See corresponding editorial on page 737 and article on page 779.

Effect of vitamin D_3 supplementation on vascular and metabolic health of vitamin D–deficient overweight and obese children: a randomized clinical trial

Kumaravel Rajakumar,[1] Charity G Moore,[2] Arshad T Khalid,[1] Abbe N Vallejo,[1] Mohamed A Virji,[3] Michael F Holick,[4] Susan L Greenspan,[5] Silva Arslanian,[1] and Steven E Reis[5]

[1]Department of Pediatrics, University of Pittsburgh School of Medicine, University of Pittsburgh Medical Center Children's Hospital of Pittsburgh, Pittsburgh, PA, USA; [2]Department of Physical Therapy, University of Pittsburgh, Pittsburgh, PA, USA; [3]Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA; [4]Department of Medicine, Boston University Medical Center, Boston, MA, USA; and [5]Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

ABSTRACT

Background: Obese children are vulnerable to vitamin D deficiency and impaired cardiovascular health; vitamin D replenishment might improve their cardiovascular health.

Objectives: The aims were to determine, in vitamin D–deficient overweight and obese children, whether supplementation with vitamin D_3 1000 or 2000 IU/d is more effective than 600 IU/d in improving arterial endothelial function, arterial stiffness, central and systemic blood pressure (BP), insulin sensitivity (1/fasting insulin concentration), fasting glucose concentration, and lipid profile and to explore whether downregulation of adipocytokines and markers of systemic inflammation underlies vitamin D effects.

Methods: We conducted a randomized, double-masked, controlled clinical trial in 225 10- to 18-y-old eligible children. Change in endothelial function at 6 mo was the primary outcome.

Results: Dose–response increases in serum 25-hydroxyvitamin D concentrations were significant and tolerated without developing hypercalcemia. Changes at 3 and 6 mo in endothelial function, arterial stiffness, systemic-systolic BP, lipids, and inflammatory markers did not differ between children receiving 1000 or 2000 IU vitamin D and children receiving 600 IU. Some secondary outcomes differed between groups. Compared with the 600-IU group, central-systolic, central-diastolic, and systemic-diastolic BP was lower at 6 mo in the 1000-IU group [−2.66 (95% CI: −5.27, −0.046), −3.57 (−5.97, −1.17), and −3.28 (−5.55, −1.00) mm Hg, respectively]; insulin sensitivity increased at 3 and 6 mo and fasting glucose concentration declined at 6 mo (−2.67; 95% CI: −4.88, −0.46 mg/dL) in the 2000-IU group.

Conclusions: Correction of vitamin D deficiency in overweight and obese children by vitamin D_3 supplementation with 1000 or 2000 IU/d versus 600 IU/d did not affect measures of arterial endothelial function or stiffness, systemic inflammation, or lipid profile, but resulted in reductions in BP and fasting glucose concentration and in improvements in insulin sensitivity. Optimization of children's vitamin D status may improve their cardiovascular health. This trial was registered at clinicaltrials.gov as NCT01797302. *Am J Clin Nutr* 2020;111:757–768.

Keywords: vitamin D deficiency, cholecalciferol, 25-hydroxyvitamin D, obesity, children, endothelial function, arterial stiffness, blood pressure, fasting blood glucose, insulin sensitivity

Introduction

Vitamin D deficiency in children is associated with markers of poor cardiometabolic health such as elevated blood pressure (BP) and elevated fasting glucose concentrations (1–4). Furthermore, low vitamin D concentrations are associated with markers of subclinical arteriosclerosis, including arterial endothelial dysfunction (5) and increased arterial stiffness (6) that are predictors for future cardiovascular events (7, 8). Obese children have a higher risk of vitamin D deficiency, systemic inflammation, and cardiometabolic risk factors (9–11). However, randomized clinical trials (RCTs) (12, 13) have not shown conclusively that vitamin D supplementation is associated with reductions in cardiometabolic risk in children, likely due to variations in study design and subject selection (14–16). We postulated that vitamin D replenishment in obese children may improve their overall cardiometabolic health through beneficial effects on immunomodulation, vascular function, and glucose homeostasis. Accordingly, we conducted a vitamin D RCT to *1*) determine whether administration of 1000 IU or 2000 IU vitamin D_3/d is more effective than 600 IU vitamin D_3/d—the current RDA

(17)—in improving endothelial function, arterial stiffness, central and systemic BP, insulin sensitivity, and metabolic markers (fasting glucose concentration and lipid profile) in vitamin D–deficient overweight and obese children and *2*) explore whether downregulation of inflammatory mediators underlies the effects of vitamin D on vascular and metabolic health. Our a priori primary interest was in comparing each higher dose (1000 IU and 2000 IU) with the lower dose (600 IU) at 6 mo. Our primary outcome measure was change in brachial artery flow-mediated dilation percentage (FMD%) at 6 mo. FMD% is a recognized measure of endothelial function (18, 19).

Methods

Study design and participants

We conducted a double-masked, randomized, controlled clinical trial of 600 versus 1000 versus 2000 IU of oral vitamin D_3 daily for 6 mo in 10- to 18-y-old overweight or obese vitamin D–deficient children residing in Pittsburgh, Pennsylvania, during August 2013 through August 2018. Children were recruited mainly from the Primary Care Center of Children's Hospital of Pittsburgh (CHP) of the University of Pittsburgh Medical Center (UPMC). Our recruitment was aided by advertisements through the University of Pittsburgh Clinical and Translational Science Institute (CTSI) Pediatric PittNet, a practice-based research network of 27 community pediatric practices in the Pittsburgh area. The study was approved by the University of Pittsburgh Human Research Protection Office. We obtained parental consents and participants' assent or consent prior to enrollment.

We screened 10- to 18-y-old overweight or obese children (BMI ≥85th percentile) who were free of conditions or treatments that could affect glucose homeostasis, BP, cholesterol concentrations, or vitamin D and calcium metabolism. Eligible participants were vitamin D–deficient (serum 25-hydroxyvitamin D [25(OH)D] <20 ng/mL) and had normal serum calcium (10–14 y: 8.8-10.8 mg/dL; ≥15 y, 8.4–10.2 mg/dL) during a screening assessment and who had fasting glucose concentrations of <125 mg/dL and, in the case of postmenarchial girls, had a negative urine pregnancy test at the time of randomization. For the purpose of this study, serum 25(OH)D <20 ng/mL was defined as vitamin D deficiency (20). Randomization and 3- and 6-mo follow-up study visits and procedures were conducted at the CTSI Montefiore Clinical and Translational Research Center (CTRC), where all venipunctures and vascular tests were completed after children had fasted for ≥8 h, except in 1 instance in which fasting duration was 4 h.

Randomization and intervention.

Participants were stratified by race and obesity status and randomly assigned, in blocks of 3, to receive either 600 IU or 1000 IU or 2000 IU of vitamin D_3 daily for 6 mo. The study statistician generated the randomization list using SAS software. Vitamin D_3 tablets (Douglas Laboratories) were identical in appearance and were dispensed in identical-looking bottles. Masking and labeling of bottles were carried out by the UPMC Investigation Drug Service research pharmacist. Treatment assignments remained concealed to participants, parents, and the investigators throughout the trial. Study tablets were manufactured in 3 batches. The average vitamin D_3 contents of the 600-, 1000-, and 2000-IU tablets across the 3 batches, analyzed as described previously (21), were 754, 1086, and 2142 IU, respectively. The average end-of-shelf-life vitamin D_3 contents of the tablets in the second batch were 500, 888, and 1822 IU, respectively.

Compliance.

Compliance was assessed by tablet count at 3 and 6 mo and was validated by an electronic medication event monitoring system (MEMs 6 Track Cap; AARDEX).

Study measurements

Anthropometry, skin color, sunlight exposure, diet, activity, and body composition.

Weight, height, and waist circumference (WC) were measured 3 times each and averaged at each visit. Sun-reactive skin type and melanin index from forehead, underarm, and hand (22);

This study was supported by the following grants: *1*) National Heart, Lung, and Blood Institute of the NIH R01HL112985 (to KR), *2*) Office of Dietary Supplements (ODS) of the Office of Director of the NIH R01HL112985 (to KR), *3*) R01HL112985 supplement from the ODS of the Office of Director of the NIH (to KR), *4*) NIH UL1 TR001857 (University of Pittsburgh Clinical and Translational Research Center and Pediatric PittNet), *5*) a Children's Hospital of Pittsburgh Research Advisory Committee seed grant (to KR), *6*) an IRG Award, Nancy E Taylor Foundation for Chronic Diseases (to ANV), and *7*) NIH P30 AG024827 (to SLG). The content of this manuscript is solely the responsibility of the authors and does not necessarily represent the official views of the NIH or the other funding sponsors.

