HHS Public Access

Author manuscript

Pediatr Obes. Author manuscript; available in PMC 2017 August 01.

Published in final edited form as:

Pediatr Obes. 2016 August ; 11(4): 279–284. doi:10.1111/ijpo.12059.

Effect of Vitamin D_3 Treatment on Endothelial Function in Obese Adolescents

A Javed, MBBS[1], **I. J. Kullo, M.D**[2], **P. Babu Balagopal, PhD**[3], and **S Kumar, MD**[1]

[1]Division of Pediatric Endocrinology, Department of Pediatric and Adolescent Medicine, Mayo Clinic, Rochester, MN, USA

[2]Division of Cardiovascular Diseases, Department of Internal Medicine, Mayo Clinic, Rochester, MN, USA

[3]Nemours Children's Clinic, Division of Biomedical Research, Jacksonville, FL, USA

Abstract

Background—Obesity in children is associated with vitamin D deficiency and endothelial dysfunction. It is not known if treatment with vitamin D improves endothelial function in obese adolescents.

Objective—To determine whether treatment with vitamin D3 improves endothelial function in obese adolescents.

Methods—Nineteen obese adolescents, 13–18 years of age, with 25-hydroxy vitamin D (25(OH)D) levels < 75 nmol/L were treated with 100,000 IU vitamin D_3 orally once a month for 3 months in an open-label, single-center prospective trial. Endothelial function was assessed by flow-mediated dilatation (FMD) of the brachial artery at study entry and 1 month after the 3rd dose of vitamin D_3. Biochemical parameters, including calcium, fasting lipids, glucose, insulin, and high-sensitivity C-reactive protein were also obtained.

Results—Mean 25(OH)D levels increased from 55.9±12.2 nmol/L to 86.9±16.7 nmol/L (P<.01). There was no correlation between 25(OH)D levels and brachial artery FMD. The brachial artery FMD (%) did not change significantly following vitamin D_3 treatment (9.5±3.53 vs 10.3±3.83, P=.83). Serum PTH declined from 3.8±1.5 pmol/L to 3.1±1 pmol/L (P=.01). The remainder of biochemical measurements did not show a significant change.

Conclusions—Treatment with vitamin D_3, 100,000 IU once a month for three months was effective in increasing 25(OH)D levels in obese adolescents but did not impact endothelial function.

Corresponding Author. Seema Kumar, MD, Division of Pediatric Endocrinology, Department of Pediatric and Adolescent Medicine, Mayo Clinic, Rochester, MN, USA, Phone: (507) 284-3300; Fax: (507) 284-0727, kumar.seema@mayo.edu.

The trial was registered on clinical trials.gov (NCT01746264).

Conflict of Interest Statement

The authors have no conflict of interest to declare.

Authors' Contributions to the Manuscript

S.K. designed the research, and S.K. and A.J. conducted the research. A.J. analyzed data; A.J, S.K., P.B., and I.J.K. interpreted the data and wrote the paper. A.J. wrote the first draft of the manuscript. S. K. had primary responsibility for final content. All authors have read and approved the final manuscript.

Keywords

vitamin D, adolescents; childhood obesity; endothelial function; flow-mediated dilatation

Introduction

The prevalence of obesity has tripled in the last three decades, and currently 21% of American adolescents are obese and 35% are either obese or overweight[1]. As a consequence of the high rates of childhood obesity, insulin resistance, dyslipidemia, and type 2 diabetes, which lead to premature atherosclerosis, are being increasingly reported in children[2]. In fact, the process of atherosclerosis, the pathologic basis for clinical cardiovascular disease (CVD), originates early in the clinical course of obesity and progresses silently to clinical manifestations later in life[3,4].

