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Vitamin D3 supplementation in obese, African-American, vitamin D deficient adolescents

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A B S T R A C T

Objectives: Obese, African-American (AA) adolescents are at increased risk for vitamin D deficiency. The primary objective of this pilot study was to examine the effect of vitamin D supplementation upon 25-hydroxy vitamin D (25OHD) levels in obese, AA adolescents.

Methods: A randomized, double-blinded, controlled pilot study included 26 obese (BMI ≥ 95%ile), vitamin D deficient (25OHD < 20 ng/mL), pubertal AA adolescents (ages 12–17). Subjects received cholecalciferol 1000 IU or 5000 IU daily for 3 months. Serum 25OHD, vitamin D binding protein, parathyroid hormone, and cardiometabolic risk markers were obtained at baseline and post-treatment.

Results: Of 39 subjects enrolled, 26 (67%) were vitamin D deficient (mean 25OHD 12.0 ± 3.8 ng/mL) at baseline and were randomized, with 22 completing the study. Sex, age, season, pubertal stage, BMI, insulin resistance (HOMA-IR) and 25OHD were similar at baseline between the 1000 IU and 5000 IU groups. Post-treatment, 25OHD increased less in the 1000 IU group (5.6 ng/mL, p = 0.03) vs. the 5000 IU group (15.6 ng/mL, p = 0.002). 83% of the 5000 IU group and 30% of the 1000 IU group reached post-treatment 25OHD ≥ 20 ng/mL (p = 0.01); 50% of the 5000 IU group, but no subject from the 1000 IU group, achieved 25OHD ≥ 30 ng/mL (p = 0.009). We detected no group differences in mineral metabolites or cardiometabolic risk markers following supplementation.

Conclusions: Cholecalciferol dosing in excess of the current Institute of Medicine dietary reference intakes was required to achieve 25OHD levels ≥20 ng/mL in obese, AA adolescents. Supplementation of 5000 IU may be required to achieve the desired goal.

Introduction

Vitamin D deficiency, defined as 25-hydroxy vitamin D (25OHD) < 20 ng/mL, is common. Dark skin pigmentation, obesity, poor dietary intake, and low sunlight exposure are risk factors. In children and adolescents, the prevalence of vitamin D deficiency is greatest among obese African-Americans (AA) (87%), compared to Latino (52%) and White (27%) peers [1]. 74% of children do not consume the Estimated Average Requirement for vitamin D, and only 7% of non-Hispanic AA children take dietary supplements with vitamin D [2]. In developing vitamin D dietary reference intakes (DRIs) for children, the Institute of Medicine (IOM) acknowledged the limited available evidence for non-skeletal outcomes [3]. Pediatric recommendations for vitamin D intake largely target rickets prevention and are unable to address how much vitamin D is required to 1) optimize bone mineral accrual and 2) address potential nontraditional vitamin D associations with immune disease [4–7], insulin resistance [8,9], muscle function [10,11], and cardiovascular disease [12–15].

In fact, defining vitamin D "sufficiency" is also problematic. The IOM [3] and Pediatric Endocrine Society [16] define 25OHD sufficiency as ≥20 ng/mL and deficiency as < 20 ng/ml, whereas the Endocrine Society defines vitamin D sufficiency as ≥30 ng/mL, insufficiency as

Abbreviations: 25OHD, 25-hydroxy vitamin D; AA, African-American; AAP, American Academy of Pediatrics; BG, blood glucose; BMI, body mass index; CHOP, The Children's Hospital of Philadelphia; CMR, cardiometabolic risk; CTRC, Clinical and Translational Research Center; CV, coefficient of variation; DRIs, dietary reference intakes; DXA, dual X-ray absorptiometry; ELISA, enzyme-linked immunosorbent assay; FMI, fat mass index; FA, fat area; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; IOM, Institute of Medicine; IU, international units; LDL-C, low-density lipoprotein cholesterol; NMR, nuclear magnetic resonance; PTH, parathyroid hormone; SD, standard deviation; TC, total cholesterol; TG, triglycerides; VDBP, vitamin D binding protein

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20–29 ng/mL, and deficiency as < 20 ng/mL [17]. The 2011 IOM DRIs focus on the amount of vitamin D intake that is required to maintain 25OHD ≥ 20 ng/dL, reported as 600 IU in adolescents, with no screening recommendations given [3]. The Endocrine Society recommends a maintenance dose of 600–1000 IU daily [17]. This Society also recommends 25OHD screening for "at risk" groups, such as obese and AA individuals, and at least 6 weeks of supplementation with 2000 IU vitamin D_2 or D_3 daily to treat vitamin D deficiency in children. They also note that individuals with risk factors such as obesity may require vitamin D doses 2–3 times higher [17]. However, these guidelines have not been tested in obese AA adolescents.