Supplemental Tables 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

De-identified data described in the manuscript may be available to academic researchers whose proposals for secondary use of the data are approved by the lead investigators of this clinical trial after 14 August, 2022. Data use should be consistent with the University of Pittsburgh Institutional Review Board approval. A data use agreement will be established between the University of Pittsburgh and the academic researcher's institution. The lead investigator and biostatistician will review the data requests to ensure the goals of the study can be obtained from the original study design, data elements, and the proposed statistical analysis. Once approved, and a Data Use Agreement executed, no restrictions will be placed on the types of analyses. De-identified data will be shared solely for academic research purposes. Following the official execution of a Data Use Agreement signed by the University of Pittsburgh Office of Compliance and the representative of the institution of the requesting researcher, data will be made available in either Excel tables or SAS datasets through secure email or pitt.box.com.

Address correspondence to KR (e-mail: kumaravel.rajakumar@chp.edu).

Abbreviations used: AIx-75, Augmentation Index at heart rate of 75 beats/min; BP, blood pressure; CHP, Children's Hospital of Pittsburgh; CTRC, Clinical and Translational Research Center; CTSI, Clinical and Translational Science Institute; FMD%, brachial artery flow-mediated dilation percentage; hsCRP, high-sensitivity C-reactive protein; MEMs, medication event monitoring system; PTH, parathyroid hormone; PWV, pulse-wave velocity; RCT, randomized clinical trial; UPMC, University of Pittsburgh Medical Center; WC, waist circumference; 25(OH)D, 25-hydroxyvitamin D; VITAL, Vitamin D and omega-3 trial.

Received June 5, 2019. Accepted for publication December 19, 2019.

First published online January 17, 2020; doi: https://doi.org/10.1093/ajcn/nqz340.

self-assessed pubertal status (23); dietary intake of calcium, vitamin D, and macronutrients (24–26); characteristics of sunlight exposure (27); and physical activity were recorded at enrollment (28, 29). At enrollment and at 6 mo, percentage of total body fat, total body fat, total lean mass, and total bone mineral content were assessed by DXA whole-body scan using a Discovery Densitometer (Hologic, Inc).

Laboratory data, BP, endothelial function, and arterial stiffness were obtained at enrollment and at 3 and 6 mo.

Laboratory analyses.

Serum concentrations of 25(OH)D, parathyroid hormone (PTH), glucose, and lipids were measured at the UPMC Clinical Chemistry Laboratory. Serum 25(OH)D was assayed by a LC–tandem MS system as previously described (30). PTH was measured using a chemiluminescent immunoassay with an overall interassay CV of <5.5%; imprecision of this assay is similar throughout the assay range of 3–2500 pg/mL. Plasma adiponectin, leptin, insulin, and C-peptide were measured in SA's laboratory at CHP, as described previously (31, 32). The 1/fasting insulin concentration was used as a surrogate for insulin sensitivity as before (33). Plasma TNF-α, high-sensitivity C-reactive protein (hsCRP), and IL-6 were measured in ANV's laboratory at CHP through a multiplex platform using the Luminex system with previously validated protocols (34–36).

Blood pressure.

Measurements were obtained by a CTRC vascular technician prior to vascular health assessments, and after 10 min of participant rest and acclimatization while supine, using a CONTEC Medical Systems model CONTEC08A automated digital oscillometric device (cuff 22–32 cm). Measurements were taken 3 times, 1 min apart, and averaged. In a small subset of extremely obese children, Welch Allyn Connex® Spot Monitor automated digital oscillometric device with a GE Critikon Blood Pressure Cuff Sensa-Cuf 2491 Large Adult Long (cuff: 31–40 cm) was used instead.

Endothelial function.

Brachial artery diameter was measured using a high-resolution ultrasound machine (GE, Vivid 7; GE Healthcare) equipped with a 9-L linear transducer preset to a dedicated vascular scanning protocol. We then occluded brachial arterial flow at the upper forearm using a 5-cm-wide occlusive cuff (Hokanson SC5) inflated to a pressure of 50 mm Hg above the systolic BP or to 200 mm Hg, whichever was greater, by a rapid-release sphygmomanometer (Hokanson DS 400) for 5 min. Post-cuff-release diameter measurements during the reactive hyperemic phase, obtained at 60, 120, and 180 s, were used to calculate FMD%.

Arterial stiffness indices.

Carotid-femoral pulse-wave velocity (PWV), aortic Augmentation Index at a heart rate of 75 beats/min (AIx-75), and central systolic and diastolic BP were measured using arterial tonometry (SphygmoCor CVMS V9, CPVH System, model EM3; AtCor Medical).

Sample size and statistical analysis

Sample size.

Sample-size estimates were based on showing a clinically important relative improvement by ≥25% in baseline FMD% after 6 mo of vitamin D replenishment in children in the 1000- and 2000-IU groups when compared with children in the 600-IU group (2 comparisons: 600-IU group vs 1000-IU group; 600-IU group vs 2000-IU group). We assumed that our participants' baseline mean FMD% would be 6.5% with a SD of 2.6%, based on previous studies in obese and overweight children (37–39). A sample size of 67 in each treatment group at the end of the trial would have 90% power (2-sided 2-sample comparison of means, $\alpha = 0.05/2 = 0.025$ adjusted for each high dose vs 600 IU at 6 mo) to detect a 25% relative improvement in FMD% (37, 39). To provide for a projected drop-out rate of 20% by the end of the trial, we proposed to recruit 252 children into the trial. Midway through the study, the sponsor requested a revised sample size based on recruitment, higher than expected attrition, and project timeline. We projected to recruit ∼90% of the original sample size based on the study timeline. With $n = 225$, assuming 25% attrition, we would have 84% power to detect the original hypothesized difference assuming the same SD.

Statistical analysis

Analysis was based on intention-to-treat for all available data. Participants were analyzed in the group to which they were assigned regardless of adherence. For all continuous primary and secondary outcomes, we used linear mixed models with group-by-time interactions and constrained equal baselines using all available data for each participant (baseline, 3 mo, 6 mo in the outcome vector) accounting for repeated measures per participant with compound symmetry (40–44). Use of these models assumes data are missing at random. For the primary outcome at 6 mo, the mean differences were calculated along with simultaneous 97.5% CIs for comparisons of children receiving each of the higher daily doses (i.e., 1000 and 2000 IU) with children receiving 600 IU ($\alpha = 0.05/2 = 0.025$, corresponding to the α used for the sample size analysis due to having 2 separate hypotheses tested for a higher dose vs the control of 600 IU). Mean group differences in the primary outcome at 3 mo and secondary outcomes were calculated with 95% CIs that were not adjusted for multiple comparisons. If the group-by-time interaction was significant, we tested for differences in mean changes across the 3 groups stratified by time using overall F tests from the linear mixed model and secondary tests of trend for those that were significant ($\alpha = 0.05$). All models controlled for randomization covariates (race and obesity status). We used SAS software, version 9.4.

Results

Participant flow through the clinical trial is shown in **Figure 1**. We studied 225 overweight or obese vitamin D–deficient children. Their mean ± SD age was 13.6 ± 2.3

