



Low Serum Vitamin D Levels Are Associated With Increased Arterial Stiffness in Youth With Type 2 Diabetes

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OBJECTIVE

Adult studies demonstrate that low vitamin D (25[OH]D) is an independent risk factor for arterial stiffness. Similar studies have not been conducted in youth with type 2 diabetes mellitus (T2DM). The objective was to elicit the association between 25[OH]D and arterial stiffness in obese youth with and without T2DM. We hypothesized that 25[OH]D would be inversely correlated with arterial stiffness indices, including pulse wave velocity (PWV), augmentation index (Aix), and brachial distensibility (BrachD).

RESEARCH DESIGN AND METHODS

Cross-sectional analysis was conducted in Cincinnati, OH, from 2004 to 2010. 25[OH]D, PWV, Aix, and BrachD were measured in 190 youth with T2DM, 190 obese control subjects without T2DM, and 190 lean control subjects without T2DM. Multivariate analyses were conducted to elicit the independent association between 25[OH]D and arterial stiffness indices by group.

RESULTS

The mean age was 17.9 ± 3.4 years, 55% were African American, and 34% were male. The mean 25[OH]D levels were 21.27, 14.29, and 14.13 ng/mL in lean individuals, obese individuals, and obese individuals with T2DM, respectively ($P < 0.01$). PWV, Aix, and BrachD worsened from lean to obese to T2DM ($P < 0.01$). General linear models found that 25[OH]D level was independently associated with PWV in lean individuals and with Aix in the group with T2DM such that a 3 ng/mL increase in 25[OH]D was associated with an Aix decrease of 1% (baseline Aix = $5.7 \pm 12.0\%$).

CONCLUSIONS

25[OH]D is inversely associated with some measures of arterial stiffness in lean adolescents and obese adolescents with T2DM but not in obese normoglycemic adolescents. Future studies are needed to determine if supplemental 25[OH]D is important for cardiovascular health.

Cardiovascular disease (CVD) is the leading cause of mortality in people with type 2 diabetes mellitus (T2DM) (1). In fact, >50% of patients with T2DM die of coronary artery disease (2,3). Early vascular disease can be detected using pulse wave velocity (PWV), augmentation index (Aix), and brachial distensibility (BrachD), measures of arterial stiffness (4,5). Adolescents with T2DM and obese youth have been shown to

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have greater arterial stiffness than their lean (age-, sex-, and race-matched) counterparts, suggesting the risk for a premature onset of CVD (6).

25-Hydroxyvitamin D (25[OH]D) has an established role in calcium and phosphorus metabolism and bone health. Recent discovery of 25[OH]D receptors and the enzyme that converts 25[OH]D to its active form in vascular smooth muscle and endothelial cells points to a role in vascular health as well (7). 25[OH]D has been shown to have antiproliferative effects in both vascular endothelial and smooth muscle cells. Furthermore, 25[OH]D limits monocyte and macrophage differentiation and the release of inflammatory cytokines, blunting the inflammatory response, a known mediator of vascular disease (8). Finally, to support the causality of this association, vitamin D receptor^{-/-} mice exhibit site-specific accelerated atherogenesis (9). As a result, recent studies have focused on the association between low 25[OH]D and vascular health.

Studies in healthy, obese, and T2DM adults have found that low serum 25[OH]D levels are associated with increased arterial stiffness (10–13). The role of 25[OH]D in arterial stiffness has not been examined in high-risk youth, namely those with obesity and those with T2DM who are at risk for low levels of 25[OH]D secondary to decreased sun exposure and excessive storage of 25[OH]D in the adipose tissue (14).

The purpose of this study was to examine the association between serum 25[OH]D levels and arterial stiffness in youth and to determine if the relationship between 25[OH]D levels and arterial stiffness differs by group. We hypothesized that low serum 25[OH]D levels would be associated with increased arterial stiffness independent of traditional cardiovascular risk factors.

