective: The aim was to determine 25-hydroxyvitamin D [25(OH)D] in response to 2000 IU vitamin D supplementation over time; to evaluate the relation between 25(OH)D concentrations and total body fat mass by dual-energy x-ray absorptiometry; and to determine whether vitamin D supplementation improves arterial stiffness measured by pulse wave velocity (PWV).

Design: We conducted a randomized, blinded, controlled clinical trial.

Setting and Participants: Forty-nine normotensive black boys and girls, aged 16.3 ± 1.4 yr, were randomly assigned to either the control group (400 IU/d; n = 24) or the experimental group (2000 IU/d; n = 25).

Results: Plasma 25(OH)D values at baseline and at 4, 8, and 16 wk were 34.0 ± 10.6, 44.9 ± 9.4, 51.2 ± 11.1, and 59.8 ± 18.2 nmol/liter, respectively, for the control group; and 33.1 ± 8.7, 55.0 ± 11.8, 70.9 ± 22.0, and 85.7 ± 30.1 nmol/liter, respectively, for the experimental group. The experimental group *vs.* the control group reached significantly higher 25(OH)D concentrations at 8 and 16 wk, respectively. Partial correlation analyses indicated that total body fat mass at baseline was significantly and inversely associated with 25(OH)D concentrations in response to the 2000-IU supplement across time. Furthermore, carotid-femoral PWV increased from baseline (5.38 ± 0.53 m/sec) to posttest (5.71 ± 0.75 m/sec) in the control group ($P = 0.016$), whereas in the experimental group carotid-femoral PWV decreased from baseline (5.41 ± 0.73 m/sec) to posttest (5.33 ± 0.79 m/sec) ($P = 0.031$).

Conclusion: Daily 2000 IU vitamin D supplementation may be effective in optimizing vitamin D status and counteracting the progression of aortic stiffness in black youth. Plasma 25(OH)D concentrations in response to the 2000 IU/d supplementation are negatively modulated by adiposity.
(*J Clin Endocrinol Metab* 95: 4584–4591, 2010)

The southeastern region of the United States has a sunny climate and relative proximity to the equator, which should favor plentiful cutaneous production of vitamin D. However, we have recently reported that black youth (14–18 yr of age) residing in Augusta, Georgia (~33° North latitude) are not ensured adequate vitamin D status indicated by circulating 25-hydroxyvitamin D [25(OH)D] in every season (1). Furthermore, low 25(OH)D concen-

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2010-0606 Received March 15, 2010. Accepted June 14, 2010.
First Published Online July 21, 2010

Abbreviations: BMI, Body mass index; BP, blood pressure; DBP, diastolic BP; 25(OH)D, 25-hydroxyvitamin D; PWV, pulse wave velocity; SBP, systolic BP.

trations have been found to be correlated with adiposity and a variety of adverse cardiovascular and metabolic risk factors in the pediatric population (2, 3). Therefore, randomized clinical trials are urgently needed to determine whether there is a relation between vitamin D and cardiovascular risk factors.

The current recommended dietary adequate intake of vitamin D, 200 IU/d, has been called into question (4). In fact, the American Academy of Pediatrics increased its recommendation for vitamin D intake for children and adolescents in October 2008 to 400 IU/d (5). However, Rajakumar *et al.* (6) demonstrated that 400 IU of vitamin D_3 daily for 1 month was still inadequate to raise the blood levels of 25(OH)D to at least 75 nmol/liter in 6- to 10-yr-old black children. Additionally, the mean 25(OH)D levels in black children (2–12 yr of age) residing in The Netherlands who received 400 IU vitamin D per day for 3 months in the summer reached only 40 nmol/liter (7). It is estimated that 1000 to 2000 IU is necessary to satisfy the body's needs for most people (8). A dose of vitamin D_3 up to 2000 IU daily is recognized as the tolerable upper intake level (9). Heaney *et al.* (10–12) suggest that individuals with 25(OH)D between 20 and 40 nmol/liter may require a daily dose of 2200 IU/d to reach the level of sufficiency of 75–80 nmol/liter. However, whether 2000 IU/d constitutes an effective dose to improve vitamin D status in black youth remains unknown.

Thus, the purpose of this randomized clinical trial in black youth using 400 IU/d vitamin D supplement as a control was to: 1) dynamically characterize 25(OH)D concentrations in response to 2000 IU vitamin D supplementation over time; 2) determine whether 2000 IU achieves optimal vitamin D status; 3) evaluate the relationship between adiposity and 25(OH)D concentrations; and 4) determine whether vitamin D supplementation improves arterial stiffness measured by pulse wave velocity (PWV).

Subjects and Methods

Subjects

Subjects were recruited from local high schools in Richmond County (Augusta, GA). The inclusion criteria were: black (African-American) as self-reported by the participants (and the parents if under 18 yr); aged 14–18 yr; apparently healthy based on self-reports from the participants (and parents if under 18 yr); nonhypertensive based on blood pressure (BP) screening (<95th percentile for age, sex, and height) (13); no medications; no difficulty swallowing pills; able to provide blood samples (*e.g.* no history of vasovagal response and veins that collapse); no pregnancy; and no vitamin supplement. The protocol was approved by the Human Assurance Committee of the Medical College of Georgia. Written informed parental consent and subject assent were obtained before testing.