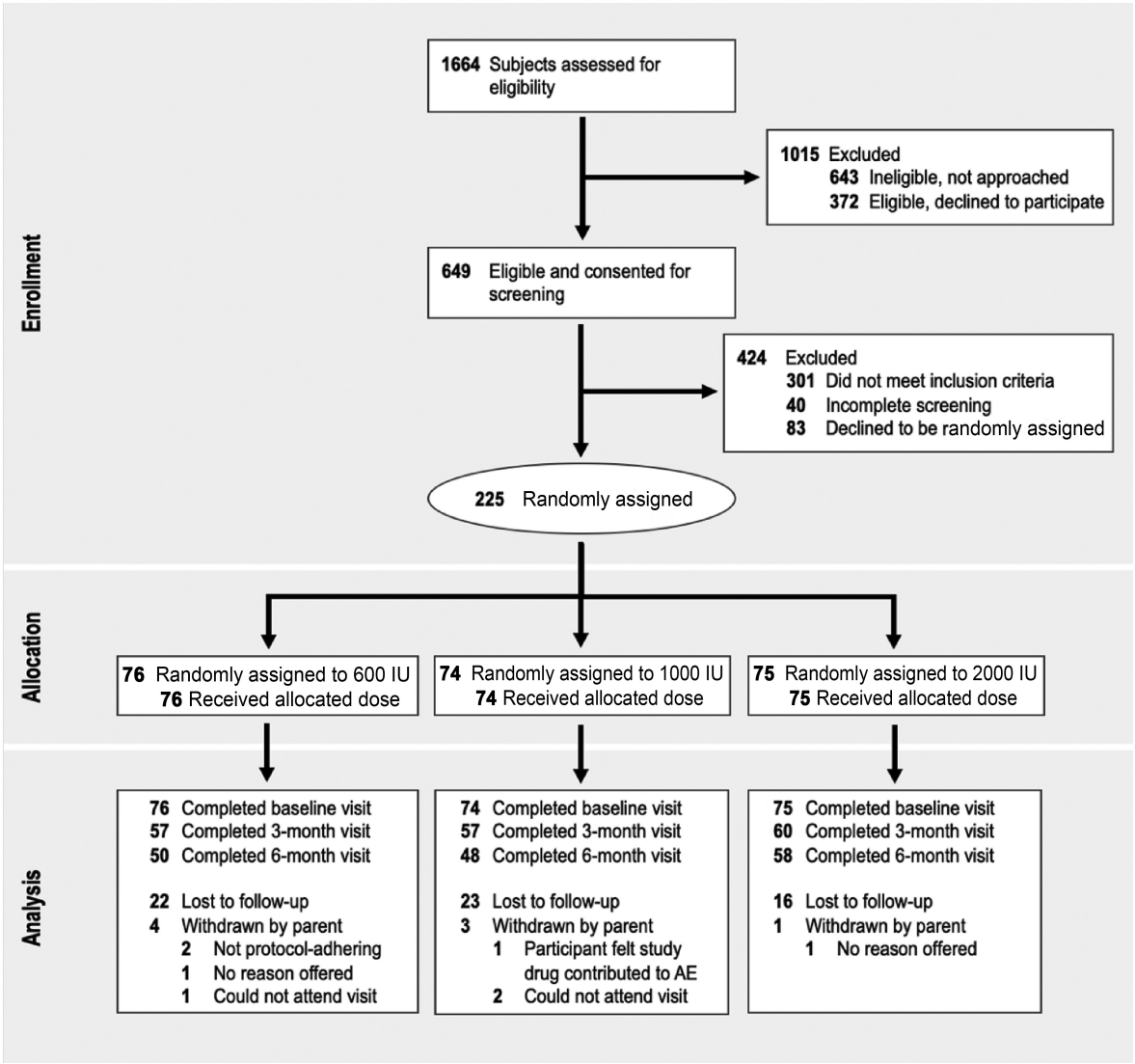

FIGURE 1 Participant flow through various stages of this randomized controlled clinical trial. AE, adverse event.

y, 35% were male, 94% were black, and their mean BMI percentile was 95.8 ± 3.8. Mean serum 25(OH)D concentration was 14.3 ± 3.7 ng/mL. Baseline characteristics of the treatment groups were similar (**Table 1**). Attrition at 6 mo was overall higher than expected (31%), ranging from 23% in the 2000-IU group to 36% in the 1000-IU group (Fisher's exact $P = 0.15$). Characteristics of children who completed and who failed to complete 6-mo follow-up visits were similar (**Supplemental Table 1**).

Effects of vitamin D_3 supplementation

Effect on 25(OH)D and PTH concentrations.

The data shown in **Figure 2** demonstrate that vitamin D_3 supplementation resulted in significant dose–response increases in mean serum 25(OH)D concentrations at 3 and 6 mo. These doses were tolerated without development of hypercalcemia. Mean 25(OH)D concentrations increased to >20 ng/mL in each of the 3 groups; the mean concentration reached >30 ng/mL only in the 2000-IU group. PTH concentrations were significantly lower than baseline concentrations at 3 mo in the 600-IU group, at 6 mo in the 1000-IU group, and at 3 and 6 mo in the 2000-IU group. Although the overall change in PTH concentrations was significant ($P < 0.0001$), the changes in PTH concentrations over time did not differ between the 3 treatment groups (group × time interaction, $P = 0.39$).

Effect on primary and secondary outcomes.

Endothelial function and arterial stiffness. No differences were observed in FMD%, PWV, and AIx-75 at 3 and 6 mo between each of the higher-dose groups (1000 IU and 2000 IU) when compared with the 600-IU group. Accounting for potential nonignorable missingness did not change the FMD% findings (clinicaltrials.gov identifier: NCT01797302; Study Protocol, Section 9.5 Data Analysis) (**Table 2**).

Central and systemic BP. Changes in central-diastolic and systemic-diastolic BP differed across groups, with lower means in the 1000-IU group than in the 600-IU group at 6 mo.

TABLE 1 Baseline characteristics of enrolled subjects[1]

	All subjects ($n = 225$)	600 IU ($n = 76$)	1000 IU ($n = 74$)	2000 IU ($n = 75$)
Demographic characteristics				
Male	78 (34.7)	22 (28.9)	29 (39.2)	27 (36.0)
Black	211 (93.8)	71 (93.4)	70 (94.6)	70 (93.3)
Hispanic	10 (4.4)	2 (2.6)	1 (1.4)	7 (9.3)
Age, y	13.6 ± 2.3	13.5 ± 2.3	13.5 ± 2.2	13.9 ± 2.4
Anthropometric measurements[2]				
Weight, kg	80.4 ± 20.9	81.4 ± 23.7	79.4 ± 20.1	80.4 ± 18.8
Height, cm	162.1 ± 9.9	161.7 ± 10.0	162.1 ± 10.0	162.5 ± 9.8
BMI, kg/m^2	30.3 ± 6.3	30.7 ± 6.9	30.0 ± 6.1	30.3 ± 5.8
BMI percentile	95.8 ± 3.8	95.8 ± 3.9	96.0 ± 3.4	95.6 ± 4.0
Percentage of 95th BMI percentile	113.4 ± 21.3	114.9 ± 22.6	112.7 ± 20.6	112.6 ± 20.7
Overweight (BMI 85th–<95th percentile)	75 (33.3)	23 (30.3)	24 (32.4)	28 (37.3)
Obese (BMI ≥95th percentile)	150 (66.7)	53 (69.7)	50 (67.6)	47 (62.7)
Tanner stage				
I	7 (3.1)	3 (3.9)	2 (2.7)	2 (2.7)
II	20 (8.9)	6 (7.9)	8 (10.8)	6 (8.0)
III	41 (18.2)	12 (15.8)	14 (18.9)	15 (20.0)
IV	74 (32.9)	23 (30.3)	30 (40.6)	21 (28.0)
V	83 (36.9)	32 (42.1)	20 (27.0)	31 (41.3)
Skin type				
I (easy burn, no tan)	3 (1.3)	0 (0)	2 (2.7)	1 (1.3)
II (easy burn, slight tan)	12 (5.3)	6 (7.9)	1 (1.4)	5 (6.7)
III (burn, then tan)	18 (8.0)	7 (9.2)	4 (5.4)	7 (9.3)
IV (no burn, good tan)	105 (46.7)	30 (39.5)	39 (52.7)	36 (48.0)
V (never burn, marked tan)	87 (38.7)	33 (43.4)	28 (37.8)	26 (34.7)
Melanin index				
Forehead	57.4 ± 13.6	57.6 ± 13.9	57.8 ± 12.6	56.9 ± 14.3
Hand	61.3 ± 12.8	61.7 ± 13.3	61.8 ± 11.9	60.5 ± 13.1
Underarm	58.5 ± 12.5	58.5 ± 12.7	59.1 ± 12.1	57.8 ± 12.9
Summertime sunlight exposure				
Duration >2 h	156 (69.3)	57 (75.0)	49 (66.2)	50 (66.7)
Sunscreen use, yes	34 (15.1)	8 (10.5)	15 (20.3)	11 (14.7)
Travel to sunny location, yes	31 (13.8)	10 (13.2)	7 (9.5)	14 (18.7)
Laboratory data				
Calcium, mg/dL	9.7 ± 0.3	9.7 ± 0.3	9.8 ± 0.3	9.7 ± 0.3
Phosphorus, mg/dL	4.1 ± 0.6	4.2 ± 0.7	4.2 ± 0.7	4.0 ± 0.6
Albumin, g/dL	4.5 ± 0.2	4.5 ± 0.3	4.5 ± 0.2	4.5 ± 0.3
25(OH)D, ng/mL	14.3 ± 3.7	14.3 ± 4.3	14.4 ± 3.4	14.2 ± 3.5
PTH, pg/mL	49.8 ± 19.6	51.7 ± 20.6	45.7 ± 18.0	52.0 ± 19.9
Dietary intake				
Calcium, mg/d	927 (643, 1339)	905 (701, 1287)	987 (647, 1374)	913 (599, 1391)
Vitamin D, IU/d	192 (122, 307)	192 (124, 288)	197 (121, 285)	189 (122, 331)
Calories per day	2232 (1653, 3303)	2352 (1685, 3311)	2334 (1610, 3141)	1984 (1639, 3267)
Protein, g/d	76.0 (57.6, 107.7)	78.4 (60.2, 109.6)	79.9 (55.9, 103.7)	71.9 (57.3, 123.0)
Fat, g/d	83.1 (61.5, 119.5)	85.1 (62.9, 124.5)	87.0 (60.4, 115.6)	74.7 (60.4, 122.1)
Saturated fat, g/d	26.0 (18.3, 35.8)	26.3 (18.3, 32.5)	27.0 (17.9, 36.6)	25.2 (18.4, 35.7)
Carbohydrates, g/d	299 (214, 441)	324 (221, 415)	312 (206, 440)	273 (212, 447)
Short food-frequency questionnaire				
Calcium, mg/d	1120 (654, 1869)	1028 (651, 1846)	1156 (628, 1849)	1157 (663, 1893)
Vitamin D, IU/d	335 (157, 509)	333 (165, 510)	363 (154, 509)	350 (168, 493)
Physical activity				
Active, h/wk	9.8 (4.9, 17.6)	12 (6, 18)	9 (5, 17)	9 (4, 17)
Sedentary, h/wk	44 (30, 65)	44 (30, 63)	44 (28, 70)	44 (30, 62)

[1] Values are n (%), mean ± SDs, or median (25th percentile, 75th percentile). 600 IU, $n = 73$; 1000 IU, $n = 73$; 2000 IU, $n = 72$ for melanin index measures. 600 IU, $n = 74$; 1000 IU, $n = 73$; 2000 IU, $n = 73$ for dietary intake. 600 IU, $n = 71$; 1000 IU, $n = 70$; 2000 IU, $n = 72$ for short food-frequency questionnaire. 600 IU, $n = 75$; 1000 IU, $n = 73$; 2000 IU, $n = 75$ for physical activity. PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D.