We performed a pilot supplementation trial in obese, vitamin D-deficient AA adolescents. The study aim was to compare the effects of three months of daily supplementation with cholecalciferol, 1000 IU vs 5000 IU, upon total 25OHD, VDBP, and parathyroid hormone (PTH). The secondary, exploratory aim was to assess the impact of supplementation on cardiometabolic risk (CMR) factors. We hypothesized that increases in 25OHD would be greater in subjects taking 5000 IU cholecalciferol than in subjects taking 1000 IU, that 5000 IU would be associated with greater rates of 25OHD > 20 ng/mL and > 30 ng/mL at follow-up, and that greater increases in 25OHD would be associated with favorable changes in CMR markers.

Methods

Participants

Subjects were recruited by newspaper advertisement, flyers in obesity and endocrine clinics, and ongoing research studies. Verbal consent was obtained before telephone screening. Potential subjects were then screened in person to confirm eligibility according to the following inclusion criteria: 1) age 12–17.9 years; 2) AA; 3) Body Mass Index (BMI) ≥95th%ile; 4) pubertal; and 5) commitment to adherence. Subsequent inclusion criterion for randomization was 25OHD < 20 ng/mL. Exclusion criteria included: 1) chronic medical conditions; 2) medication use with growth, nutrition, bone health, vitamin D metabolism, or insulin sensitivity effects; and 3) hypercalcemia or hypercalciuria history.

Baseline visits were conducted from August 2011 to June 2012, and follow-up visits were conducted from January to September 2012 at The Children's Hospital of Philadelphia (CHOP) Clinical and Translational Research Center (CTRC). Written informed consent and age-appropriate assent were obtained before subject participation. The CHOP Institutional Review Board approved the study.

Randomization

Subjects with 25OHD < 20 ng/mL meeting study criteria were randomized to cholecalciferol 1000 or 5000 IU daily for 12 weeks. Randomization was performed by the CHOP Investigational Pharmacy using a set of identical, opaque, tamper-evident, sequentially-numbered envelopes, and was stratified by sex. Both subjects and investigators were blinded to group assignment. To ensure allocation concealment, a randomly permuted block design with varying block sizes was used.

Measures

With the exception of dual X-ray absorptiometry (DXA), which was completed only at baseline, measures were performed at baseline and at 12 weeks.

Anthropometrics

Weight was measured with the subject wearing light clothing without shoes, using a digital scale (Scaletronix, White Plains, New York). Height was measured using a wall-mounted stadiometer (Holtain Inc, Crymych, UK). BMI Z-score (BMI-Z), and percentile were calculated using the 2000 CDC growth charts [18].

Pubertal assessment

Sexual maturation was determined by a pediatric endocrinologist (AK, SNM) according to the method of Tanner [19]; testicular volume was measured according to Prader [20]. Pubertal was defined as breast stage > 1 for females and testicular volume > 3 cc for males.

Laboratory measures

After a 12-h overnight fast, a blood sample was obtained for 25OHD, PTH, VDBP, glucose (BG), insulin, lipid panel, high sensitivity C-reactive protein (hs-CRP), and adiponectin.

25OHD was measured by liquid chromatography-tandem mass spectrometry as previously described [21]; inter-assay coefficient of variation (CV): 5.59–5.60%; intra-assay CV: 3.37–4.01%. Intact PTH was measured by chemiluminescence; inter-assay CV: 5.9%; intra-assay CV: 1.2%. BG was measured in the CHOP CTRC on the Nova Stat Strip glucose monitor (Nova Biomedical, Waltham, MA). VDBP, adiponectin, and insulin were measured in duplicate in the CHOP Translational Core Laboratory (TCL) using ELISA (VDBP: R&D Systems, Minneapolis, MN; adiponectin: ALPCO Diagnostics, Salem, NH; insulin: ALPCO Diagnostics, Salem, NH). Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) was calculated as (fasting BG (mmol/l) × insulin (μU/ml))/22.5. In the University of Pennsylvania TCL, triglycerides (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were measured on a Roche COBAS c501 (Roche Diagnostics, Indianapolis, IN). Low-density lipoprotein cholesterol (LDL-C) was calculated as LDL-C = TC − HDL-C − [triglycerides/5]. Non-HDL cholesterol was calculated as TC − HDL-C. Hs-CRP was quantified by SIEMENS BNII (SIEMENS Healthcare Diagnostics, Newark, DE).