Study design

This was an open-label, investigator-blinded, single-center, and randomized clinical trial (protocol ID no. 0901159). Forty-nine black boys and girls, aged 16.3 ± 1.4 yr, were randomly assigned to either the control group (400 IU/d; n = 24) or the experimental group (2000 IU/d; n = 25). To randomize the participants, our information technology department used the Microsoft random number generator function and retained the randomization schedule. The vitamin D_3 (cholecalciferol; Nature Made, Mission Hills, CA) trial was conducted for 16 wk from February to May 2009. To ensure and assess compliance, vitamin D supplements were issued at baseline, exchanged for a new bottle at both 4 wk and 8 wk, and returned to the research staff at posttesting. Participants were asked to place the pill bottle into a brown bag, which was then stapled shut and labeled with their unique ID number and date, and pills were counted later for compliance. To remain blinded, one research assistant who was not involved in data collection coordinated the supplement assignment schedule. The same research assistant was responsible for prepackaging the vitamin D supplement into brown bags and labeling them with subjects' ID numbers. All subjects were asked not to take additional vitamin D or calcium supplements or seek excessive sun exposure during the study period. To control for potential confounding effects of changes in diet and sun exposure, and physical activity on the outcome variables, subjects completed a 2-d food intake checklist targeting sources of vitamin D and calcium specifically designed for this study by a research dietitian, a sun exposure questionnaire that used commonly asked questions for assessing sunlight exposure (14), and the Physical Activity Questionnaire for Adolescents (PAQ-A) (15) at study entry and completion.

All the anthropometrics including height, weight, body mass index (BMI), and fasting blood and overnight urine samples were obtained at baseline and at 4, 8, and 16 wk. Body weight, height, and waist circumference were evaluated by using established protocols (16). At baseline and 16 wk, total body scans were assessed by dual-energy x-ray absorptiometry (QDR-4500W; Hologic, Waltham, MA) to measure total body fat mass (kilograms) (17).

Outcome variables

Plasma 25(OH)D, serum PTH, and overnight urine calcium were measured at baseline and at 4, 8, and 16 wk. Plasma 25(OH)D was measured by the enzyme immunoassay (Immunodiagnostic Systems, Fountain Hills, AZ). The intra- and interassay coefficients of variation were 5.6 and 6.4%, respectively. Analytical reliability of 25(OH)D assays was monitored through participation in DEQAS (Vitamin D External Quality Assessment Scheme). Bioactive intact PTH was measured by the ELISA kits (Immutopics, Inc., San Clemente, CA). Overnight urine calcium was measured by BioVision's Colorimetric Calcium Assay Kit.

At baseline and 16 wk, systolic BP (SBP) and diastolic BP (DBP) measurements (Dinamap1864 SX; Criticon, Inc., Tampa, FL) were taken three times at 5, 7, and 9 min after a 5-min relaxation period. The average was used to represent SBP and DBP. At baseline and 16 wk, carotid-radial PWV, carotid-femoral PWV, and carotid-dorsalis-pedis (foot) PWV were measured noninvasively with applanation tonometry (Millar Instruments, Houston, TX) (18) and commercially available acquisition and analysis software (SphygmoCor; AtCor Medi-

cal, Sydney, Australia). The PWV was then automatically calculated from measurements of pulse transit time and the distance traveled by the pulse between the two recording sites: PWV = distance (meters)/transit time (seconds). The same investigator who was blinded to the treatments performed PWV measurement both at baseline and posttest. The within-observer variability (mean difference ± SD of two measurements) was 0.15 ± 1.01 m/sec for carotid-radial PWV, 0.08 ± 1.10 m/sec for carotid-femoral PWV, and 0.17 ± 1.17 m/sec for carotid-dorsalis-pedis (foot) PWV.

Vitamin D status

Vitamin D deficiency was classified as 25(OH)D no greater than 50 nmol/liter, vitamin D sufficiency as 25(OH)D of at least 75 nmol/liter, and insufficiency as more than 50 but less than 75 nmol/liter (10, 19, 20).

Statistical analyses

Descriptive statistics are presented as mean ± SD if not stated otherwise. Data were analyzed using the intent-to-treat principle based on treatment assignment and not on treatment receipt. Differences in baseline group descriptive characteristics were compared by independent *t* tests if data were distributed normally and Mann-Whitney *U* tests in nonparametric distributions. Group differences for categorical variables were tested by Fisher's exact tests.

Responses of 25(OH)D, PTH, and serum/urine calcium to the two treatments (400 IU or 2000 IU vitamin D_3) were analyzed using repeated measures analysis of covariance (two groups by four time points). Age and sex were considered as potential confounders. PTH had a skewed distribution, and thus, it was log-transformed before analyses (normal distribution was confirmed after transformation). If a group by time interaction was observed, analyses of simple main effects were conducted to identify specific group differences. Tukey *post hoc* tests were performed to identify individual differences within groups across time. Similarly, responses of PWV parameters, SBP, and DBP to the two treatments were also analyzed using repeated measures ANOVA (two groups by two time points). Finally, partial correlation analyses, controlling for age and sex, were conducted to examine the relationship between total fat mass and 25(OH)D concentrations in the 400-IU and 2000-IU groups separately. All tests were conducted two-sided, and a P value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 18.02 for Mac OS X (PASW Statistics, Chicago, IL).