Obesity is a risk factor for vitamin D insufficiency, and severity of obesity is inversely correlated with 25-hydroxy vitamin D [25(OH)D] levels[5]. Currently, there is lack of consensus on what optimal 25(OH)D levels should be for health benefits[6, 7]. Because vitamin D receptors are expressed by virtually all tissues, including vascular smooth muscle cells and cardiomyocytes, a link between vitamin D deficiency and cardiovascular disease has been suggested[8]. The metabolic and cardiovascular implications of low vitamin D status in obese children and adolescents are not very well characterized. A positive correlation between 25(OH)D levels and high-density lipoprotein (HDL) cholesterol and an inverse correlation between 25(OH)D levels and fasting glucose amongst children have been reported[9]. Endothelial dysfunction is an early marker of cardiovascular disease in obese children[10]. Although vitamin D supplementation in vitamin D deficient adults has been shown to improve endothelial function[11], there is scarcity of data on the effect of vitamin D treatment on endothelial function in vitamin D deficient or insufficient obese children[12]. The primary purpose of this trial was to investigate the effects of treatment with vitamin D on endothelial function, measured by flow-mediated dilatation (FMD) of the brachial artery, in obese adolescents with 25(OH)D levels <75 nmol/L. Secondarily, we also assessed the effect of vitamin D treatment on markers of cardiovascular risk such as lipid profile and fasting glucose.

Methods

The study was a pre-post, open-label, single-center clinical intervention trial registered on clinical trials.gov (NCT01746264) and approved by the Institutional Review Board of Mayo Clinic, Rochester, MN. Written consent was obtained from participants and parents.

Adolescents were recruited between February 2013 and December 2013 through local advertisements and through recruitment letters to obese adolescents seen in Pediatric Endocrinology outpatient clinics at Mayo Clinic, Rochester. The inclusion criteria were age, 13–18 years old; body mass index (BMI) ≥95th percentile for age and gender; serum 25(OH)D concentrations <75 nmol/L; and systolic (SBP) or diastolic (DBP) blood pressure <95th percentile for age, sex, and height. Age- and gender-specific BMI percentiles and z scores were calculated using the standards recommended by the Centers for Disease Control

and Prevention[13]. Participants were excluded if they had serum calcium >2.59 mmol/L; serum phosphorus >1.52 mmol/L; were pregnant or nursing; suffered from cancer, diabetes, or malabsorption disorders such as celiac disease; or were on exogenous vitamin D supplementation or calcium intake >1500 mg/day. The serum 25(OH)D cut off level of <75 nmol/L was chosen on the basis of recent expert guidelines that defined vitamin D sufficiency as 25(OH)D levels above 75 nmol/L sufficiency[8]. Nineteen participants received 100,000 IU vitamin D_3 (Cholecalciferol, 2 pills of 50,000 IU each; Bio-Tech Pharmacal, Inc., Fayetteville, AR) once a month for 3 months. The once monthly dosing regimen was chosen mainly due to the potential lack of adherence and/or compliance in the adolescents with daily medications and evidence for similar increase in 25(OH)D levels with daily, weekly or monthly dosing frequency with vitamin D3[14]. All participants enrolled completed the study. Participants were asked to forgo additional vitamin D or calcium supplements during the study period. Endothelial function, measured at baseline and at conclusion of the study, was assessed by brachial artery flow-mediated dilatation (FMD). Fasting laboratory studies obtained at baseline and at study completion included 25(OH)D, calcium, phosphorus, parathyroid hormone (PTH), glucose, insulin, lipid profile, high-sensitivity C-reactive protein (hs-CRP), and random urine calcium and creatinine. Additionally, serum 25(OH)D, serum calcium, and random urine calcium and creatinine were obtained at 1 and 2 months after starting vitamin D_3. Pill bottles were checked at the end of the trial by study team members for pill count. Participants underwent physical examination for Tanner staging of puberty[15, 16] and completed an international physical activity questionnaire (IPAQ)[17] and a short calcium questionnaire (SCQ)[18] at study entry and completion. Height, weight, and blood pressure (BP) were obtained after an overnight fast at study entry. Measurements of BP were obtained using an aneroid sphygmomanometer (Welch Allyn sphygmomanometer, model # CE0050) with the participant's arm supported and positioned at the level of the heart. All BP measurements were taken twice and >10 minutes after being seated for an interview.