Body composition

Whole body DXA scans were acquired (Hologic Discovery, Bedford, MA) employing standard positioning techniques and analyzed using Discovery software (version 13.5). Lean body mass (LBM), fat mass (FM), and visceral fat area (FA) estimates were generated. The instrument was calibrated daily with a hydroxyapatite phantom. *In vitro* CV was < 0.6%; *In vivo* CV was < 1%. FM index (FMI = FM/height^2) was calculated.

Adherence

A 12-week supply of cholecalciferol, plus 20% extra, was dispensed to each subject. Unused pills were returned. Adherence by pill count was assessed assuming that all missing pills were consumed, and by dividing missing pill number by the number of subject-specific study days. "Self-reported" adherence was assessed by dividing the number of doses recorded on the study calendar as taken, by subject-specific study day number.

Season

April–October were categorized as summer and November–March were categorized as winter [22].

Adverse events

No adverse events related to treatment were reported.

Sample size

The study was planned as a pilot, and as such the investigators recognized that they may not be able to detect small differences. The goal was to recruit 40 subjects to have 15 completers per group, which would detect between treatment * time effects upon 25OHD as small as 0.53 standard deviations (SD) with 80% power. To test the hypothesis that 80% of the 5000 IU group and 20% of the 1000 IU group would attain 25OHD > 20 ng/mL with 80% power, 19 subjects would be

needed per group; 13 subjects would be needed per group to test the hypothesis that 50% of the 5000 IU group vs 10% of the 1000 IU group would achieve 25OHD > 20 ng/mL. Because the actual sample size was smaller than planned, a post hoc power calculation was performed and determined that there was 80% power to detect a time * treatment effect size of 0.63 SD.

Statistical analysis

Mean and SD were used to summarize normally distributed continuous variables. Median, minimum, and maximum values were used to summarize continuous variables that deviated from normality. Student's *t*-test or Wilcoxon rank-sum test were used, as indicated, to compare continuous variables, and chi2 test was used to compare proportions. Between-dose group differences for outcomes of interest were analyzed by repeated measures ANOVA.

Pearson or Spearman correlations, depending upon normality, were used to examine the relationships of baseline 25OHD with continuous variables (BMI, VDBP, iPTH) as well as the relationships of 25OHD change with change in iPTH. The Stata XTREG procedure was used to conduct longitudinal, intention-to-treat, mixed effect analyses comparing 25OHD responses from baseline to 12 weeks between the two dose groups, while adjusting for age, sex, season, and BMI. The impact of VDBP on change in 25OHD was also tested in these models. The relationship of 25OHD with iPTH was also assessed using longitudinal models and included non-randomized individuals to increase power. Analyses were conducted using Stata 13 (Stata Corp., College Station, TX, USA). A p-value ≤0.05 was considered statistically significant.

Results

Of the 39 subjects who completed the baseline visit (Table 1), 26 (67%) had 25OHD < 20 ng/mL and were randomized to receive cholecalciferol 1000 (n = 12) or 5000 (n = 14) IU daily for 12 weeks (Table 1); one subject was excluded from randomization for pre-pubertal status. Twenty-two subjects completed the follow-up visit (Fig. 1). With the exception of younger age among completers (mean: 14.0 vs 16.4 years; p = 0.02), baseline characteristics of completers and subjects lost to follow-up (n = 4) were similar. Baseline 25OHD was not correlated with BMI (p = 0.37), iPTH (p = 0.17), or VDBP (p = 0.45).

Baseline characteristics were similar for the 1000 IU and 5000 IU groups (Table 1). Baseline increased iPTH (> 65 pg/mL) was present in two participants randomized to 1000 IU group but no 5000 IU group (p = 0.11). No difference in adherence was found between the 1000 IU and 5000 IU groups (pill count: 96.5 ± 1.8% vs 89.8 ± 4.2%, p = 0.19; self-report: 96.2 ± 2.4% vs 89.9 ± 4.5%, p = 0.26).