Results

Compliance

The overall compliance determined by pill count was 87%. There was no statistical difference in the overall compliance between the control group (88%) and the experimental group (85%) ($P = 0.65$). During the study, five subjects dropped out. Of them, three did not show up for testing and did not respond to our further contacts, one discontinued the high school education and became unavailable, and one was positive on the pregnancy test. As such, 44 subjects (21 in the control group, and 23 in the experimental group) from the total 49 subjects completed the study.

Baseline: clinical characteristics

The average baseline plasma level of 25(OH)D was 33.4 nmol/liter, which indicates a vitamin D deficiency (<50 nmol/liter) status in black youth. The baseline plasma 25(OH)D did not differ between the two groups as shown in Table 1. At baseline, 42 of the 44 participants had a 25(OH)D level of less than 50 nmol/liter. The two remaining participants, one in the control group and the other in the experimental group, had a 25(OH)D level above 50 nmol/liter (53.4 and 60.3 nmol/liter, respectively). In other words, none of the subjects in this study demonstrated a vitamin D sufficiency level (≥75 nmol/liter). The 25(OH)D concentration did not differ between females (32.5 ± 10.5 nmol/liter) and males (34.8 ± 8.2 nmol/liter; $P = 0.76$). The PTH concentration was lower in females (n = 20; 33.5 ± 10.0 pg/ml) than males (n = 25; 46.7 ± 21.5 pg/ml; $P = 0.02$).

Dynamic responses over time: 25(OH)D, PTH, and serum/urinary calcium

For plasma 25(OH)D levels, there was a significant group by time interaction ($P < 0.001$), even after adjusting for age and sex. Plasma 25(OH)D levels were significantly higher in the 2000-IU group compared with the 400-IU group at 8 and 16 wk, but not at baseline and 4 wk, respectively (Fig. 1A). The experimental group (2000 IU) achieved a 25(OH)D level of 85.7 ± 30.1 nmol/liter at 16 wk from 33.5 ± 9.9 nmol/liter at baseline ($P < 0.001$), effectively improving the vitamin D status from deficiency (<50 nmol/liter) to sufficiency (≥75 nmol/liter). On the other hand, although the control group (400 IU) was able to increase 25(OH)D levels significantly from 33.3 ± 10.3 nmol/liter at baseline to 59.8 ± 18.2 nmol/liter at 16 wk, the vitamin D status remained at the level of insufficiency (50–75 nmol/liter). At posttest in the 2000-IU group, 56.5% of the participants became vitamin D sufficient (≥75 nmol/liter); 39.1 and 4.4% (1 of 23) remained insufficient and deficient, respectively. The highest individual value of 25(OH)D after treatment was 162.6 nmol/liter, which is far below the defined level of toxicity (374 nmol/liter) (21). At posttest in the 400-IU group, 23.5% achieved sufficiency; 15.5 and 61% remained insufficient and deficient, respectively.

For PTH, after adjusting for age and sex, mean (SE) serum PTH values at baseline and at 4, 8, and 16 wk were 3.67 ± 0.08, 3.63 ± 0.08, 3.68 ± 0.08, and 3.49 ± 0.08 pg/ml, respectively, for the 400-IU group; and 3.60 ± 0.08, 3.50 ± 0.07, 3.63 ± 0.07, and 3.53 ± 0.07 pg/ml,

TABLE 1. Baseline clinical characteristics

Clinical characteristics	Control (400 IU)	Experimental (2000 IU)	*P* value
n	21	23	
Males/females[a]	15/7	10/13	0.08
Age (yr)	16.3 ± 1.1	16.5 ± 1.4	0.95
Height (cm)	171.6 ± 6.4	166.6 ± 7.8	0.04
Weight (kg)	69.2 ± 14.4	75.7 ± 31.3	0.39
Total body fat mass (kg)	14.5 ± 7.7	20.7 ± 15.4	0.10
BMI percentile	61.6 ± 33.4	67.8 ± 30.9	0.53
SBP (mm Hg)	114.9 ± 7.8	111.3 ± 10.4	0.20
DBP (mm Hg)	69.4 ± 6.5	68.2 ± 12.3	0.17
Plasma 25(OH)D (nmol/liter)	34.0 ± 10.6	33.1 ± 8.7	0.76
Serum PTH (pg/ml)[b]	40.4 (16.8–104.9)	32.6 (23.3–75.4)	0.21
Serum calcium (mg/dl)	7.95 ± 1.76	7.55 ± 1.54	0.29
Serum sodium (mmol/liter)	142.6 ± 1.6	141.4 ± 1.7	0.28
Serum potassium (mmol/liter)	4.3 ± 0.3	4.4 ± 0.3	0.57
Serum creatinine (mg/liter)	10.3 ± 2.7	10.7 ± 2.7	0.68
Overnight urine calcium (mg/dl)	8.83 ± 4.52	8.97 ± 4.53	0.92
Energy intake (kcal/d)[b]	1723 (380–4380)	1498 (1058–5333)	0.36
Calcium intake (mg/d)[b]	648 (282–2497)	520 (334–1780)	0.34
Vitamin D intake (IU/d)[b]	133 (19–750)	111 (39–433)	0.37

Values are expressed as means ± SD or median (range). All results not marked were based upon independent *t* tests.