Endothelial Function Assessment

Endothelial function was assessed by a high-resolution Doppler ultrasonography examination of the right brachial artery. Before the visit, participants were asked to forgo strenuous exercise for 24 hours and fast for 12 hours. The participants were requested to avoid caffeinated beverages for 24 hours prior to their appointment. Documentation of medications, vitamins or supplements, or use of tobacco was made at time of enrollment. FMD was measured per recommendations of the American Society of Cardiology by two technicians who had received standardized training to perform brachial artery FMD measurements. The basal diameter of the right brachial artery was measured at rest. Next, the cuff of a sphygmomanometer was placed on the forearm and inflated to 50 mm Hg above the participant's SBP for a period of 5 minutes. The cuff was then deflated. Brachial artery diameter (BAD) was measured 45–90 seconds after deflation. FMD was calculated as the maximal percentage increase in BAD from baseline after the release of cuff occlusion. Multiple measurements were taken along the vessel and then averaged. The increase in resting brachial blood flow was calculated as the maximum flow recorded in the first 15 seconds after cuff deflation and was expressed as a percentage increase from baseline reactive hyperemia index (RHI).

Laboratory testing

25(OH)D was measured performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Total 25(OH)D concentration of each sample was calculated using internal standards, $25(OH)D_2$ and $25(OH)D_3$. Calcium was measured by Photometric, O-Cresolphthalein assay (Roche Diagnostics, Indianapolis, IN). Phosphorus was measured by Photometric, Ammonium Molybdate assay (Roche Diagnostics, Indianapolis, IN). PTH was measured by a two-site chemiluminescent immunometric assay on the Immulite automated immunoassay system (Diagnostic Products Corp. Los Angeles, CA; NKA Siemens Medical Solutions Diagnostics, Flanders, NJ). Serum insulin was measured using commercial electrochemiluminescence immunoassay kits (Roche E Modular, Roche Diagnostics, Indianapolis, IN). Plasma glucose was measured by hexokinase enzymatic assay (Roche Glucose Reagent; Roche Diagnostics, Indianapolis, IN). The Homeostatic Model Assessment-Insulin Resistance (HOMA-IR) index was calculated as: HOMA-IR = fasting serum glucose (mmol/L)×fasting insulin (μU/mL)/22.5. Total cholesterol, HDL cholesterol, and triglyceride levels were measured by an enzymatic colorimetric assay (Roche Diagnostics, Indianapolis, IN). Low-density lipoprotein (LDL) cholesterol was calculated as: LDL = Total cholesterol − HDL cholesterol − Triglycerides/5. hs-CRP was measured using particle-enhanced immunonephelometry (Siemens Healthcare Diagnostics, Deerfield, IL).

Urine calcium and creatinine were measured using Inductively Coupled Plasma-Optical Emission Spectrometry and enzymatic colorimetric assay, respectively.

Sample size calculation—A sample size of 19 participants was deemed to give 80% power to detect at least a correlation coefficient of 0.61 between variables using 0.05 as significance level.

Statistical analyses

Descriptive statistics were presented as mean ± SD if not stated otherwise. All variables were tested for normality. The assumptions of linear regression were verified by reviewing error terms. Changes in parameters were assessed using paired *t* tests or Wilcoxon signed rank test for normal and non-normal distribution of data, respectively. Simple correlation analysis was used to study association between change in 25(OH)D levels and other variables. Responses of FMD, RHI, 25(OH)D, and other biochemical parameters to treatment with vitamin D_3 were assessed using paired *t* tests. Univariate and multivariate regression models to assess predictors of change in 25(OH)D and other biochemical variables with consideration of BMI and baseline 25(OH) levels (serum 25(OH)D concentrations less than 50 nmol/L or between 50 and 75 nmol/L) as potential confounders were also performed. Statistical analysis was performed using JMP 10.0 SAS Institute Inc.