RESEARCH DESIGN AND METHODS

Study Population

The population for this analysis was drawn from individuals who participated in the National Institutes of Health–funded study Cardiovascular Disease in Adolescents with Type 2 Diabetes (T2CVD) at Cincinnati Children's Hospital Medical Center from 2004 to 2010 (15,16). In brief, the T2CVD study consisted of three groups: 221 individuals

with T2DM, 250 obese individuals without T2DM, and 261 lean individuals (non-obese individuals without T2DM). All participants were between 10 and 24 years old at recruitment. Individuals with diabetes were islet cell antibody negative (glutamic acid decarboxylase, ICA512, and insulin autoantibodies), had no evidence of another specific type of diabetes, and did not require insulin in the basal state to prevent diabetic ketoacidosis. Each participant with T2DM was matched to at least one lean (BMI <85th percentile) and one obese control (BMI ≥95th percentile) by age, race, and sex. All obese subjects underwent a 2-h oral glucose tolerance test to rule out subclinical T2DM according to American Diabetes Association guidelines (17). Pregnant females were excluded from the study. Prior to enrollment in T2CVD, written informed consent was obtained from subjects ≥18 years old or the parent or guardian for subjects <18 years old. Written assent was also obtained for subjects <18 years old according to the guidelines established by the institutional review board at Cincinnati Children's Hospital. The institutional review board granted a waiver of consent for the current study.

The study population for the current study was established using power calculations. We determined that a total sample size of 570 (190 subjects from each of the three groups) would be able to detect group differences of at least 6 ± 20 ng/mL in 25[OH]D with 90% power at a significance level of 0.05. Therefore, we randomly selected 190 subjects from each of the three subgroups.

Data Collection in T2CVD

After a 10-h overnight fast, participants in the T2CVD study were seen at an in-person study visit (6,15). Anthropometric measurements, venipuncture, blood pressure (BP), and arterial stiffness data were collected. Trained personnel obtained two measures of height (stadiometer; Veeder-Rood, Elizabethtown, NC) and weight (electronic scale; Health O Meter), with the average of each used in analyses. BMI was calculated as kilograms per meter squared. Waist circumference was measured to the nearest 0.5 cm using a flexible metal research tape twice and averaged according to a

standardized research protocol from the National Heart, Lung, and Blood Institute (NHLBI) Growth and Health Study. Systolic and diastolic BP were measured according to the standards of the Fourth Report on Blood Pressure in Children (18). DXA was performed with a Hologic 4500A (Hologic, Bedford, MA). Standards correlating X-ray beam attenuation to amount of lean and fat mass have been developed and validated against the hydrodensitometry method, which has previously been established as the most valid measurement of lean body mass and fat mass (19). Percent body fat was calculated as total body fat mass/total body mass $\times 100$.

Laboratory Data Collected in T2CVD

Plasma glucose was measured using a Hitachi model 704 glucose analyzer (Roche Hitachi, Indianapolis, IN) with intra-assay and interassay coefficients of variation of 1.2 and 1.6%, respectively. Plasma insulin was measured by radioimmunoassay using an anti-insulin serum raised in guinea pigs, ¹²⁵I-labeled insulin (Linco, St. Louis, MO), and a double antibody method to separate bound from free tracer. Assays of fasting plasma lipid profiles were performed in a laboratory that is NHLBI/Centers for Disease Control and Prevention standardized, with the LDL cholesterol concentration calculated using the Friedewald equation. C-reactive protein (CRP) was measured using a high-sensitivity ELISA. HbA_{1c} was measured in red blood cells using high-performance liquid chromatography methods.

Present Study

For the current study, the following laboratory measures were performed on serum samples that had been stored at -80°C . 1) 25[OH]D was measured on a Diasorin Liaison automated instrument by direct competitive chemiluminescence immunoassay for quantitative determination of total 25[OH]D in serum. 25[OH]D is a stable compound, and the assay is known to be reliable in frozen samples (20). 2) Basic metabolic profile was performed to ensure normal calcium-phosphorus metabolism, renal function, albumin, and creatinine. Each was measured on the Roche 311 chemistry auto-analyzer. Glomerular filtration rate (GFR) was then calculated using the Schwarz formula $[\text{GFR (mL/min/1.73 m}^2) = (0.41 \times \text{height in cm})/\text{serum creatinine}$

in mg/dL]. None of the subjects had GFR <60 mL/min/1.73 m², and hence none were excluded from the study based on their renal function.

Vascular Measures Collected in T2CVD

Vascular function testing was conducted after 5 min of rest in the supine position. Alx and PWV were obtained using the SphygmoCor device (SphygmoCor SCOR-PVx System; Atcor Medical, Sydney, Australia). BrachD was measured using the DynaPulse Pathway instrument (Pulse Metric, Inc.).