[a] Tests of significance between groups were based on Fisher's exact test.

[b] Tests of significance between groups were based on Mann-Whitney *U* tests.

respectively, for the 2000-IU group. We did not observe a significant time × group interaction ($P = 0.10$). There was not a significant time effect ($P = 0.19$) on serum PTH in the groups. Lastly, the magnitude of difference in PTH levels over 16 wk was not significantly different between groups ($P = 0.60$). Urine and serum calcium levels did not change across all the time points, either in the control group or in the experimental group (all P values > 0.05).

Evaluation of the relationship between 25(OH)D and adiposity, and 25(OH)D and PTH

At pretest, total body mass at baseline in the entire sample (n = 44) was not correlated with 25(OH)D ($r = -0.09$; $P = 0.56$). In the control group, total body fat mass at baseline was not significantly correlated with 25(OH)D concentrations at any of the four time points (Table 2). However, in the experimental group, total body fat mass at baseline was consistently and inversely correlated with 25(OH)D concentrations in response to the 2000-IU treatment at 4, 8, and 16 wk. In addition, in the 2000-IU group, total body fat mass at posttest was also inversely correlated with 25(OH)D concentrations at posttest. We did not observe any correlations between PTH and total body fat mass at any time points in either the control group or the experimental group (all P values < 0.05).

Pre- and postintervention: PWV, SBP, and DBP

Of the total 44 subjects, 35 were available for PWV. A significant group by time interaction was observed for carotid-femoral PWV ($P = 0.019$) (Table 3), even after adjusting for age and sex. In the control group (n = 17), carotid-femoral PWV significantly increased from baseline (5.38 ± 0.53 m/sec) to posttest (5.71 ± 0.75 m/sec), whereas in the experimental group (n = 18), carotid-femoral PWV significantly decreased from baseline (5.41 ± 0.73 m/sec) to posttest (5.33 ± 0.79 m/sec). Although not statistically significant, similar trends were observed for both the carotid-radial and carotid-foot, such that the control group exhibited increases from baseline to posttest, whereas the experimental group exhibited decreases from baseline to posttest. SBP and DBP remained unchanged over time in both the control and experimental groups (all P values > 0.05).

Sun exposure, physical activity, and dietary calcium and vitamin D intake

The study was conducted during a regular school time of the year (February to May), and therefore, adolescent subjects participated in their regular school activities. No differences ($P > 0.05$) in sun exposure, vitamin D intake, and physical activity from pretest to posttest were detected in either the control group or the experimental group.

Discussion

We have conducted a 16-wk randomized clinical trial of vitamin D supplementation, 400 IU (control) *vs.* 2000 IU (experimental), in black youth. The major findings of this