Results

The baseline characteristics of the participants are provided in Table 1. Nineteen out of twenty eight participants, screened, fulfilled eligibility criteria. Out of the nine participants not meeting inclusion criteria, five had serum 25 (OH)D just above 75 nmol/L (mean 77

nmol/L), two participants met eligibility criteria based on screening visit but did not return for the baseline visit and two participants were just under the 95th percentile for BMI.

The mean age (years) of the 19 participants enrolled was 15.8±1.7 and mean BMI (kg/m^2) was 36.1±6.03; the majority of the participants were non-Hispanic white (89.5%). All but two participants (one male and one female) were in Tanner stage V. None of the participants had impaired fasting glucose at baseline (Table 1). Mean serum 25(OH)D was 55.9±12.2 nmol/L and six participants (31.6%) had serum 25(OH)D levels <50 nmol/L (40.4±5.3). The overall compliance determined by pill count was 95%. One participant took 50,000 IU instead of 100,000 IU for the second dose of vitamin D_3. There were no dropouts in the study.

Mean 25(OH)D concentrations increased from 55.9±12.2 nmol/L to 86.9±16.7 nmol/L (P<.01) after 3 months of once monthly treatment. Mean 25(OH)D concentrations increased to 73.4±16 nmol/L and 84.4±16.2 nmol/L after 1 and 2 months of once monthly treatment respectively. 25(OH)D levels increased to above 75 nmol/L in 15/19 (79%) of participants after 3 months. Predictors of increase in serum 25(OH)D concentrations were lower baseline BMI (P=.01) and lower baseline serum 25(OH)D concentration (P=.047). Serum PTH declined from a baseline mean of 3.8±1.5 pmol/L to follow-up mean of 3.1±1 pmol/L (P=.01). However, none of the participants had a suppressed PTH. There were no changes in serum calcium concentration or in random urinary calcium to creatinine ratio following vitamin D_3 treatment (P=.20 and P=.32, respectively).

Mean baseline BAD (mm) was 3.63±0.43. Mean FMD (%) was 9.5±3.53 at baseline, and there was no correlation between baseline brachial FMD and 25(OH)D levels (P=.68). RHI (%) at baseline was 449.8±243.53, and there was no correlation between 25(OH)D levels and RHI (P=.72). The FMD and RHI did not differ between participants with serum 25(OH)D levels <50 nmol/L and those with 25(OH)D concentrations between 50 and 75 nmol/L (P=.63). There was no change in FMD or RHI following vitamin D_3 treatment (P=.60 and P=.66, respectively; Table 1). There was no change in FMD or RHI even after exclusion of the four participants in whom the 25(OH)D levels had not increased to above 75 nmol/L.

There was no change in body weight, BMI, waist or hip circumference, SBP, or DBP over the follow up period (all P values >.20) (Table 1). There was an increase in total cholesterol and serum triglyceride concentrations from baseline to follow up (P<.01 for both)., The increase in 25(OH)D levels did not predict change in total cholesterol or triglyceride levels (all P values >.05). There was no change in LDL cholesterol, HDL cholesterol, hs-CRP, fasting glucose, insulin, and HOMA-IR (Table 1).

Mean daily calcium intake (mg/day) at baseline for the cohort was 1102.7±304.3 and decreased from baseline to follow up (P=.02). The mean physical activity score at baseline (metabolic equivalent of task [MET]-minutes/week) was 1786.6±1506.2, placing most participants (68%) in the sedentary to moderate activity categories[17]. Physical activity scores did not change (P=.22) during the 3-month intervention period.

Discussion

We performed an open-label, prospective trial to study the effects of treatment with vitamin D3 at a dose of 100,000 IU once monthly for three months on endothelial function in obese adolescents with 25(OH)D levels <75 nmol/L. In addition, we assessed the effect of vitamin D3 treatment on markers of cardiovascular risk including lipid profile, fasting glucose and hs-CRP.

Our study demonstrated that once-monthly treatment with 100,000 IU of vitamin D_3 over a period of three months did not have an effect on endothelial function in obese adolescents, despite a post-treatment increase in 25(OH)D levels. To our knowledge, this is the first study that has examined the impact of vitamin D treatment on endothelial function in obese adolescents with 25(OH)D levels less than 75 nmol/L.