A SphygmoCor tonometer was used to measure Alx, a measure of arterial stiffness and pulse wave reflections (6). The tonometer was placed over the right radial artery and three measures of Alx were collected. A single value, calculated as an average of three Alx measurements, was used in the analysis. The pressure waves were calibrated using mean arterial BP and diastolic BP obtained in the same arm. The device then analyzed the pulse wave using a validated generalized transfer function. Since Alx is affected by heart rate (HR), all Alx values were adjusted to a standard HR of 75 bpm. Reproducibility studies in our laboratory demonstrated intraclass correlation coefficients between 0.7 and 0.9.

The SphygmoCor tonometer was also used to measure PWV, an additional measure of arterial stiffness (21). The distance from a proximal artery (carotid) to the distal artery (femoral) was measured to the nearest 0.1 cm twice, averaged, and entered into the software. A tonometer was used to collect proximal and distal arterial waveforms gated by the R-wave on a simultaneously recorded electrocardiogram. PWV then was calculated as the distance from the carotid to distal path length divided by the time delay measured between the carotid and femoral waveforms reported in meters per second. Three recordings of PWV were obtained on each subject and were averaged. Repeat measures show a coefficient of variation of <5.2%.

The DynaPulse Pathway instrument (Pulse Metric, Inc.) was used to measure BrachD (22). This device derives brachial artery pressure curves from distensibility arterial pressure signals obtained from a standard cuff sphygmomanometer. Three measures of brachial artery distensibility were obtained and

averaged. A lower BrachD indicates a higher arterial stiffness. Repeat measures in our laboratory show a coefficient of variation of <9.6%.

Statistical Analysis

All analyses were performed with Statistical Analyses Software (SAS, version 9.3). Mean values for demographic, anthropometric, and laboratory data were obtained by group. Variance-stabilizing measures to transform nonnormal values were performed on HbA_{1c}, glucose, HDL cholesterol, triglycerides (TGs), CRP, creatinine, and 25[OH]D. χ^2 analyses were performed to determine group differences for categorical variables. One-way ANOVA and ANCOVA were performed to test for mean differences between the groups. Bivariate correlations were calculated between arterial stiffness measures and potential covariates. General linear models (GLMs) were constructed using significant covariates from correlation analyses to elucidate independent determinants of arterial stiffness. Separate models were created for each group (lean, obese, and T2DM) because 25[OH]D has been shown to have different effects based on degree of adipose tissue. Each model potentially included age,

sex, race, height, waist-to-height ratio, percent body fat (from DXA), HR (not for Alx), systolic BP z score, diastolic BP z score, TG-to-HDL ratio (as a measure of small dense LDL particles), CRP, glucose, and insulin. Waist to height was chosen over BMI because it has been shown to be a superior measure of adiposity compared with BMI, especially in people with diabetes (23,24). All models contained 25[OH]D. We also adjusted for potential seasonal variation in blood draw (April through September and October through March), since 25[OH]D levels have been shown to be lower in the winter months. In the final model, nonsignificant ($P > 0.05$) variables were removed.

RESULTS

Table 1 lists the demographic, anthropometric, and laboratory data for all participants stratified by study group. There were no significant differences among the three groups in age, sex, and race. Serum 25[OH]D levels were higher in lean youth compared with their obese and T2DM counterparts ($P < 0.01$) with no difference in serum 25[OH]D levels between obese youth and youth with T2DM. The 25[OH]D concentration was <20 ng/mL in 50% of lean youth and