25OHD

Mean 25OHD at follow-up was 18.8 ng/mL in the 1000 IU group (p = 0.0006) and 28.8 ng/mL in the 5000 IU group (p < 0.0001); maximum post-treatment 25OHD was 44.2 ng/mL (Table 2). Eighty-three percent of the 5000 IU group and 30% of the 1000 IU group achieved post-treatment 25OHD ≥ 20 ng/mL (p = 0.01). 50% of the 5000 IU group, but no 1000 IU subject achieved 25OHD ≥ 30 ng/mL (p = 0.009) (Fig. 2). In longitudinal models adjusting for covariates (sex, summer season, BMI, age), 12 weeks of 5000 IU conferred an average 25OHD increase of 15.6 ng/ml vs 5.6 ng/ml with 1000 IU (Table 3).

PTH

On average iPTH remained unchanged in both the 1000 IU (p = 1.0) and 5000 IU (p = 0.88) groups, and no between group differences in iPTH were identified, Table 2. Over the 12-week intervention, the one participant in the 5000 IU group suspected of non-adherence developed elevated PTH (109 pg/mL with 25OHD = 5.2). While no relationship between iPTH and 25OHD was found at baseline, longitudinal models that included all baseline and follow-up data found a near-significant negative relationship between iPTH and 25OHD (β-coefficient = −0.50; 95%CI: −1.0 to 0.01; p = 0.055).

Vitamin D binding protein

VDBP was not different between 25OHD deficient and "non-deficient" subjects at baseline (p = 0.7), was not related to baseline 25OHD (p = 0.27), was not significantly different following supplementation (p = 0.90), and was not related to follow-up 25OHD (p = 0.38).

Vitamin D and cardiometabolic risk markers

No differences in cardiometabolic markers were found between subjects with and without 25OHD < 20 ng/mL at baseline (Table 1) or following vitamin D treatment (Table 2). Additionally, longitudinal analyses identified no relationships between 25OHD or iPTH and changes in cardiometabolic outcomes following 12 weeks of vitamin D replacement (analyses not shown).

Discussion

Vitamin D plays a key role in bone mineral metabolism and has been implicated in cardiometabolic health. However, optimal 25OHD concentration and the cholecalciferol dose required to achieve it have yet to be defined. Limited data are available to guide either vitamin D dietary intake or supplementation in obese, African-American adolescents, a group at particular risk for vitamin D deficiency. Here, we present preliminary data describing the impact of cholecalciferol supplementation upon total 25OHD, VDBP, PTH, and CMR factors in obese, AA adolescents with vitamin D deficiency.

Defining optimal vitamin D status has been complicated by limited data, particularly in obese, AA youth. Current vitamin D intake recommendations are based upon bone outcomes, but prevention of rickets is not synonymous with optimizing bone mineral accrual during childhood. While adolescents are not at high risk for vitamin D deficient rickets, they are in a stage of rapid growth and bone accrual, critical for "peak bone mass" achievement. Vitamin D deficiency may hinder bone mineral acquisition. Paradoxically, AA adolescents have higher bone density than Whites [23,24,4] despite lower 25OHD. Moreover, obese individuals have greater bone mass than non-obese individuals, although the extent to which the compensatory increase in bone mass sufficiently protects against fractures is unknown. Additionally, the extent to which 25OHD > 20 ng/mL or even > 30 ng/mL optimizes bone density is not known.

In the classic model, vitamin D deficiency is associated with increased PTH. This increased PTH ultimately serves to maintain plasma calcium by 1) increasing active vitamin D production, 2) mobilizing calcium from bone, and to some extent, enhancing renal calcium reabsorption, while fostering urinary phosphate wasting. Bone mineral mobilization and phosphate wasting ultimately compromise bone mineral density. Ideally, a vitamin D intervention would lower PTH, and a plateauing in PTH at some 25OHD threshold would help identify "optimal" 25OHD. Neither cross-sectional data from Hill et al. [25] nor longitudinal data from the Rajakumar et al. [26] study in which PTH were obtained randomly throughout the day were able to identify such a threshold. On average, PTH did not decrease substantially in our study—echoing findings in AA youth during a 6-month randomized trial of cholecalciferol 1000 IU daily vs placebo in which baseline PTH was similar to levels found in our cohort [26]. Despite lack of apparent change in PTH despite cholecalciferol 5000 IU daily, 25OHD was negatively associated with iPTH in our longitudinal analyses. A mid-puberty increase in PTH has been considered an adaptive process to enhance calcium absorption during the accelerated bone accrual of