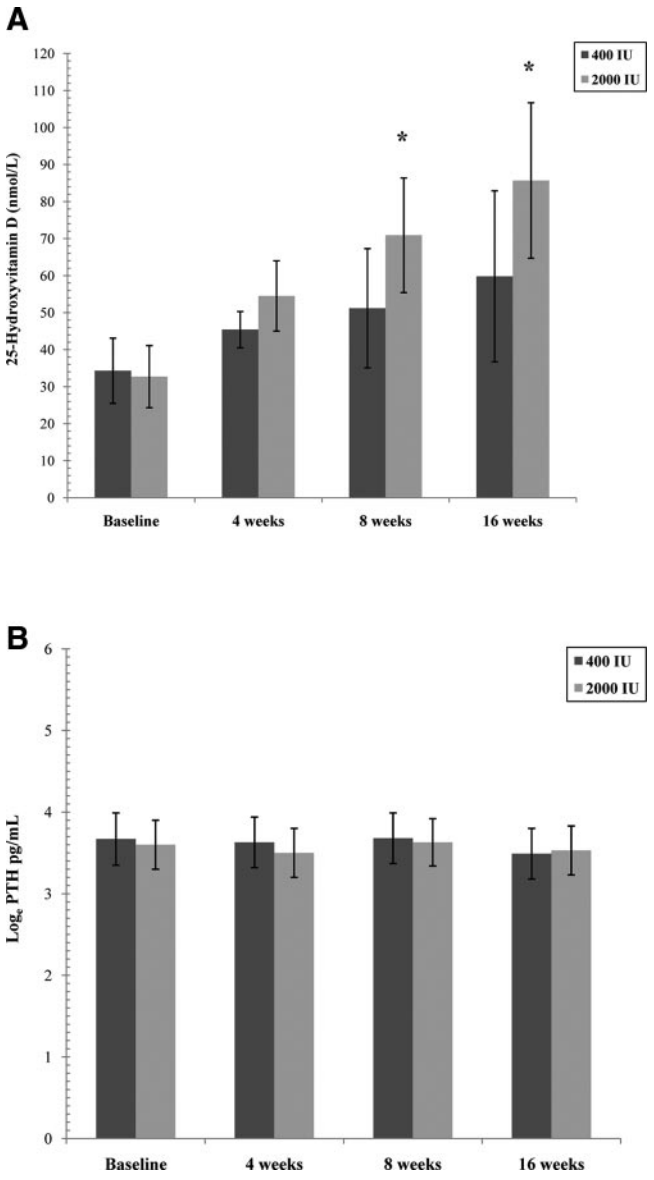

FIG. 1. A, The dynamic changes of 25(OH)D concentrations in response to the 16-wk treatment of 400 and 2000 IU in black adolescents. A significant group by time interaction was observed ($P < 0.001$). Analyses of simple main effects specifically identified between-group differences at 8 wk ($P = 0.003$) and 16 wk ($P < 0.001$), but not at baseline ($P = 0.68$) or 4 wk ($P = 0.15$), respectively. Finally, *post hoc* tests indicated that within either the 400-IU group or the 2000-IU group, 25(OH)D concentrations significantly increased over time (all P values < 0.01). All the tests were adjusted for age and sex. The *dark bars* and the *gray bars* represent 25(OH)D concentrations (means ± SD) in response to 400 and 2000 IU/d across time, respectively. Note: On average, the 2000-IU group reached vitamin D sufficiency at 8 wk, whereas the 400-IU group was still insufficient at 16 wk. B, The dynamic changes of PTH concentrations in response to the 16-wk treatment of 400 and 2000 IU in black adolescents. Results presented are mean (95% confidence interval), adjusted for age and sex. There was neither a significant time nor group effect of the vitamin D supplementation on $\log_e$ PTH over 16 wk (P values < 0.05). The *dark bars* and the *gray bars* represent PTH concentrations in response to 400 and 2000 IU/d across time, respectively.

clinical trial include: 1) 2000 IU/d vitamin D supplement may achieve the optimal vitamin D status; 2) adiposity negatively impacts the adequacy of vitamin D intervention; and 3) vitamin D supplementation could favorably improve indices of aortic stiffness. The present study is the first clinical trial of vitamin D intervention to: 1) use 2000 IU in black subjects; and 2) include cardiovascular risk factors as outcomes in youth.

2000 *vs.* 400 IU

Our randomized clinical trial demonstrates for the first time that on average, 2000 IU can achieve a vitamin D sufficiency [25(OH)D > 75 nmol/liter] over a time period of 16 wk in black youth with baseline 25(OH)D concentrations between 20 and 40 nmol/liter. Given that no signs of toxicity were present and more than 40% of the participants still remained insufficient or deficient after supplementation, the current tolerable upper intake level, 2000 IU/d (9), might need to be reconsidered in black youth. We noted that the vitamin D status still remained at the level of insufficiency (50–75 nmol/liter) after the treatment of 400 IU, which is in accordance with previous findings (6, 7). A study of 340 children ages 10 to 17 yr in Lebanon found that increasing the intake of oral vitamin D 10-fold, from the currently recommended dose of 200 to 2000 IU/d, was required to reach a 25(OH)D level of 30 ng/ml or 75 nmol/liter (22). Tolerance of vitamin D supplementation at doses close or equal to 2,000 IU has been demonstrated in French schoolboys supplemented with 100,000 IU oral vitamin D three times over 6 months (~1,700 IU/d) and Lebanese schoolchildren given 14,000 IU/wk for a year (equal to 2000 IU/d) (22, 23). These data support the notion that the current recommendation for the minimum daily intake of 400 IU of vitamin D by the American Academy of Pediatrics might need to be revised in black youth. On the other hand, the magnitude of decrease in PTH levels was not significantly different between the two vitamin D treatment groups in our study. This suggests that 2000 IU/d did not excessively suppress PTH in black youth, which provides further evidence that a revision of the current tolerable upper intake level should be considered. Furthermore, we also did not observe any correlations between PTH and adiposity in the time course of the clinical trial, which is in line with some of the previous findings (24). The feedback between PTH and 25(OH)D, *e.g.* the inhibitory pattern of PTH in response to various vitamin D doses and the effects of adiposity on the relationship between PTH and 25(OH)D, are poorly understood (25, 26). These warrant further investigations in the pediatric population, particularly in black youth.

TABLE 2. Correlations between total body fat mass and 25(OH)D concentrations

	Total body fat mass			
	Control group (400 IU, n = 21)		Experimental group (2000 IU, n = 23)	
25(OH)D	Baseline	16 wk	Baseline	16 wk
Baseline	r = −0.22; $P = 0.34$		r = −0.26; $P = 0.23$	
4 wk	r = −0.16; $P = 0.48$		r = −0.63; $P = 0.001$	
8 wk	r = −0.01; $P = 0.95$		r = −0.52; $P = 0.010$	
16 wk	r = −0.08; $P = 0.73$	r = −0.15; $P = 0.54$	r = −0.46; $P = 0.03$	r = −0.46; $P = 0.03$

Total body fat mass was measured by dual-energy x-ray absorptiometry at pretest and posttest. Partial correlation analyses, controlling for age and sex, were conducted in the control group and the experimental group separately. The total body fat mass at baseline was consistently and inversely associated with 25(OH)D concentrations across time in response to the 2000-IU supplement. In the 2000-IU group, the total body fat mass at posttest was also inversely associated with 25(OH)D concentrations at posttest.