Table 1—Characteristics of the study population

	Lean (n = 191)	Obese (n = 190)	T2DM (n = 189)	P values
Age (years)	17.6 ± 3.7	18.0 ± 3.4	18.1 ± 3.2	n/s
Sex (n, % female)	122 (63)	128 (67)	122 (64)	n/s
Race (n, % African American)	98 (51)	115 (60)	103 (54)	n/s
Height (cm) ^a	165.7 ± 10.7	166.7 ± 10.4	169.4 ± 9.9	<0.01
Weight (kg) ^b	58.4 ± 11.5	100.8 ± 19.7	103.5 ± 26	<0.01
BMI (kg/m ²) ^b	21.1 ± 2.5	36.2 ± 6.0	35.9 ± 7.8	<0.01
Systolic BP (mmHg) ^e	107.4 ± 10.2	117.1 ± 11.2	121.7 ± 12.3	<0.01
Diastolic BP (mmHg) ^b	59.6 ± 13.0	66.7 ± 12.1	67.1 ± 13.0	<0.01
HbA _{1c} (%) ^a	5.4 (5.1, 5.5)	5.5 (5.2, 5.7)	8.2 (5.9, 10.3)	<0.01
HbA _{1c} (mmol/mol)	36 (32, 37)	37 (33, 39)	66 (41, 89)	<0.01
Glucose (mg/dL) ^a	89.5 (85.5, 94.2)	92.6 (88.3, 96.5)	155.6 (94.3, 204)	<0.01
Insulin (mU/mL) ^e	11.3 (8.3, 13.2)	22.8 (13.4, 27.3)	26.3 (13.6, 32.7)	<0.01
LDL cholesterol (mg/dL) ^b	90.0 (72, 105)	103.5 (84, 121)	107.7 (83, 132)	<0.01
HDL cholesterol (mg/dL) ^c	57.5 (49, 63)	46.3 (41, 52)	44.1 (36, 50)	<0.01
TGs (mg/dL) ^e	71.3 (48, 84)	106.1 (63, 123)	141.3 (77, 169)	<0.01
CRP (mg/L) ^b	0.9 (0.2, 0.9)	4.1 (1.2, 6.3)	4.6 (1.3, 6.9)	<0.01
Calcium (mg/dL)	9.8 ± 0.8	9.7 ± 1.6	9.8 ± 0.9	n/s
Creatinine (mg/dL) ^d	0.7 ± 0.2	0.73 ± 0.2	0.64 ± 0.2	<0.01
25[OH]D (ng/mL) ^c	21.3 (14, 26.7)	14.3 (8.8, 18.2)	14.1 (8.3, 18.7)	<0.01

Data are mean ± SD, n (%), or median (25th, 75th percentile). P values in the table represent the overall comparison between the three groups using ANOVA; n/s, not significant. Between-group differences are shown by: ^aT2DM >lean and obese. ^bT2DM and obese >lean. ^cLean >T2DM and obese. ^dLean and obese >T2DM. ^eT2DM >obese >lean.

80% of both obese youth and youth with T2DM. There was no difference in 25[OH]D levels by sex. African American youth had a lower 25[OH]D level compared with their Caucasian counterparts ($P < 0.01$).

The arterial stiffness measurements by group are presented in Fig. 1. All three measures of arterial stiffness worsened from lean to obese to T2DM ($P < 0.01$). Negative Alx in the lean group indicates that wave reflection happened late in the cardiac cycle, consistent with more pliable (less stiff) arteries.

The univariate associations between 25[OH]D levels and arterial stiffness were examined by the study group. There was a negative correlation between serum 25[OH]D and Alx in lean and T2DM only (both $r = -0.24$, $P < 0.01$) and between 25[OH]D and PWV in lean ($r = -0.26$), obese ($r = -0.27$), and T2DM ($r = -0.36$), all $P < 0.01$. Univariate correlations between 25[OH]D and Alx and PWV are shown in Fig. 2A and B, respectively. BrachD was not correlated with 25[OH]D by group (data not shown), and therefore GLMs were not pursued.

GLM analysis (Table 2) showed that serum 25[OH]D level was an independent risk factor for Alx in the T2DM group only such that an increase in serum 25[OH]D by 3 ng/mL was associated with a 1% decrease in Alx. Other independent determinants of Alx in the group with T2DM were age, height, and diastolic BP z score. In combination, the above risk factors explained ~20% of the variance for Alx in the group with T2DM.

The GLM analysis for PWV showed that serum 25[OH]D level was an independent

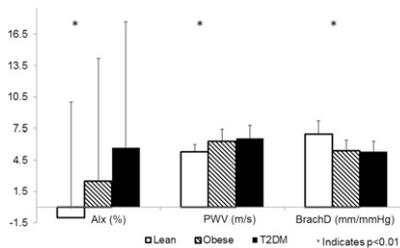


Figure 1—Comparison of the vascular measurements by group. Data are mean and SD, where the white bar is the lean group, the hatched bar is the obese group, and the black bar is the group with T2DM. The asterisks indicate a P value of < 0.01 between the three groups by ANOVA. For Alx and PWV a higher value indicates worse arterial stiffness. For BrachD a lower value indicates worse arterial stiffness.