[2] Anthropometric data shown in Table 1 were obtained during screening visits.

Although the overall group × time interaction in central-systolic BP was not significant, central-systolic BP differed between the 1000-IU group and the 600-IU group at 6 mo, with a lower mean in the 1000-IU group than in the 600-IU group (mean difference: −2.66; 95% CI: −5.27, −0.046 mm Hg; no adjustment for multiple comparisons). Between-group differences in mean changes in systemic-systolic BP were not significant.

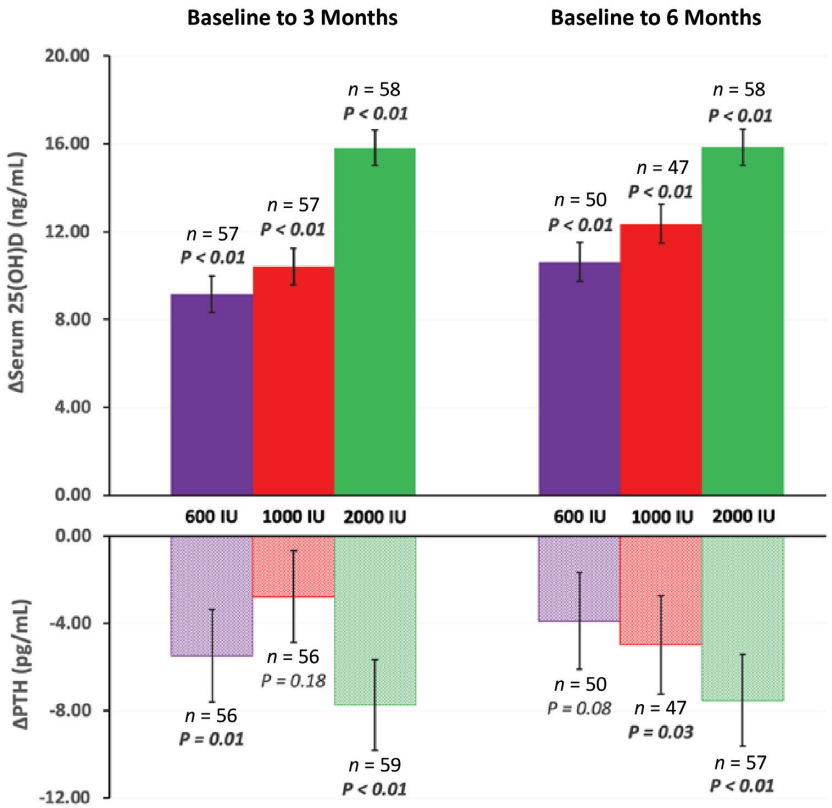

FIGURE 2 Within-group changes in mean concentrations of 25(OH)D and PTH from baseline (Δ) at 3 and 6 mo. Results are presented as means ± SEMs in bar graphs. Adjusted within-group differences, corresponding *P* values, and tests for interaction were derived using linear mixed models with fixed effects of group × time interaction, adjusting for race and obesity status (randomization stratification factors) and restricting baseline means to be equal. 25(OH)D: group × time interaction, $P < 0.0001$; PTH: group × time interaction, $P = 0.39$. PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D.

Lipid profile. No between-group differences were detected in mean changes in lipid profile indices.

Fasting glucose. No overall group × time interaction was detected in fasting glucose concentrations. However, at 6 mo, the mean fasting glucose concentration was lower in the 2000-IU group than in the 600-IU group (mean difference: −2.67; 95% CI: −4.88, −0.46 mg/dL; no adjustment for multiple comparisons).

Insulin sensitivity. No overall group × time interaction was detected in insulin sensitivity (1/fasting plasma insulin concentration). However, without adjustment for multiple comparisons, the 2000-IU group had higher values than the 600-IU group at 3 mo (mean difference: 0.014; 95% CI: 0.0015, 0.026) and at 6 mo (mean difference: 0.015; 95% CI: 0.0019, 0.027).

Changes in anthropometry and body composition

Changes in BMI differed between groups. BMI was lower in the 2000-IU group than in the 600-IU group at 6 mo. No overall group × time interaction was detected in BMI *z* score, WC, waist-to-height ratio, percentage of total body fat, total body fat, total lean mass, or total bone mineral content. However, without adjustment for multiple comparisons, WC and total body fat were lower in the 2000-IU group than in the 600-IU group at 6 mo (mean difference: −2.46 [95% CI: −4.48, −0.44] cm and -1248.42 [95% CI: -2477.14, -19.70] g, respectively).

Limiting the analyses only to black children did not make the overall effect on the primary outcome and the significant secondary outcome measures consistently stronger (data not shown).

Exploratory measures of inflammation

No between-group differences were found in mean changes in leptin, adiponectin, hsCRP, IL-6, and TNF-α at 3 and 6 mo (**Supplemental Table 2**).

Compliance

Overall, 73% of prescribed tablets were taken at 3 and 6 mo. The proportions taken were validated through MEMs Cap readings and were similar across the 600-IU versus the 1000-IU versus the 2000-IU treatment groups at 3 mo [73% ($n = 52$) vs 68% ($n = 44$) vs 77% ($n = 50$); $P = 0.33$] and 6 mo [73% ($n = 42$) vs 73% ($n = 40$) vs 73% ($n = 52$); $P = 0.94$], respectively.

TABLE 2 Primary and secondary outcomes[1]

		600 IU ($n = 76$)		1000 IU ($n = 74$)		2000 IU ($n = 75$)		Adjusted difference,[2] mean (95% CI)		
	Visit	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	1000 IU–600 IU	2000 IU–600 IU	Overall,[3,4] P
Vascular measures										
FMD,[5] %	Baseline	76	7.66 (4.47)	74	6.82 (5.58)	75	7.09 (4.63)	—	—	0.88
	3-mo visit	57	7.64 (5.51)	57	8.14 (4.50)	60	7.79 (4.34)	0.70 (−1.05, 2.44)	0.35 (−1.37, 2.07)	
	6-mo visit	50	7.31 (5.14)	47	8.27 (5.92)	58	7.61 (5.15)	0.85 (−1.31, 3.01)	0.31 (−1.74, 2.36)	
Pulse-wave velocity, m/s	Baseline	66	4.78 (0.76)	68	4.91 (0.80)	70	4.99 (0.74)	—	—	0.85
	3-mo visit	51	4.96 (0.74)	54	4.78 (0.67)	51	4.93 (0.68)	− 0.099 (−0.33, 0.13)	− 0.097 (−0.33, 0.14)	
	6-mo visit	45	4.88 (0.75)	43	4.85 (0.63)	51	4.85 (0.76)	− 0.021 (−0.27, 0.23)	− 0.092 (−0.33, 0.15)	
Augmentation index at 75 beats/min	Baseline	71	3.70 (9.4)	74	2.54 (9.23)	74	3.80 (9.34)	—	—	0.61
	3-mo visit	55	2.89 (11.01)	55	3.11 (10.44)	58	2.40 (8.73)	0.75 (−2.35, 3.85)	− 0.45 (−3.51, 2.61)	
	6-mo visit	44	1.74 (10.29)	46	2.22 (10.97)	57	3.75 (8.44)	0.23 (−3.16, 3.62)	1.74 (−1.50, 4.97)	
Central-systolic BP, mm Hg	Baseline	70	97.16 (9.23)	73	97.77 (8.36)	74	98.18 (6.57)	—	—	0.24
	3-mo visit	55	96.85 (8.26)	55	96.08 (8.31)	58	98.07 (6.20)	− 1.06 (−3.46, 1.33)	0.63 (−1.72, 2.99)	
	6-mo visit	44	98.50 (7.84)	46	96.05 (8.19)	57	98.45 (7.20)	− 2.66 (−5.27, −0.046)*	− 0.54 (−3.04, 1.95)	
Central-diastolic BP, mm Hg	Baseline	70	66.79 (8.09)	73	67.64 (7.47)	74	68.12 (5.78)	—	—	0.0117
	3-mo visit	55	67.35 (6.70)	55	65.77 (7.47)	58	68.12 (6.15)	− 2.14 (−4.34, 0.052)	0.22 (−1.95, 2.39)	0.063
	6-mo visit	44	68.57 (6.58)	46	65.33 (8.27)	57	68.69 (5.87)	− 3.57 (−5.97, −1.17)*	− 0.25 (−2.54, 2.05)	0.004
Systemic-systolic BP, mm Hg	Baseline	76	114.57 (10.28)	74	115.35 (10.36)	75	115.20 (7.58)	—	—	0.53
	3-mo visit	57	113.70 (9.84)	58	113.21 (8.95)	60	115.00 (7.96)	− 0.61 (−3.28, 2.06)	0.83 (−1.81, 3.47)	
	6-mo visit	50	115.70 (9.55)	48	114.88 (11.76)	58	114.71 (8.45)	− 1.13 (−3.99, 1.73)	− 1.54 (−4.28, 1.20)	
Systemic-diastolic BP, mm Hg	Baseline	76	65.97 (7.99)	74	66.70 (7.39)	75	67.15 (5.74)	—	—	0.0256
	3-mo visit	57	66.12 (6.65)	58	64.88 (7.02)	60	67.02 (6.02)	− 1.71 (−3.83, 0.40)	0.37 (−1.73, 2.47)	0.12
	6-mo visit	50	67.30 (6.53)	48	64.73 (8.44)	58	67.57 (5.81)	− 3.28 (−5.55, −1.00)*	− 0.35 (−2.53, 1.82)	0.008
Biochemical measures										
LDL cholesterol, mg/dL	Baseline	76	91.64 (22.94)	74	86.88 (24.63)	75	91.51 (25.39)	—	—	0.98
	3-mo visit	57	90.49 (22.45)	57	87.53 (24.96)	60	90.87 (27.00)	0.44 (−5.39, 6.27)	0.28 (−5.48, 6.04)	
	6-mo visit	50	89.24 (23.15)	48	86.83 (23.57)	58	90.59 (29.53)	1.33 (−4.88, 7.54)	1.73 (−4.24, 7.70)	
HDL cholesterol, mg/dL	Baseline	76	46.49 (10.24)	74	45.41 (9.58)	75	44.95 (8.98)	—	—	0.70
	3-mo visit	57	45.19 (8.84)	57	45.11 (8.56)	60	44.55 (8.41)	− 0.43 (−2.44, 1.59)	0.99 (−1.00, 2.98)	
	6-mo visit	50	46.26 (10.78)	48	46.17 (9.94)	58	44.95 (9.08)	− 0.012 (−2.15, 2.13)	0.30 (−1.76, 2.36)	
Triglycerides, mg/dL	Baseline	76	72.66 (34.13)	74	77.14 (32.96)	75	76.16 (31.21)	—	—	0.82
	3-mo visit	57	77.07 (30.68)	57	75.91 (39.28)	60	76.82 (22.85)	− 2.39 (−11.73, 6.95)	− 4.70 (−13.93, 4.52)	
	6-mo visit	50	78.30 (31.07)	48	84.29 (39.93)	58	81.74 (33.07)	2.51 (−7.46, 12.49)	0.049 (−9.52, 9.61)	