Adiposity

Adiposity has been negatively associated with 25(OH)D concentrations in cross-sectional studies in youth (27–29). To our knowledge, there have only been two interventional studies investigating the relationship between adiposity and vitamin D in the literature. In a study from Australia, Lee *et al.* (30) supplemented 17 elderly hospital inpatients [25(OH)D < 15 nmol/liter] with 10,000 IU vitamin D_3 per day orally for 1 wk. Serum 25(OH)D concentrations achieved after 1 wk of vitamin D_3 replacement were found to be negatively correlated with BMI ($r^2 = 0.63$; $P < 0.01$). These results indicate that adequacy of vitamin D replacement in severe deficiency is likely dependent on BMI. Rajakumar *et al.* (6) conducted an open-label, nonrandomized study in Pittsburgh in which 21 obese [baseline 25(OH)D concentrations at 55.4 ± 24.0 nmol/liter] and 20 nonobese [baseline 25(OH) concentrations at 64.6 ± 27.9 nmol/liter] preadolescent black children were treated with 400 IU of vitamin D_3 for 4 wk. They found that vitamin D deficiency occurred in 12 of 21 (57%) obese children *vs.* eight of 20 (40%) nonobese children at baseline and persisted in five of 21 (24%) obese children *vs.* two of 18 (11%) nonobese children after treatment. When the cohort was stratified by the baseline 25(OH)D concentrations, there were differences in the response to treatment in the obese and nonobese children. However, this present study did not observe any significant correlations between total body fat mass and 25(OH)D in the 400-IU group. This could be due to the disparities in the subjects' age, baseline 25(OH)D levels, the study design, different measures of adiposity (BMI *vs.* fat mass), and the time length of the supplement between the two studies (4 *vs.* 16 wk). Nevertheless, we found that the adequacy of the treatment of 2000 IU/d for 16 wk was consistently and negatively modulated by fat mass across all the time points. Conceivably, the sequestration of vitamin D in body fat stores and the metabolism of 25(OH)D affected by adiposity could explain the differential treatment effects (31). Our results indicate that youth with greater fat mass might require higher doses of vitamin D supplementation to achieve vitamin D repletion.

Cardiovascular risk

Few clinical trials investigating cardiovascular risks in relation to vitamin D have been performed. A study conducted during the winter in Scotland among 87 patients with type 2 diabetes and vitamin D deficiency showed that a single dose of 100,000 IU vitamin D_2 improved 25(OH)D level by 15.3 nmol/liter and improved flow-mediated vasodilatation of the brachial artery by 2.3%, an independent predictor of future cardiovascular events (32). In addition, Tarcin *et al.* (33) recently studied 23 asymptomatic, vitamin D-deficient, Turkish young adults (age, 23.3 ± 3.0 yr) with 25(OH)D levels of less than 25 nmol/liter. After receiving 300,000 IU im monthly for 3 months (equivalent to ~3,300 IU/d for 12 wk), there was a significant treatment effect of 3.4% (from 7.0 ± 3.2 to

TABLE 3. PWV and vitamin D supplementation

	Control (400 IU; n = 17)		Experimental (2000 IU; n = 18)		Time × group interaction
PWV	Baseline	16 wk	Baseline	16 wk	*P* value
Carotid-femoral	5.38 ± 0.53	5.71 ± 0.75	5.41 ± 0.73	5.33 ± 0.79	0.019
Carotid-radial	7.77 ± 1.64	7.92 ± 0.89	7.83 ± 1.14	7.81 ± 0.98	0.93
Carotid-distal (foot)	6.87 ± 0.64	7.22 ± 0.79	6.75 ± 0.64	6.71 ± 0.63	0.46

A significant group by time interaction was observed ($P = 0.019$) for carotid-femoral PWV, even after controlling for age and sex. In the control group (n = 17), carotid-femoral PWV increased from baseline to posttest ($P = 0.016$), whereas in the experimental group (n = 18), carotid-femoral PWV decreased from baseline to posttest ($P = 0.031$).

10.4 ± 3.35%) on flow-mediated dilation. In our 16-wk clinical trial in black normotensive youth, 2000 IU daily vitamin D_3 supplement appeared to counterbalance the progression of carotid-femoral PWV, the "gold standard" measurement of arterial stiffness in the central vasculature (*e.g.* aorta). Our results are in accordance with the previous cross-sectional observations that 25(OH)D concentrations were inversely correlated with aortic PWV in patients with end-stage renal disease and hemodialysis (34) and carotid artery atherosclerotic plaque in black adults (35). The vasculoprotective function of vitamin D might result from direct and/or indirect effects on vascular cells, renoprotective effects, suppression of the renin-angiotensin-aldosterone system, effects on calcium metabolism, counterbalance of inflammation and oxidative stress, and improvement of glucose metabolism and insulin sensitivity (36–38). Our data suggest that sufficient supplementation of vitamin D could offer a means to elicit favorable functional alterations in the arterial system and in cardiovascular function in general.