It has been postulated that vitamin D might exert protective effects on the vasculature through direct and indirect effects on renal and vascular cells as well as on mediators of inflammation and oxidative stress and calcium metabolism[12, 19, 20] A link between vitamin D insufficiency and endothelial activation in obese white children was suggested by elevated levels of soluble vascular adhesion molecule-1 in obese white children with 25(OH)D <50 nmol/L[21]. We found no correlation between 25(OH)D levels and brachial FMD in obese children. Additionally there was no change in brachial artery FMD following vitamin D_3 treatment. Our data of a lack of correlation between 25(OH)D levels and endothelial function is consistent with those reported by Pacifico et al.[19], where they assessed endothelial function using brachial artery FMD and carotid intima media thickness in white children and adolescents. However, Dong et al. have demonstrated that 2000 IU/day of vitamin D_3, as compared to 400 IU/day, can stop the progression of arterial stiffness as assessed by pulse wave velocity (PWV) of the carotid femoral vasculature in African American adolescents[12]. Several differences in characteristics of the participants in our study and the one by Dong and colleagues exist. While the participants in our study were obese and non-Hispanic white, Dong et al. included both normal weight and obese African American adolescents with much lower 25(OH)D levels (mean, 33.9 nmol/L compared to the 54.9 nmol/L in our participants). It is important to note that the participants receiving vitamin D_3 2000 IU/day in the study by Dong et al. had a much greater incremental increase in 25(OH)D levels (from 33.5 nmol/L at baseline to 85.7 nmol/L at 16 weeks) in comparison to their own controls receiving 400 IU/day (from 34 nmol/L at baseline to 59.8 nmol/L at 16 weeks) and to our participants (mean of 55.9 nmol/L at baseline to 86.9 nmol/L at 12 weeks). Another important difference related to the method of assessment of vascular function (FMD in our study versus PWV in the study by Dong and colleagues). Data in adults also conflict with some studies demonstrating an improvement in endothelial function with vitamin D supplementation[22] and others showing no improvement[23].

Our findings of an increase in total cholesterol following treatment of adolescents with vitamin D are not particularly surprising and are consistent with those seen reported in adults[24].. Vitamin D receptors are found ubiquitously, including in adipose tissue, and 25(OH)D plays an important role in lipid metabolism via several mechanisms including induction of an increase in lipoprotein lipase activity[25], increased lipogenesis and lipolysis

and enhanced intestinal calcium absorption, which could reduce the formation of calcium-fatty soaps in the gut and increase the absorption of fat.

Our study also demonstrates the short-term safety and efficacy of once-monthly vitamin D_3 treatment in obese adolescents; while serum 25(OH)D increased to above 75 nmol/L in almost 80% of participants, there was no evidence for hypercalcemia or hypercalciuria. Obese adolescents in particular have been a difficult group to treat, often needing multiple and higher doses of vitamin D.

Our study has several strengths. The ethnic homogeneity of our population (the vast majority of our participants being non-Hispanic whites) was an advantage, as ethnicity can modify the association of vitamin D status with metabolic factors predisposing to endothelial dysfunction[26]. Further we included a broad range of metabolic markers in addition to the endothelial function measurement. Other strengths included our extensive experience performing brachial artery FMD assessments to determine endothelial function[27] and the measurement of 25(OH)D levels using LC-MS/MS. The LC-MS/MS assay compares favorably with the Diasorin radioimmunoassay (concordance correlation coefficient = 0.97 and mean bias = 1.1 micrograms/L or 2.7 nmo/L[28].

We did not specifically ask the participants to attempt weight reduction during the duration of the trial, and therefore weight reduction was not a confounding factor.

One of the main limitations of our study was the lack of well-defined cut offs for FMD in the pediatric population. However, FMD has been used in the research setting in healthy pediatric participants compared to participants with various conditions, including adiposity, type 1 diabetes, dyslipidemia, family history of CVD, inflammation, as well as after various interventions such as exercise and use of statins, antihypertensive, and dietary supplements[29]. Another limitation of our study was the lack of direct measurement of large artery stiffness (PWV).