Table 1
Baseline characteristics of obese, African-American adolescents prior to randomization to 1000 or 5000 IU of cholecalciferol.[a]

Baseline Characteristics	Vitamin D deficient vs non-deficient			Randomized Subjects[b]			
	25OHD < 20 n = 26	25OHD ≥ 20 n = 12	p value	1000 IU n = 12	5000 IU n = 14		p value
Sex			0.49				0.72
% Male	46	58		50	42		
% Female	54	42		50	58		
Season			0.06				0.39
% winter (Nov–Mar)	57.7	25		66.7	33.3		
% summer (Apr–Oct)	42.3	75			50	50	
Age, years	14.4 (1.7)	14.6 (1.5)	0.73		14.7 (1.7)	14.1 (1.7)	0.34
Tanner stage			0.53				0.71
% Tanner Stage 2	7.7	16.7			0	14.3	
% Tanner Stage 3	19.2	8.3			16.7	21.4	
% Tanner Stage 4	15.4	16.7			16.7	14.3	
% Tanner Stage 5	57.7	58.3			66.7	50	
Fat mass, kg[c]	35.7 (10.9)	38.5 (9.9)	0.46		36.8 (14.3)	34.6 (6.2)	0.65
% body fat[c]	38.2 (5.8)	39.2 (6.0)	0.64		36.9 (7.8)	39.6 (2.6)	0.28
Fat mass index, kg/m^2[c]	12.9 (3.8)	13.6 (3.9)	0.54		13.1 (5.2)	12.7 (1.7)	0.82
Visceral fat, cm^2[c]	74.4 (22.9)	82.7 (25.6)	0.30		79.4 (27.9)	69.3 (16.3)	0.30
BMI, kg/m^2	33.4 (26.1, 53.4)	34.8 (28.3, 39.9)	0.54		35.4 (7.8)	36.2 (8.9)	0.80
BMI-Z	2.28 (0.44)	2.34 (0.34)	0.66		2.2 (0.49)	2.4 (0.4)	0.51
BMI percentile	98.5 (94, 99)	99.0 (95, 99)	0.17		97.3 (2.0)	98.0 (1.4)	0.33
25OHD, ng/mL[d]	12.0 (3.7)	24.1 (3.8)	< 0.0001		12.0 (4.5)	12.2 (3.4)	0.68
VDBP, mg/dL	10.4 (5.2, 23.9)	8.0 (5.0, 21.1)	0.73		13.7 (6.2)	10.1 (5.1)	0.12
PTH, pg/mL[d]	39.9 (14.6, 102)	35.4 (10.3, 85)	0.68		47.1 (21.3, 102)	37.5 (14.6, 64.7)	0.24
HOMA-IR	3.9 (1.9, 8.8)	3.3 (0.9, 10.1)	0.17		4.7 (2.4)	4.4 (2.1)	0.72
Glucose, mg/dL[d]	91.1 (8.8)	91.4 (10.7)	0.93		92 (8.8)	90.4 (8.9)	0.64
Insulin, μIU/mL[d]	19.9 (9.0)	15.0 (9.2)	0.13		20.8 (10.5)	19.1 (7.7)	0.64
Adiponectin, ng/mL	3.0 (1.5, 6.6)	3.3 (1.5, 7.4)	0.37		3.0 (1.5, 6.6)	2.9 (1.7, 5.2)	0.78
hs-CRP, mg/L[d,e]	1.6 (0.15, 15.3)	3.7 (0.5, 8.3)	0.34		0.7 (0.15, 12.7)	1.9 (0.3, 14.3)	0.35
HDL-C, mg/dL[d]	43 (26, 84)	51 (33, 72)	0.17		44.5 (36, 84)	42.5 (26, 71)	0.64
LDL-C, mg/dL[d]	95.3 (25.4)	103.7 (35.7)	0.41		87.2 (22.2)	102.3 (26.7)	0.13
Total cholesterol, mg/dL[d]	155.9 (27.9)	166.0 (46.4)	0.41		147.5 (23.6)	163.1 (30.1)	0.16
Triglycerides, mg/dL[d]	78.3 (27.1)	65.4 (22.9)	0.16		76 (27.8)	80.2 (27.4)	0.70
Triglyceride:HDL ratio	1.9 (0.9)	1.4 (0.6)	0.08		1.8 (0.9)	2 (1)	0.50

Abbreviations: 25OHD, 25-hydroxy vitamin D; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particle; HOMA-IR, homeostatic model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; LDL-P, low-density lipoprotein particle; PTH, parathyroid hormone; VDBP, vitamin D binding protein; VLDL-P, very low-density lipoprotein particle.