(*Continued*)

TABLE 2 (*Continued*)

		600 IU ($n = 76$)		1000 IU ($n = 74$)		2000 IU ($n = 75$)		Adjusted difference,[2] mean (95% CI)		
	Visit	*n*	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)	1000 IU–600 IU	2000 IU–600 IU	Overall,[3,4] *P*
Total cholesterol, mg/dL	Baseline	76	152.72 (27.48)	74	147.72 (27.27)	75	151.71 (26.64)	—	—	0.97
	3-mo visit	57	151.11 (26.41)	57	147.77 (27.11)	60	150.78 (28.33)	− 0.50 (−6.87, 5.88)	0.041 (−6.26, 6.34)	
	6-mo visit	50	151.18 (27.80)	48	149.88 (25.15)	58	151.88 (30.11)	1.76 (−5.02, 8.55)	1.76 (−4.75, 8.28)	
Non–HDL cholesterol, mg/dL	Baseline	76	106.24 (25.78)	74	102.31 (26.57)	75	106.76 (25.54)	—	—	0.95
	3-mo visit	57	105.91 (25.14)	57	102.67 (27.19)	60	106.23 (27.18)	0.017 (−5.83, 5.86)	− 0.91 (−6.68, 4.86)	
	6-mo visit	50	104.92 (24.86)	48	103.71 (25.87)	58	106.93 (29.52)	1.87 (−4.35, 8.09)	1.52 (−4.45, 7.50)	
Fasting blood glucose, mg/dL	Baseline	76	88.72 (6.30)	74	89.49 (7.18)	75	89.19 (7.04)	—	—	0.0945
	3-mo visit	57	89.89 (6.11)	57	88.00 (6.95)	60	88.52 (7.14)	− 1.87 (−4.02, 0.29)	− 2.09 (−4.22, 0.032)	
	6-mo visit	50	90.44 (6.04)	48	88.48 (7.05)	58	88.41 (6.33)	− 1.90 (−4.20, 0.41)	− 2.67 (−4.88, −0.46)*	
Insulin, μU/mL	Baseline	75	21.23 (16.12)	73	19.08 (10.43)	73	22.57 (26.73)	—	—	0.68
	3-mo visit	53	25.21 (17.24)	54	20.99 (12.33)	57	24.72 (30.88)	− 2.25 (−6.35, 1.86)	− 2.16 (−6.23, 1.91)	
	6-mo visit	47	24.78 (20.12)	44	22.26 (11.68)	56	23.95 (30.00)	− 2.30 (−6.68, 2.08)	− 2.63 (−6.81, 1.56)	
1/Fasting insulin	Baseline	75	0.07 (0.05)	73	0.07 (0.04)	73	0.08 (0.05)	—	—	0.12
	3-mo visit	53	0.06 (0.03)	54	0.06 (0.04)	57	0.07 (0.05)	0.0096 (−0.0028, 0.022)	0.014 (0.0015, 0.026)*	
	6-mo visit	47	0.06 (0.04)	44	0.06 (0.04)	56	0.08(0.06)	0.0087 (−0.0045, 0.022)	0.015 (0.0019, 0.027)*	
C-peptide, ng/mL	Baseline	75	1.91 (1.21)	73	1.96 (1.28)	73	1.87 (1.15)	—	—	0.79
	3-mo visit	53	2.24 (1.34)	54	2.16 (1.25)	57	2.11 (1.18)	− 0.11 (−0.40, 0.18)	− 0.15 (−0.44, 0.14)	
	6-mo visit	47	2.15 (1.38)	44	2.05 (1.26)	56	2.15 (1.32)	− 0.061 (−0.37, 0.25)	0.015 (−0.28, 0.31)	
Anthropometric measures										
BMI, kg/m^2	Baseline	76	30.68 (7.06)	74	29.8 (6.14)	75	30.27 (5.78)	—	—	0.0094
	3-mo visit	57	30.23 (6.55)	58	30.53 (6.03)	60	31.01 (6.05)	− 0.094 (−0.61, 0.42)	0.026 (−0.49, 0.54)	0.89
	6-mo visit	50	30.62 (6.78)	48	30.99 (6.41)	58	30.82 (6.12)	0.19 (−0.36, 0.74)	− 0.59 (−1.12, −0.062)*	0.01
BMI *z* score	Baseline	76	1.90 (0.51)	74	1.87 (0.47)	75	1.86 (0.47)	—	—	0.051
	3-mo visit	57	1.85 (0.50)	58	1.91 (0.43)	60	1.90 (0.49)	− 0.0035 (−0.058, 0.051)	− 0.0021 (−0.056, 0.052)	
	6-mo visit	50	1.84 (0.52)	48	1.94 (0.44)	58	1.84 (0.52)	0.021 (−0.036, 0.079)	− 0.054 (−0.11, 0.0017)	
Waist circumference, cm	Baseline	76	88.82 (15.49)	74	88.95 (12.29)	75	89.42 (13.56)	—	—	0.094
	3-mo visit	57	88.92 (15.03)	58	89.36 (12.02)	60	90.17 (14.14)	− 1.83 (−3.80, 0.13)	− 1.62 (−3.57, 0.33)	
	6-mo visit	50	89.60 (14.84)	48	90.04 (12.72)	58	89.94 (14.16)	− 1.19 (−3.29, 0.90)	− 2.46 (−4.48, −0.44)*	
Waist-to-height ratio	Baseline	76	0.51 (0.14)	74	0.50 (0.12)	75	0.49 (0.10)	—	—	0.31
	3-mo visit	57	0.50 (0.12)	58	0.50 (0.11)	60	0.51 (0.10)	0.0060 (−0.0075, 0.019)	0.0083 (−0.0050, 0.022)	
	6-mo visit	50	0.51 (0.12)	48	0.50 (0.11)	58	0.51 (0.10)	0.0094 (−0.0049, 0.024)	− 0.00078 (−0.015, 0.013)	

(*Continued*)

TABLE 2 (*Continued*)