Other limitations of our study included the short duration of intervention, relatively small sample size, and the open label and non-randomized study design. These study limitations may have led to reduced power to detect differences. It is not clear if other treatment protocols such as those with daily or weekly vitamin D doses may have a different effect on endothelial function. Larger multicenter studies are warranted to confirm our findings. We also acknowledge the small number of participants with 25(OH)D <50 nmol/L in our study, particularly considering the Institute of Medicine 2011 report that concluded that 25(OH)D levels of 50 nmol/L cover the requirements of at least 97.5% of the population[7]. The lack of a placebo arm and the short duration of the follow up were other inherent limitations.

In conclusion, in this pilot study in obese adolescents, treatment with vitamin D3 100,000 IU taken once a month for a period of three months did not result in improvements in endothelial function. Larger studies examining the impact of vitamin D treatment in adolescents with vitamin D deficiency and established endothelial dysfunction are warranted.

Acknowledgments

This publication was made possible by CTSA Grant Number UL1 TR000135 from the National Center for Advancing Translational Sciences (NCATS), a component of the National Institutes of Health (NIH). Its contents are solely the responsibility of the authors and do not necessarily represent the official view of NIH.

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What is already known about this subject

1. Childhood obesity predisposes to adverse cardiovascular outcomes in adulthood.
2. Obese children have been noted to have endothelial dysfunction, an early marker of cardiovascular disease.
3. Vitamin D deficiency is common in obese youth and may be associated with adverse cardiovascular outcomes.

What this study adds

1. There was no association between 25(OH)D levels and endothelial function as measured by brachial artery flow mediated dilatation in obese adolescents.
2. There was no effect of vitamin D3 treatment on endothelial function in obese adolescents.

Table 1

Comparison of Baseline and Follow Up Measures

Characteristic or Measurement	Baseline	Follow up	Change from Baseline to Follow up (P value)[b]
BMI, kg/m^2	36.1(6.03)	36.4 (6.11)	.32
IPAQ score	1786.6(1506.2)	2799.1 (3834.6)	.21
SCQ score, mg/day*	1102.7(304.3)	975.5 (282.2)	.02
25(OH)D, nmol/L	55.9(12.2)	86.9±16.7	<.001
Serum PTH, pmol/L	4.1 (1.6)	3.3(1.1)	.0123
Fasting glucose, mmol/L	4.9(0.28)	4.97(0.26)	.09
Fasting insulin pmol/L	225.71(127.93)	245.16(150.01)	.27
HOMA-IR	7.11(4.18)	7.9(5.10)	.18
hs-CRP, nmol/L	47.62(35.43)	40(25.7)	.33
LDL cholesterol, mmol/L	1.95(0.66)	2.15(0.81)	.05
HDL cholesterol, mmol/L	1.2(0.28)	1.16(0.23)	.41
Total cholesterol, mmol/L	3.69(0.71)	4.03(0.87)	<.01
Triglycerides, mmol/L	1.19(0.57)	1.58(0.81)	<.01
Urine calcium to creatinine	67.8(59.2)	87.7(73.2)	.32
Mean FMD (%)[c]	9.5(3.52)	10.4(3.82)	.59
Mean RHI (%)[c]	449.3(243.5)	513.9(325.6)	.47

Abbreviations: 25(OH)D, 25-hydroxy vitamin D; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); hs-CRP, high-sensitivity C-reactive protein; FMD, flow-mediated dilatation; HDL, high-density lipoprotein; HOMA-IR, Homeostatic Model Assessment-Insulin Resistance; IPAQ, international physical activity questionnaire; LDL, low-density lipoprotein; RHI, reactive hyperemia index; SCQ, short calcium questionnaire; PTH, parathyroid hormone

[a] Values are presented as mean (SD) unless otherwise indicated.

[b] Wilcoxon Signed rank test

[c] n=18

P value represents significant change from baseline to follow up