[a] Results are shown as mean (standard deviation) or median (minimum, maximum), based on the necessity of parametric or nonparametric analyses, respectively.

[b] All randomized participants had 25OHD < 20 ng/mL at baseline.

[c] Not all participants had DXA scan due to weight limit. For DXA, n = 22 for participants with 25OHD < 20 and n = 12 for participants with 25OHD ≥ 20. n = 11 for 1000 IU group and n = 11 for 5000 IU group.

[d] Laboratory unit conversions from Conventional to Systme International: 25OHD: multiply ng/mL by 2.496 to get nmol/L. PTH: multiply pg/mL by 0.1061 to get pmol/L. Glucose: multiply mg/dL by 0.0555 to get mmol/L. Insulin: multiply μIU/mL by 6.945 to get pmol/L. hs-CRP: multiply mg/L to 9.524 to get nmol/L. HDL-C, LDL-C, and Total Cholesterol: multiply mg/dL by 0.0259 to get mmol/L. Triglycerides: multiply mg/dL by 0.0113 to get mmol/L.

[e] The lower limit of detection for the hs-CRP assay was 0.16; results reported as < 0.16 were assigned a value of 0.15.

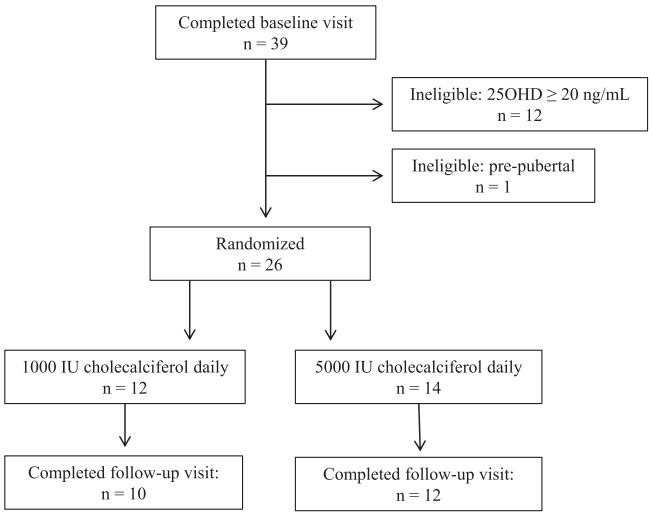

Fig. 1. Flow diagram of study subjects.

adolescents [27]; this finding suggests a 25OHD independent mechanism is operative and may help explain the limited impact of vitamin D supplementation upon PTH in our study.

The current study was initiated before the release of the 2011 IOM DRIs; at that time, the IOM recommended 400 IU daily for adolescents. Supplementation doses used in this study were 1) greater than the 2011 IOM intake recommendations for teenagers of 600 IU daily, but 2) consistent with Endocrine Society guidelines suggesting that the recommended treatment dose for vitamin D deficiency (2000 IU daily) may need to be doubled or tripled in specific populations (i.e. 4000–6000 IU daily in obesity). Our findings suggest that the general recommendation of 600 IU daily or even 1000 IU daily is ineffective for achieving the currently defined desirable 25OHD level in obese, AA vitamin D deficient adolescents. Furthermore, given that only 50% of the 5000 IU treatment group in our study reached 30 ng/ml, depending on the definition of sufficiency used, doses even higher than the Endocrine Society's recommendation of 4000–6000 IU daily for treating deficiency in "at risk" populations may be required. Of note, our study specifically enrolled vitamin D-deficient individuals. The impact of vitamin D supplementation in subjects with 25OHD ≥ 20, and the supplementation required to maintain 25OHD in an acceptable range in a

Table 2
Outcomes of Interest Before and After Treatment with 1000 or 5000 IU Cholecalciferol for 12 Weeks[a]