	Visit	600 IU ($n = 76$)		1000 IU ($n = 74$)		2000 IU ($n = 75$)		Adjusted difference,[2] mean (95% CI)		
		n	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)	1000 IU–600 IU	2000 IU–600 IU	Overall,[3,4] *P*
Percent body fat by DXA	Baseline	73	34.00 (8.19)	74	32.91 (7.97)	74	33.47 (8.85)	—	—	0.19
	6-mo visit	45	33.89 (7.99)	44	34.70 (7.20)	52	32.62 (9.99)	−0.30 (−1.29, 0.69)	−0.86 (−1.81, 0.085)	
Total body fat, g	Baseline	73	29,129.32 (13,907.96)	74	26,844.62 (11,703.27)	74	27,796.98 (12,059.81)	—	—	0.19
	6-mo visit	45	28,960.22 (13,272.12)	44	29,290.23 (11,732.59)	52	28,569.82 (13,101.01)	−374.74 (−1653.79, 904.31)	−1248.42 (−2477.14, −19.70)*	
Total lean mass, g	Baseline	73	51,055.42 (12,677.32)	74	51,103.94 (12,379.65)	74	50,900.39 (10,447.92)	—	—	0.32
	6-mo visit	45	51,538.95 (11,866.33)	44	51,461.98 (11,625.88)	52	53,818.52 (10,478.48)	−137.21 (−1162.44, 888.01)	−707.16 (−1692.11, 277.78)	
Total bone mineral content, g	Baseline	73	2155.34 (525.04)	74	2154.34 (504.67)	74	2154.34 (504.67)	—	—	0.70
	6-mo visit	45	2202.02 (481.40)	44	2188.34 (449.50)	52	2188.34 (449.50)	−13.70 (−54.26, 26.87)	−15.46 (−54.43, 23.51)	

[1]BP, blood pressure; FMD%, brachial artery flow-mediated dilation percentage.
[2]Adjusted between-group (2000–600 IU and 1000–600 IU) differences were estimated using linear mixed models with fixed effects of group × time interaction, adjusting for race and obesity status (randomization stratification factors) and restricting baseline means to be equal. *Indicates significant adjusted between-group (2000–600 IU or 1000–600 IU) differences in mean concentrations.
[3]*P* values of group × time interaction and, if significant ($P < 0.05$), overall *F* test for group (2000 IU vs 1000 IU vs 600 IU) differences at 3 mo and 6 mo were derived from linear mixed models with fixed effects of group × time interaction adjusting for race and obesity status (randomization stratification factors) and restricting baseline means to be equal.
[4]Dose–response at 6 mo: central-diastolic BP, $P = 0.83$; systemic diastolic BP, $P = 0.75$; BMI, $P = 0.029$.
[5]FMD% between-group difference in mean concentration represented with 97.5% CI at 6 months.

Adverse effects

The occurrence of adverse events did not differ between treatment groups. Adverse effects possibly related to study intervention consisted of nausea in 1 participant and abdominal pain in 2 participants.

Discussion

We found that correction of vitamin D deficiency in overweight and obese children by means of vitamin D_3 supplementation (1000 IU or 2000 IU/d vs 600 IU/d) did not affect measures of arterial endothelial function or stiffness, lipid profile indices, or systemic inflammation. However, vitamin D supplementation resulted in reductions in BP and fasting glucose concentrations and led to improvements in insulin sensitivity. Of note, we found that BP was significantly reduced in the 1000-IU group but not in the 2000-IU group.

Studies reporting the effect of vitamin D supplementation on endothelial function and arterial stiffness in children are limited (13, 45). Our failure to find an effect may indicate an actual lack of effect, or failure to achieve a threshold concentration of 25(OH)D required for affecting these indices, or variations among participants in vitamin D metabolism or genetics affecting pharmacokinetics or function of vitamin D (46, 47). Our findings of the lack of effect of vitamin D supplementation on endothelial function are consistent with those of an open-label trial by Javed et al. (13). Dong et al. (45) conducted a 16-wk RCT of 400 versus 2000 IU vitamin D_3/d in 49 14- to 18-y-old vitamin D–deficient black youth (BMI percentile: 64.7 ± 32.1). In that trial, mean 25(OH)D concentrations increased from 13.4 ± 3.8 ng/mL to 23.9 ± 7.3 and 34.3 ± 12 ng/mL in the 400-IU and 2000-IU groups, respectively. Measures of arterial stiffness improved in the 2000-IU group; no impact on BP was found in either group. As noted earlier, our failure to find an effect on arterial stiffness may have been the result of the smaller increases in 25(OH)D concentration in our trial.

Studies examining the effects of vitamin D on BP in children also are limited (45, 48), and in adults they have yielded inconsistent results, probably from differences in trial design (49–53). In a study by Khayyatzadeh et al. (48) in 940 Iranian girls aged 12 to 18 y old, once-weekly supplementation with 50,000 IU vitamin D_3 for 9 wk increased their mean serum 25(OH)D concentration from 9.4 ± 8.8 to 36.4 ± 15.4 ng/mL and reduced their diastolic BP, fasting glucose concentrations, WC, and total- and LDL-cholesterol concentrations. In a subset of 580 of the girls, increases in 25(OH)D concentrations were associated with reductions in hsCRP concentrations (54). Our findings on BP and glucose are consistent with these earlier findings. In our study sample, the reductions in PTH, improvements in insulin sensitivity, and possible downregulation of the renin-angiotensin-aldosterone system (55, 56) may explain the lowering of BP at 3 and 6 mo. Our findings of null effects on CRP and other markers of systemic inflammation could be ascribed to our trial's relatively lower doses of vitamin D intervention and smaller increases in 25(OH)D concentrations. Our findings of reductions in central-systolic BP and fasting glucose concentrations and improvements in insulin sensitivity should be interpreted with caution given

the lack of adjustment for multiplicity and considered as hypothesis-generating and deserve further exploration for confirmation.

In a small 6-mo placebo-controlled trial among 35 overweight and obese children aged 9 to 19 y, administration of 4000 IU vitamin D_3/d increased mean 25(OH)D concentration from 19.6 to 39.1 ng/mL and reduced fasting glucose concentrations and insulin resistance, without an impact on inflammatory markers, similarly to our findings (57).

In 2 RCTs conducted in, respectively, in 44 and 47 predominantly white 12- to 18-y-old obese children with 25(OH)D concentrations averaging ~25 ng/mL, supplementation with 2000 IU vitamin D_3/d for 12 wk increased 25(OH)D concentrations to 31 and 27 ng/mL, respectively, but had no impact on glucose homeostasis or on lipids (58, 59). Similarly, in a 12-wk RCT among 323 black children and white children aged 9 to 13 y (normal weight: 55%; mean 25(OH)D concentration: 28 ± 0.4 ng/mL) receiving 0, 400, 1000, 2000, or 4000 IU vitamin D_3/d, dose–response increases in 25(OH)D concentrations showed no effect on insulin resistance (12). Higher representations of white children and the inclusion of vitamin D–replete (25[OH]D >20 ng/mL) children in those studies (12, 58, 59), in contrast to our study's higher number of black children and inclusion only of vitamin D–deficient children, may explain the contrasting findings regarding glucose homeostasis between those studies and our study.

Supplementation-induced changes in BMI, WC, and total body fat at 6 mo in our cohort are clinically irrelevant, as there were no changes in waist-to-height ratio, percentage of total body fat, or total lean mass at 6 mo.

Our study had several strengths, such as the inclusion of only vitamin D–deficient children; enrollment of a substantial number of black children, a population at risk of both vitamin D deficiency and cardiometabolic risk factors (11, 27, 60–62); 3 fixed doses of vitamin D_3 intervention over 6 mo; utilization of state-of-the-art tests of vascular health; and ascertainment of body composition with DXA. Limitations of our study are the relatively high rate of attrition (30%) and the relatively low rate of pill-count–based compliance (73%), which could have impacted the magnitude of increase in serum 25(OH)D concentrations and the consequent responsive changes in our primary and secondary outcome variables.

In conclusion, vitamin D_3 supplementation (1000 or 2000 IU/d vs 600 IU/d) in overweight and obese vitamin D–deficient children induced increases in 25(OH)D concentrations and resulted in lowering of BP and fasting glucose concentration and improvements in insulin sensitivity. Vitamin D supplementation did not affect arterial endothelial function or stiffness. In the VITAL (Vitamin D and omega-3 trial) study, supplementation with 2000 IU vitamin D_3/d for 5 y in adults aged ≥50 y had no impact on cardiovascular events compared with placebo (63). Notwithstanding the VITAL trial's findings, our findings suggest that vitamin D status optimization, through its beneficial effects on BP and glucose homeostasis, may have a primary preventive role in improving the long-term cardiovascular health of overweight and obese children. Because our study sample consisted mainly of black children, future studies should evaluate the use of vitamin D supplementation as a strategy for addressing racial disparities in cardiovascular disease.

We thank the research coordinators (Flora Olabopo and Arshad Khalid); research pharmacist (Staci Ziobert); University of Pittsburgh's NIH-funded office-based research network (Pediatric PittNet); and Montefiore CTRC for their contributions toward the success of this project. We also express our gratitude to Jack L Paradise, Professor Emeritus of Pediatrics, University of Pittsburgh School of Medicine, for his invaluable editorial suggestions. In addition, we thank and acknowledge Catherine Gordon, Professor of Pediatrics, Harvard Medical School; Lisa Bodnar, Professor of Epidemiology, University of Pittsburgh Graduate School of Public Health and School of Medicine; Satish Iyengar, Professor, Department of Statistics, University of Pittsburgh; and William A Neal, Professor Emeritus of Pediatrics, West Virginia University School of Medicine for their time and effort to serve on the data and safety monitoring board for this trial.