Nevertheless, cautions should be taken to interpret our data. First, because of the randomization, there were more males than females in the control group; however, results remained consistent even after adjusting for sex as a confounding factor in our analyses. Second, our randomized clinical trial was single-blinded (investigators-blinded) rather than double-blinded. The majority of the youth were unaware of which group (400 or 2000 IU) they were assigned to, and more than 60% did not read the labels on the bottle of the pills, according to our posttest survey, thus reducing the magnitude of this limitation. Third, not having a true placebo may be viewed as a limitation; however, our data are in support of previous findings that 400 IU of vitamin D does not increase 25(OH)D to the sufficient level. In addition, the compliance rates did not differ between the control group and experimental group. Fourth, our relatively small sample size could be another limitation, such that clinical trials with a larger sample size are warranted. Fifth, our data were obtained in black youth, which should not be extrapolated to the general pediatric population. Last, we examined normotensive youth because they are unlikely to have developed significant target organ damage, which can mask or compromise the normal response pattern. The treatment effect of 2000 IU on carotid-femoral PWV was relatively small; however, such small favorable changes in adolescence might impact the development of cardiovascular diseases later in adulthood.

In conclusion, the present study indicates that the current adequate intake of vitamin D should be revised upward in black youth (39). Of importance, atherosclerosis begins in youth, and therefore primary prevention of atherosclerosis should begin in adolescence (40). More clinical trials are needed to help understand the role of vitamin D deficiency implicated in the pathogenesis of arterial stiffness as a nontraditional risk factor and to provide interventional evidence for the necessity of maintaining vitamin D sufficiency as early as adolescence.

Acknowledgments

Address all correspondence and requests for reprints to: Yanbin Dong, Georgia Prevention Institute, Department of Pediatrics, Medical College of Georgia, 1120 15th Street, HS-1640, Augusta, Georgia 30912. E-mail: ydong@mail.mcg.edu.

This work was supported by an intramural grant of the Diabetes and Obesity Discovery Institute at the Medical College of Georgia. Cholecalciferol (vitamin D_3) is available over the counter, and we purchased it online. The company had no role in the study design, study conduct, data collection or analysis, interpretation of the data, or preparation, review, or approval of the manuscript.

Clinical Trial Registration: Vitamin D Supplement Study for Adolescents (VIP), NCT00909454, http://www.clinicaltrials.gov/NCT00909454.

Disclosure Summary: The authors have nothing to declare.

References

1. **Dong Y, Pollock N, Stallmann-Jorgensen IS, Gutin B, Lan L, Chen TC, Keeton D, Petty K, Holick MF, Zhu H** 2010 Low 25-hydroxyvitamin D levels in adolescents: race, season, adiposity, physical activity, and fitness. Pediatrics 125:1104–1111
2. **Kumar J, Muntner P, Kaskel FJ, Hailpern SM, Melamed ML** 2009 Prevalence and associations of 25-hydroxyvitamin D deficiency in US children: NHANES 2001–2004. Pediatrics 124:362–370
3. **Reis JP, von Muhlen D, Miller 3rd ER, Michos ED, Appel LJ** 2009 Vitamin D status and cardiometabolic risk factors in the United States adolescent population. Pediatrics 124: 371–379
4. **Holick MF** 2008 The vitamin D deficiency pandemic and consequences for nonskeletal health: mechanisms of action. Mol Aspects Med 29:361–368
5. **Wagner CL, Greer FR** 2008 Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. Pediatrics 122:1142–1152
6. **Rajakumar K, Fernstrom JD, Holick MF, Janosky JE, Greenspan SL** 2008 Vitamin D status and response to vitamin D(3) in obese vs. non-obese African-American children. Obesity (Silver Spring) 16: 90–95
7. **Stellinga-Boelen AA, Wiegersma PA, Storm H, Bijleveld CM, Verkade HJ** 2007 Vitamin D levels in children of asylum seekers in The Netherlands in relation to season and dietary intake. Eur J Pediatr 166:201–206
8. **Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B** 2006 Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr 84:18–28
9. **Yates AA, Schlicker SA, Suitor CW** 1998 Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D and fluoride. J Am Diet Assoc 98:699–706
10. **Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R** 2005 Estimates of optimal vitamin D status. Osteoporos Int 16:713–716