	1000 IU group			5000 IU group			Difference between groups[b] p value
	Baseline n = 12	Follow-up n = 10	Within group p value	Baseline n = 14	Follow-up n = 12	Within group p value	
25OHD, ng/mL	11.7 (4.1)	18.8 (3.9)	0.0006	12.3 (3.5)	28.8 (11.4)	< 0.0001	< 0.0001
VDBP, mg/dL	13.7 (6.2)	13.4 (5.7)	0.9	10.1 (5.1)	10.3 (5.4)	0.91	0.28
PTH, pg/mL	47.1 (21.3, 102)	47.5 (23.9, 71.6)	1	37.5 (14.6, 64.7)	32.5 (13.3, 109)	0.88	0.74
BMI-Z	2.2 (1.6, 2.9)	2.3 (1.4, 2.9)	0.74	2.3 (0.4)	2.3 (0.4)	0.97	0.99
HOMA-IR	4.1 (1.9, 8.7)	4.3 (2.1, 20.8)	0.69	4.4 (2.1)	4.3 (1.9)	0.89	0.54
Adiponectin, ng/mL	3 (1.5, 6.6)	3 (1.5, 7.8)	0.74	3.1 (1.0)	3.4 (1.4)	0.63	0.33
hs-CRP, mg/L[c]	0.7 (0.15, 12.7)	0.9 (0.2, 7.1)	0.95	1.9 (0.3.14.3)	2.3 (0.15, 20.3)	0.82	0.35
HDL-C, mg/dL	44.5 (36, 84)	46.5 (35, 89)	0.74	44.8 (11.7)	45.3 (10.7)	0.92	0.62
LDL-C, mg/dL	87.2 (22.2)	83.5 (24.3)	0.72	102.3 (26.7)	108.5 (24.9)	0.55	0.70
Total cholesterol, mg/dL	147.5 (23.6)	149.6 (26.3)	0.85	163 (30.1)	172 (27.5)	0.42	0.68
Triglycerides, mg/dL	76 (27.8)	82.4 (33.2)	0.63	80.2 (27.4)	93.1 (41.4)	0.35	0.20
Triglyceride:HDL ratio	1.8 (0.9)	1.8 (1.0)	0.84	2 (0.5, 3.7)	1.7 (0.5, 5.7)	0.68	0.22

[a] Results are shown as mean (SD) or median (min, max), based on the necessity of parametric or nonparametric analyses, respectively. For expansion of abbreviations and conversion factors from Conventional to System International, please refer to Table 1.
[b] Between-group differences from baseline to follow-up were analyzed by repeated measures ANOVA, using the group x time interaction term.
[c] The lower limit of detection for the hs-CRP assay was 0.16; results reported as < 0.16 were assigned a value of 0.15.

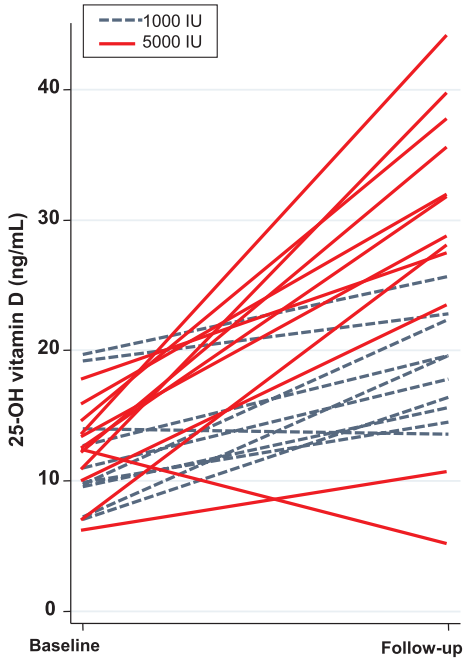

Fig. 2. Subjects in the 5000 IU group experienced greater increases in 25OHD than the 1000 IU group, $p < 0.001$.

Table 3
Results of a longitudinal regression model examining the effects of vitamin D supplementation upon total 25-hydroxy vitamin D in vitamin D deficient obese, African American adolescents adjusting for covariates.

	Partial β Coefficient (ng/mL)	95% Confidence Interval	p value
Body Mass Index	−0.15	(−0.35, 0.04)	0.1
1000 IU treatment effect	5.6	(0.7, 10.4)	0.03
5000 IU treatment effect	15.6	(3.6, 16.4)	0.002
Age	−0.9	(−2.0, 0.3)	0.1
Female sex	−6.2	(−9.7, −2.6)	0.001
Summer season	4.0	(0.5, 7.5)	0.025
Constant	31.5	(13.9, 49.0)	< 0.001

previously deficient individual were not studied.