The authors' responsibilities were as follows—KR, CGM, ANV, SA, SLG, and SER: designed the trial; KR, ATK, CGM, ANV, SA, and SER: conducted the trial; CGM: performed the statistical analysis; KR, CGM, and ATK: wrote the manuscript; KR, CGM, ATK, ANV, SA, SLG, MAV, MFH, and SER: critically revised the manuscript for important intellectual content; MAV and MFH: provided material and technical support; KR: had primary responsibility for the final content; and all authors: read and approved the final manuscript. MFH is a consultant for Quest Diagnostics. All other authors report no conflicts of interest.

References

1. Delvin EE, Lambert M, Levy E, O'Loughlin J, Mark S, Gray-Donald K, Paradis G. Vitamin D status is modestly associated with glycemia and indicators of lipid metabolism in French-Canadian children and adolescents. J Nutr 2010;140(5):987–91.
2. Kao KT, Abidi N, Ranasinha S, Brown J, Rodda C, McCallum Z, Zacharin M, Simm PJ, Magnussen CG, Sabin MA. Low vitamin D is associated with hypertension in paediatric obesity. J Paediatr Child Health 2015;51(12):1207–13.
3. Moore CE, Liu Y. Elevated systolic blood pressure of children in the United States is associated with low serum 25-hydroxyvitamin D concentrations related to body mass index: National Health and Examination Survey 2007–2010. Nutr Res 2017;38:64–70.
4. Reis JP, von Muhlen D, Miller ER 3rd, Michos ED, Appel LJ. Vitamin D status and cardiometabolic risk factors in the united states adolescent population. Pediatrics 2009;124(3):e371–9.
5. Tarcin O, Yavuz DG, Ozben B, Telli A, Ogunc AV, Yuksel M, Toprak A, Yazici D, Sancak S, Deyneli O, et al. Effect of vitamin D deficiency and replacement on endothelial function in asymptomatic subjects. J Clin Endocrinol Metab 2009;94(10):4023–30.
6. Al Mheid I, Patel R, Murrow J, Morris A, Rahman A, Fike L, Kavtaradze N, Uphoff I, Hooper C, Tangpricha V, et al. Vitamin D status is associated with arterial stiffness and vascular dysfunction in healthy humans. J Am Coll Cardiol 2011;58(2):186–92.
7. Maruhashi T, Soga J, Fujimura N, Idei N, Mikami S, Iwamoto Y, Iwamoto A, Kajikawa M, Matsumoto T, Oda N, et al. Endothelial dysfunction, increased arterial stiffness, and cardiovascular risk prediction in patients with coronary artery disease: FMD-J (Flow-Mediated Dilation Japan) Study A. J Am Heart Assoc 2018;7(14):e008588.
8. Yeboah J, Crouse JR, Hsu FC, Burke GL, Herrington DM. Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: the Cardiovascular Health Study. Circulation 2007;115(18):2390–7.
9. Dong Y, Pollock N, Stallmann-Jorgensen IS, Gutin B, Lan L, Chen TC, Keeton D, Petty K, Holick MF, Zhu H. Low 25-hydroxyvitamin D levels in adolescents: race, season, adiposity, physical activity, and fitness. Pediatrics 2010;125(6):1104–11.
10. Pacifico L, Anania C, Osborn JF, Ferraro F, Bonci E, Olivero E, Chiesa C. Low 25(OH)D3 levels are associated with total adiposity, metabolic syndrome, and hypertension in Caucasian children and adolescents. Eur J Endocrinol 2011;165(4):603–11.
11. Rajakumar K, de las Heras J, Chen TC, Lee S, Holick MF, Arslanian SA. Vitamin D status, adiposity, and lipids in black American and Caucasian children. J Clin Endocrinol Metab 2011;96(5):1560–7.

12. Ferira AJ, Laing EM, Hausman DB, Hall DB, McCabe GP, Martin BR, Hill Gallant KM, Warden SJ, Weaver CM, Peacock M, et al. Vitamin D supplementation does not impact insulin resistance in black and white children. J Clin Endocrinol Metab 2016;101(4):1710–8.
13. Javed A, Kullo IJ, Balagopal PB, Kumar S. Effect of vitamin D3 treatment on endothelial function in obese adolescents. Pediatr Obes 2016;11(4):279–84.
14. Al Mheid I, Quyyumi AA. Vitamin D and cardiovascular disease: controversy unresolved. J Am Coll Cardiol 2017;70(1):89–100.
15. Mazidi M, Karimi E, Rezaie P, Vatanparast H. The impact of vitamin D supplement intake on vascular endothelial function; a systematic review and meta-analysis of randomized controlled trials. Food Nutr Res 2017;61(1):1273574.
16. Veloudi P, Jones G, Sharman JE. Effectiveness of vitamin D supplementation for cardiovascular health outcomes. Pulse (Basel) 2017;4(4):193–207.
17. Institute of Medicine. Dietary Reference Intake for Calcium and Vitamin D. Washington (DC): The National Academies Press; 2011.
18. Donald AE, Charakida M, Falaschetti E, Lawlor DA, Halcox JP, Golding J, Hingorani AD, Smith GD, Deanfield JE. Determinants of vascular phenotype in a large childhood population: the Avon Longitudinal Study of Parents and Children (ALSPAC). Eur Heart J 2010;31(12):1502–10.
19. Urbina EM, Williams RV, Alpert BS, Collins RT, Daniels SR, Hayman L, Jacobson M, Mahoney L, Mietus-Snyder M, Rocchini A, et al. Noninvasive assessment of subclinical atherosclerosis in children and adolescents: recommendations for standard assessment for clinical research: a scientific statement from the American Heart Association. Hypertension 2009;54(5):919–50.
20. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM, Endocrine S. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011;96(7):1911–30.
21. Chen TC, Turner AK, Holick MF. A method for the determination of the circulating concentration of vitamin D. J Nutr Biochem 1990;1(5):272–6.
22. Khalid AT, Moore CG, Hall C, Olabopo F, Rozario NL, Holick MF, Greenspan SL, Rajakumar K. Utility of sun-reactive skin typing and melanin index for discerning vitamin D deficiency. Pediatr Res 2017;82(3):444–51.
23. Rajakumar K, Fernstrom JD, Janosky JE, Greenspan SL. Vitamin D insufficiency in preadolescent African-American children. Clin Pediatr (Phila) 2005;44(8):683–92.
24. Nucci AM, Russell CS, Luo R, Ganji V, Olabopo F, Hopkins B, Holick MF, Rajakumar K. The effectiveness of a short food frequency questionnaire in determining vitamin D intake in children. Dermatoendocrinol 2013;5(1):205–10.
25. Rockett HR, Breitenbach M, Frazier AL, Witschi J, Wolf AM, Field AE, Colditz GA. Validation of a youth/adolescent food frequency questionnaire. Prev Med 1997;26(6):808–16.
26. Rockett HR, Wolf AM, Colditz GA. Development and reproducibility of a food frequency questionnaire to assess diets of older children and adolescents. J Am Diet Assoc 1995;95(3):336–40.
27. Rajakumar K, Moore CG, Yabes J, Olabopo F, Haralam MA, Comer D, Bogusz J, Nucci A, Sereika S, Dunbar-Jacob J, et al. Effect of vitamin D3 supplementation in black and in white children: a randomized, placebo-controlled trial. J Clin Endocrinol Metab 2015;100(8):3183–92.
28. Gortmaker SL, Cheung LW, Peterson KE, Chomitz G, Cradle JH, Dart H, Fox MK, Bullock RB, Sobol AM, Colditz G, et al. Impact of a school-based interdisciplinary intervention on diet and physical activity among urban primary school children: eat well and keep moving. Arch Pediatr Adolesc Med 1999;153(9):975–83.
29. Wolf AM, Hunter DJ, Colditz GA, Manson JE, Stampfer MJ, Corsano KA, Rosner B, Kriska A, Willett WC. Reproducibility and validity of a self-administered physical activity questionnaire. Int J Epidemiol 1994;23(5):991–9.
30. Michel H, Olabopo F, Wang L, Nucci A, Greenspan SL, Rajakumar K. Determinants of 25-hydroxyvitamin D concentrations in infants and toddlers. Curr Nutr Food Sci 2015;11(2):124–30.
31. Kim JY, Bacha F, Tfayli H, Michaliszyn SF, Yousuf S, Arslanian S. Adipose tissue insulin resistance in youth on the spectrum from normal weight to obese and from normal glucose tolerance to impaired glucose tolerance to type 2 diabetes. Diabetes Care 2019;42(2):265–72.
32. Kim JY, Michaliszyn SF, Nasr A, Lee S, Tfayli H, Hannon T, Hughan KS, Bacha F, Arslanian S. The shape of the glucose response curve during an oral glucose tolerance test heralds biomarkers of type 2 diabetes risk in obese youth. Diabetes Care 2016;39(8):1431–9.
33. George L, Bacha F, Lee S, Tfayli H, Andreatta E, Arslanian S. Surrogate estimates of insulin sensitivity in obese youth along the spectrum of glucose tolerance from normal to prediabetes to diabetes. J Clin Endocrinol Metab 2011;96(7):2136–45.
34. Ferguson ID, Griffin P, Michel JJ, Yano H, Gaffen SL, Mueller RG, Dvergsten JA, Piganelli JD, Rosenkranz ME, Kietz DA, et al. T cell receptor-independent, CD31/IL-17A-driven inflammatory axis shapes synovitis in juvenile idiopathic arthritis. Front Immunol 2018;9:1802.
35. Shaaban CE, Aizenstein HJ, Jorgensen DR, MacCloud RL, Meckes NA, Erickson KI, Glynn NW, Mettenburg J, Guralnik J, Newman AB, et al. In vivo imaging of venous side cerebral small-vessel disease in older adults: an MRI method at 7T. AJNR Am J Neuroradiol 2017;38(10):1923–8.
36. Vallejo AN, Hamel DL Jr, Mueller RG, Ives DG, Michel JJ, Boudreau RM, Newman AB. NK-like T cells and plasma cytokines, but not anti-viral serology, define immune fingerprints of resilience and mild disability in exceptional aging. PLoS One 2011;6(10):e26558.
37. Engler MM, Engler MB, Malloy MJ, Chiu EY, Schloetter MC, Paul SM, Stuehlinger M, Lin KY, Cooke JP, Morrow JD, et al. Antioxidant vitamins C and E improve endothelial function in children with hyperlipidemia: Endothelial Assessment of Risk from Lipids in Youth (EARLY) Trial. Circulation 2003;108(9):1059–63.
38. Kelly AS, Wetzsteon RJ, Kaiser DR, Steinberger J, Bank AJ, Dengel DR. Inflammation, insulin, and endothelial function in overweight children and adolescents: the role of exercise. J Pediatr 2004;145(6):731–6.
39. Woo KS, Chook P, Yu CW, Sung RY, Qiao M, Leung SS, Lam CW, Metreweli C, Celermajer DS. Overweight in children is associated with arterial endothelial dysfunction and intima-media thickening. Int J Obes Relat Metab Disord 2004;28(7):852–7.
40. Twisk J, de Boer M, de Vente W, Heymans M. Multiple imputation of missing values was not necessary before performing a longitudinal mixed-model analysis. J Clin Epidemiol 2013;66(9):1022–8.
41. Peters SA, Bots ML, den Ruijter HM, Palmer MK, Grobbee DE, Crouse JR 3rd, O'Leary DH, Evans GW, Raichlen JS, Moons KG, et al. Multiple imputation of missing repeated outcome measurements did not add to linear mixed-effects models. J Clin Epidemiol 2012;65(6):686–95.
42. Fitzmaurice GM, Laird N, Ware JH. Applied Longitudinal Analysis. 2nd ed. Hoboken (NJ): Wiley; 2011.
43. Coffman CJ, Edelman D, Woolson RF. To condition or not condition? Analysing "change" in longitudinal randomised controlled trials. BMJ Open 2016;6(12):e013096.
44. Liu GF, Lu K, Mogg R, Mallick M, Mehrotra DV. Should baseline be a covariate or dependent variable in analyses of change from baseline in clinical trials? Stat Med 2009;28(20):2509–30.
45. Dong Y, Stallmann-Jorgensen IS, Pollock NK, Harris RA, Keeton D, Huang Y, Li K, Bassali R, Guo DH, Thomas J, et al. A 16-week randomized clinical trial of 2000 international units daily vitamin D3 supplementation in black youth: 25-hydroxyvitamin D, adiposity, and arterial stiffness. J Clin Endocrinol Metab 2010;95(10):4584–91.
46. Barry EL, Rees JR, Peacock JL, Mott LA, Amos CI, Bostick RM, Figueiredo JC, Ahnen DJ, Bresalier RS, Burke CA, et al. Genetic variants in CYP2R1, CYP24A1, and VDR modify the efficacy of vitamin D3 supplementation for increasing serum 25-hydroxyvitamin D levels in a randomized controlled trial. J Clin Endocrinol Metab 2014;99(10):E2133–7.
47. Carlberg C, Haq A. The concept of the personal vitamin D response index. J Steroid Biochem Mol Biol 2018;175:12–7.
48. Khayyatzadeh SS, Mirmoosavi SJ, Fazeli M, Abasalti Z, Avan A, Javandoost A, Rahmani F, Tayefi M, Hanachi P, Ferns GA, et al. High-dose vitamin D supplementation is associated with an improvement in several cardio-metabolic risk factors in adolescent girls: a nine-week follow-up study. Ann Clin Biochem 2018;55(2):227–35.
49. Bressendorff I, Brandi L, Schou M, Nygaard B, Frandsen NE, Rasmussen K, Odum L, Ostergaard OV, Hansen D. The effect of high dose cholecalciferol on arterial stiffness and peripheral and central blood pressure in healthy humans: a randomized controlled trial. PLoS One 2016;11(8):e0160905.