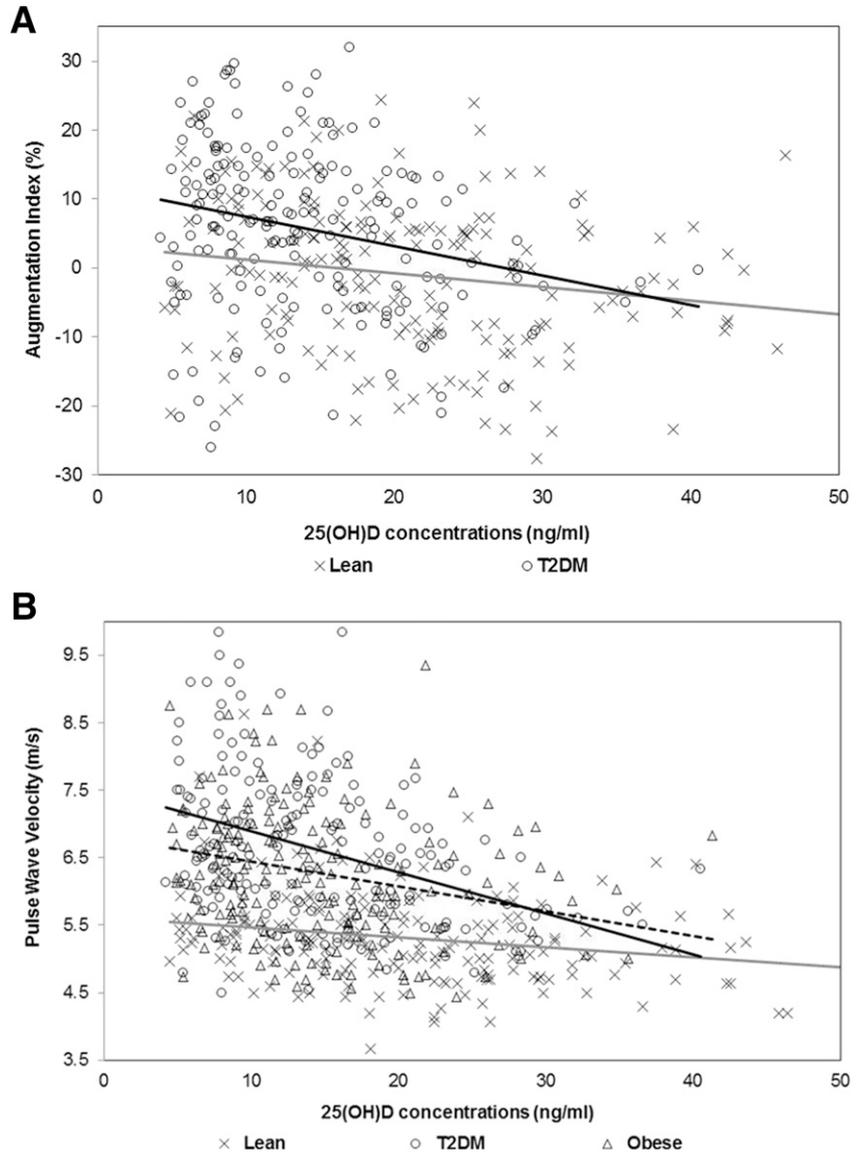


Figure 2—A: The relationship between Alx (%) and 25[OH]D concentrations by group. The gray line and solid black line are the regression lines for the lean group ($r = -0.24$, $P < 0.01$) and the group with T2DM ($r = -0.24$, $P < 0.01$), respectively. B: The relationship between PWV (m/s) and 25[OH]D concentrations by group. The gray, dashed black, and solid black lines are the regression lines for the lean group ($r = -0.24$, $P < 0.01$), obese group ($r = -0.27$, $P < 0.01$), and group with T2DM ($r = -0.36$, $P < 0.01$), respectively.

risk factor for PWV in the lean group only (Table 2), although this was a small effect. Other independent determinants of PWV in lean individuals were age, sex, systolic BP z score, TG-to-HDL ratio, and HR. This model explained ~37% of variance for PWV in the lean group. In all models, interactions between 25[OH]D and race and 25[OH]D and HbA_{1c} were tested and were nonsignificant.

CONCLUSIONS

Our study found that low serum 25[OH]D levels in youth with T2DM were negatively associated with arterial stiffness,

measured by Alx, even after adjustment for demographic and traditional CVD risk factors. Furthermore, we show that in youth with T2DM, a higher 25[OH]D level of 3 ng/mL is associated with a decrease in Alx of 1%. Considering that Alx in the group with T2DM was $5.7 \pm 12.0\%$ (mean \pm SD), an absolute change of 1% may have a large impact on future cardiovascular outcomes given that an increase in arterial stiffness is known to predict future myocardial infarction and stroke (5,25).