We also explored the potential impact of increasing 25OHD and PTH changes upon CMR markers—a clinically relevant outcome in obese AA adolescents, but did not find any significant associations. The current literature contains conflicting reports regarding the impact of vitamin D supplementation upon cardiometabolic health in populations at risk for vitamin D deficiency. Recently, the large, longitudinal Cardiovascular Risk in Young Finns Study identified an association between low 25OHD during childhood and increased carotid intima-media thickness (a surrogate marker for cardiovascular disease risk) in adulthood [28]. This study was important given the association found between low 25OHD as a child, and cardiovascular disease risk 27 years later. During a 3-month trial of 89 overweight and obese AA adults with mean baseline 25OHD of approximately 15 ng/mL, 4000 IU cholecalciferol daily was associated with improved insulin sensitivity compared to placebo [29]. In obese adolescents, improved insulin sensitivity was found with 4000 IU cholecalciferol daily vs placebo at 6, but not 3 months [30]. A 26-week Danish study of 43 obese adults randomized to 7000 IU cholecalciferol daily vs placebo found no significant changes in insulin sensitivity, lipids, inflammatory factors, adiponectin, or leptin [31].

In our pilot study, no statistically significant differences in CMR factors were found following cholecalciferol treatment between the 5000 IU vs 1000 IU treatment groups, and CMR changes were not related to changes in 25OHD or PTH over the interval. Additional study, with a longer observation period and a larger sample size, is needed to determine if vitamin D replacement can confer improved CMR profile in obese, vitamin D deficient AA adolescents.

Another important clinical factor is that 25OHD is primarily protein bound. Polymorphisms in the gene encoding vitamin D binding protein (VDBP) differ in AA and Whites, and confer differences in circulating VDBP concentrations as well as alterations in 25OHD binding [32]. Thus, the definition of optimal total 25OHD may differ in AA and White individuals. Furthermore, free or bioavailable 25OHD may be better related to bone outcomes [33]. In a cross-sectional study of U.S. adults, African-Americans had lower total 25OHD and VDBP compared to Whites, but similar calculated bioavailable 25OHD [32]. Our study included only AA adolescents; VDBP levels were measured, but VDBP polymorphisms were not determined and free 25OHD was not directly measured. However, in this exploratory study, VDBP was not associated with 25OHD at baseline or follow-up, and was not significantly different following cholecalciferol supplementation. In contrast to our longitudinal study of obese AA adolescents, a recent cross-sectional

study of non-obese AA and White adolescents found an inverse relationship between VDBP and insulin resistance as measured by HOMA [34]. Interestingly, Ashraf et al. found that increased total, free, and bioavailable 25OHD were positively associated with increased arterial stiffness specifically in AA adolescent females, while in Whites, the reverse association between vitamin D measures and arterial stiffness existed but did not reach statistical significance [35]. Further study in this area is needed.

Our study had several limitations. The sample size was relatively small and was neither designed to look at safety at the population level nor powered to detect changes in cardiometabolic markers. The study duration of 3 months may not have provided sufficient time to observe significant changes in CMR factors. However, we were able to detect significant differences in 25OHD achieved between the two treatment groups. While the use of supplements containing vitamin D was exclusionary, we did not assess dietary intake, although based upon NHANES dietary intake data [2] and the vitamin D deficient status of the cohort studied, we presume that intake was low. In addition, measurement methods for dietary intake, particularly in the adolescent and overweight/obese populations, are known to be inaccurate [36]. Finally, for the purposes of generating pilot data to guide development of a larger clinical trial, multiple comparisons were made; while no differences in CMR were identified, any that were found may have arisen just by chance. Significant strengths include the examination of obese AA adolescents, a group at particularly increased risk for vitamin D deficiency, careful selection of participants according to inclusion/exclusion criteria, and high adherence.

Many chronic medical conditions find their origins in childhood [37], and some outcomes arise only with prolonged exposure [38]. The optimal timing and duration of vitamin D intervention likely depends on the outcome being considered. Additionally, bioavailable vitamin D has emerged as an important consideration in understanding racial differences in vitamin D status. Here, preliminary data on the impact of cholecalciferol supplementation at two different doses upon total 25OHD and CMR factors in obese AA adolescents are presented, and intended to inform larger scale studies that will better define optimal vitamin D status and the supplementation doses needed to achieve it.

Clinical trial registration

This study is registered on clinicaltrials.gov (NCT01546103).

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Contributor's statement

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