50. Sollid ST, Hutchinson MY, Fuskevag OM, Figenschau Y, Joakimsen RM, Schirmer H, Njolstad I, Svartberg J, Kamycheva E, Jorde R. No effect of high-dose vitamin D supplementation on glycemic status or cardiovascular risk factors in subjects with prediabetes. Diabetes Care 2014;37(8):2123–31.
51. Witham MD, Dove FJ, Dryburgh M, Sugden JA, Morris AD, Struthers AD. The effect of different doses of vitamin D(3) on markers of vascular health in patients with type 2 diabetes: a randomised controlled trial. Diabetologia 2010;53(10):2112–9.
52. Forman JP, Scott JB, Ng K, Drake BF, Suarez EG, Hayden DL, Bennett GG, Chandler PD, Hollis BW, Emmons KM, et al. Effect of vitamin D supplementation on blood pressure in blacks. Hypertension 2013;61(4):779–85.
53. Sluyter JD, Camargo CA Jr, Stewart AW, Waayer D, Lawes CMM, Toop L, Khaw KT, Thom SAM, Hametner B, Wassertheurer S, et al. Effect of monthly, high-dose, long-term vitamin D supplementation on central blood pressure parameters: a randomized controlled trial substudy. J Am Heart Assoc 2017;6(10):e006802.
54. Tabatabaeizadeh SA, Avan A, Bahrami A, Khodashenas E, Esmaeili H, Ferns GA, Abdizadeh MF, Ghayour-Mobarhan M. High dose supplementation of vitamin D affects measures of systemic inflammation: reductions in high sensitivity c-reactive protein level and neutrophil to lymphocyte ratio (NLR) distribution. J Cell Biochem 2017;118(12):4317–22.
55. Vaidya A, Forman JP, Williams JS. Vitamin D and the vascular sensitivity to angiotensin II in obese Caucasians with hypertension. J Hum Hypertens 2011;25(11):672–8.
56. Vaidya A, Sun B, Larson C, Forman JP, Williams JS. Vitamin D3 therapy corrects the tissue sensitivity to angiotensin ii akin to the action of a converting enzyme inhibitor in obese hypertensives: an interventional study. J Clin Endocrinol Metab 2012;97(7):2456–65.
57. Belenchia AM, Tosh AK, Hillman LS, Peterson CA. Correcting vitamin D insufficiency improves insulin sensitivity in obese adolescents: a randomized controlled trial. Am J Clin Nutr 2013;97(4):774–81.
58. Javed A, Vella A, Balagopal PB, Fischer PR, Weaver AL, Piccinini F, Dalla Man C, Cobelli C, Giesler PD, Laugen JM, et al. Cholecalciferol supplementation does not influence beta-cell function and insulin action in obese adolescents: a prospective double-blind randomized trial. J Nutr 2015;145(2):284–90.
59. Nader NS, Aguirre Castaneda R, Wallace J, Singh R, Weaver A, Kumar S. Effect of vitamin D3 supplementation on serum 25(OH)D, lipids and markers of insulin resistance in obese adolescents: a prospective, randomized, placebo-controlled pilot trial. Horm Res Paediatr 2014;82(2):107–12.
60. Lefferts WK, Augustine JA, Spartano NL, Atallah-Yunes NH, Heffernan KS, Gump BB. Racial differences in aortic stiffness in children. J Pediatr 2017;180:62–7.
61. Mokwatsi GG, Schutte AE, Kruger R. Ethnic differences regarding arterial stiffness of 6–8-year-old black and white boys. J Hypertens 2017;35(5):960–7.
62. Rajakumar K, de las Heras J, Lee S, Holick MF, Arslanian SA. 25-Hydroxyvitamin D concentrations and in vivo insulin sensitivity and beta-cell function relative to insulin sensitivity in black and white youth. Diabetes Care 2012;35(3):627–33.
63. Manson JE, Cook NR, Lee IM, Christen W, Bassuk SS, Mora S, Gibson H, Gordon D, Copeland T, D'Agostino D, et al. Vitamin D supplements and prevention of cancer and cardiovascular disease. N Engl J Med 2019;380(1):33–44.