11. **Heaney RP, Davies KM, Chen TC, Holick MF, Barger-Lux MJ** 2003 Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. Am J Clin Nutr 77:204–210
12. **Heaney RP** 2005 The vitamin D requirement in health and disease. J Steroid Biochem Mol Biol 97:13–19
13. **Zhu H, Yan W, Ge D, Treiber FA, Harshfield GA, Kapuku G, Snieder H, Dong Y** 2007 Cardiovascular characteristics in American youth with prehypertension. Am J Hypertens 20:1051–1057
14. **McCarty CA** 2008 Sunlight exposure assessment: can we accurately assess vitamin D exposure from sunlight questionnaires? Am J Clin Nutr 87:1097S–1101S
15. **Janz KF, Lutuchy EM, Wenthe P, Levy SM** 2008 Measuring activity in children and adolescents using self-report: PAQ-C and PAQ-A. Med Sci Sports Exerc 40:767–772
16. **Kapuku GK, Treiber FA, Davis HC, Harshfield GA, Cook BB, Mensah GA** 1999 Hemodynamic function at rest, during acute stress, and in the field: predictors of cardiac structure and function 2 years later in youth. Hypertension 34:1026–1031
17. **Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo Jr JL, Jones DW, Materson BJ, Oparil S, Wright Jr JT, Roccella EJ** 2003 The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. JAMA 289:2560–2572
18. **Nichols W, O'Rourke MF** 1998 McDonald's blood flow in arteries: theoretical, experimental and clinical principles. 4th ed. London: Arnold
19. **Holick MF, Siris ES, Binkley N, Beard MK, Khan A, Katzer JT, Petruschke RA, Chen E, de Papp AE** 2005 Prevalence of vitamin D inadequacy among postmenopausal North American women receiving osteoporosis therapy. J Clin Endocrinol Metab 90:3215–3224
20. **Holick MF** 2006 High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc 81:353–373
21. **Vieth R** 1999 Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. Am J Clin Nutr 69:842–856
22. **Maalouf J, Nabulsi M, Vieth R, Kimball S, El-Rassi R, Mahfoud Z, El-Hajj Fuleihan G** 2008 Short- and long-term safety of weekly high-dose vitamin D3 supplementation in school children. J Clin Endocrinol Metab 93:2693–2701
23. **Guillemant J, Le HT, Maria A, Allemandou A, Pérès G, Guillemant S** 2001 Wintertime vitamin D deficiency in male adolescents: effect on parathyroid function and response to vitamin D3 supplements. Osteoporos Int 12:875–879
24. **Weaver CM, McCabe LD, McCabe GP, Braun M, Martin BR, Dimeglio LA, Peacock M** 2008 Vitamin D status and calcium metabolism in adolescent black and white girls on a range of controlled calcium intakes. J Clin Endocrinol Metab 93:3907–3914
25. **Valiña-Tóth AL, Lai Z, Yoo W, Abou-Samra A, Gadegbeku CA, Flack JM** 2010 Relationship of vitamin D and parathyroid hormone to obesity and body composition in African-Americans. Clin Endocrinol (Oxf) 72:595–603
26. **Vieth R, El-Hajj Fuleihan G** 2005 There is no lower threshold level for parathyroid hormone as 25-hydroxyvitamin D concentrations increase. J Endocrinol Invest 28:183–186
27. **Gordon CM, DePeter KC, Feldman HA, Grace E, Emans SJ** 2004 Prevalence of vitamin D deficiency among healthy adolescents. Arch Pediatr Adolesc Med 158:531–537
28. **Looker AC** 2005 Body fat and vitamin D status in black versus white women. J Clin Endocrinol Metab 90:635–640
29. **Saintonge S, Bang H, Gerber LM** 2009 Implications of a new definition of vitamin D deficiency in a multiracial US adolescent population: the National Health and Nutrition Examination Survey III. Pediatrics 123:797–803
30. **Lee P, Greenfield JR, Seibel MJ, Eisman JA, Center JR** 2009 Adequacy of vitamin D replacement in severe deficiency is dependent on body mass index. Am J Med 122:1056–1060
31. **Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF** 2000 Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 72:690–693
32. **Sugden JA, Davies JI, Witham MD, Morris AD, Struthers AD** 2008 Vitamin D improves endothelial function in patients with type 2 diabetes mellitus and low vitamin D levels. Diabet Med 25:320–325
33. **Tarcin O, Yavuz DG, Ozben B, Telli A, Ogunc AV, Yuksel M, Toprak A, Yazici D, Sancak S, Deyneli O, Akalin S** 2009 Effect of vitamin D deficiency and replacement on endothelial function in asymptomatic subjects. J Clin Endocrinol Metab 94:4023–4030
34. **London GM, Guérin AP, Verbeke FH, Pannier B, Boutouyrie P, Marchais SJ, Mëtivier F** 2007 Mineral metabolism and arterial functions in end-stage renal disease: potential role of 25-hydroxyvitamin D deficiency. J Am Soc Nephrol 18:613–620
35. **Freedman BI, Wagenknecht LE, Hairston KG, Bowden DW, Carr JJ, Hightower RC, Gordon EJ, Xu J, Langefeld CD, Divers J** 2010 Vitamin D, adiposity, and calcified atherosclerotic plaque in African-Americans. J Clin Endocrinol Metab 95:1076–1083
36. **Pilz S, Tomaschitz A, Ritz E, Pieber TR** 2009 Vitamin D status and arterial hypertension: a systematic review. Nat Rev Cardiol 6:621–630
37. **Richart T, Li Y, Staessen JA** 2007 Renal versus extrarenal activation of vitamin D in relation to atherosclerosis, arterial stiffening, and hypertension. Am J Hypertens 20:1007–1015
38. **Pittas AG, Harris SS, Stark PC, Dawson-Hughes B** 2007 The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in nondiabetic adults. Diabetes Care 30:980–986
39. **Mitka M** 2009 More evidence on low vitamin D levels fuels push to revise recommended intake. JAMA 302:2527–2528
40. **Berenson GS, Srinivasan SR, Bao W, Newman 3rd WP, Tracy RE, Wattigney WA** 1998 Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. N Engl J Med 338:1650–1656