Recent epidemiologic studies in adults have shown an inverse association

Table 2—Independent determinants of Alx and PWV

	Alx (%)			PWV (m/s)		
	Lean	Obese	T2DM	Lean	Obese	T2DM
Intercept	45.087 (<0.001)	−3.790 (0.333)	49.044 (0.003)	1.295 (<0.001)	0.807 (<0.001)	0.880 (<0.001)
Age (years)	n/s	n/s	0.696 (0.010)	0.020 (<0.001)	0.02 (<0.001)	0.024 (<0.001)
Sex (female)	n/s	6.158 (<0.001)	n/s	−0.051 (0.003)	n/s	n/s
Race (% African American)	4.475 (<0.001)	n/s	n/s	n/s	0.061 (<0.001)	0.120 (<0.001)
Height (cm)	−0.319 (0.004)	n/s	−0.303 (<0.001)	n/s	n/s	n/s
Diastolic BP z score (mmHg)	n/s	n/s	2.110 (0.003)	n/s	0.022 (0.018)	n/s
Systolic BP z score (mmHg)	n/s	3.031 (<0.001)	n/s	0.020 (0.029)	0.022 (0.024)	0.040 (<0.001)
TG-to-HDL cholesterol ratio	n/s	n/s	n/s	−0.024 (0.010)	n/s	0.009 (0.008)
HR (bpm)	n/s	n/s	n/s	0.003 (<0.001)	0.003 (0.004)	n/s
25[OH]D (ng/mL)	n/s	n/s	−0.331 (0.006)	−0.002 (0.003)	n/s	n/s
Seasonality	n/s	−3.340 (0.040)	n/s	n/s	n/s	n/s
R ²	0.17	0.13	0.20	0.37	0.54	0.50

Data are parameter estimates (*P* values). Only significant values are listed. n/s, nonsignificant variable (*P* > 0.05).

between serum levels of 25[OH]D and arterial stiffness in healthy adults (10), obese adults (13), and adults with T2DM (12). Basic science research supports the potential role for 25[OH]D in the development of atherosclerosis (26–28) by demonstrating that both vascular smooth muscle cells and vascular endothelial cells have 25[OH]D receptors and the enzyme to convert 25[OH]D to its active form 1,25-OH vitamin D (7). 25[OH]D has been shown to have anti-proliferative effects on vascular endothelial and smooth muscle cells and the ability to limit monocyte and macrophage differentiation and the release of inflammatory cytokines, a known mediator of vascular disease (8). Finally, it has been shown that vitamin D receptor^{−/−} mice exhibit site-specific accelerated atherogenesis (9). Thus, there is reasonable evidence to support that 25[OH]D has a role in the development of atherosclerosis.

We found serum levels of 25[OH]D to be lower in obese youth and youth with T2DM compared with their lean counterparts. In fact, nearly 80% of both youth with obesity and youth with diabetes had a serum 25[OH]D level of <20 ng/mL (Institute of Medicine defines that a serum concentration of >20 ng/mL 25[OH]D is needed for good bone health [29]), in contrast to only 50% of lean youth. We also noted a seasonal variation in the 25[OH]D level in all three groups, with 25[OH]D levels being lower in fall and winter (October through March) compared with spring and summer (April

through September). These results are consistent with prior studies (30,31) that show lower 25[OH]D in obese individuals due to decreased exposure to sunlight and excessive storage of 25[OH]D in the adipose tissue (14).

Our linear regression models showed that after adjustment for risk factors, serum 25[OH]D level had a significant negative association with PWV in lean individuals and with Alx in participants with T2DM. In the obese group, although we found no association between 25[OH]D levels and arterial stiffness, it should be noted that Alx was associated with season (higher Alx in spring/summer vs. fall/winter), which may reflect changes in 25[OH]D levels with season. The reasons for the differential associations between 25[OH]D and arterial stiffness are unclear, but we hypothesize some potential reasons.

First, each measure of arterial stiffness, although all reliable, assesses a different aspect of the vasculature. This is the advantage of using the three independent measurements. PWV, a measure of central arterial stiffness, is considered the gold standard measure of subclinical arterial stiffness in both adults and children and has been shown to predict future cardiovascular events and mortality (32,33). Alx is a mixed measure of arterial stiffness that is influenced by central stiffness (PWV) and peripheral wave reflections (21) and has also been shown to predict all-cause mortality in adults with end-stage renal disease (34) and hypertension (5). BrachD is a

nonultrasound measure of stiffness (arterial compliance) in a medium muscular artery (4) and is highly correlated with cardiovascular risk factors (4). Differential associations between risk factors and arterial stiffness have been documented previously (6,35), because although the arterial stiffness measures are correlated, each assesses a different property of the arterial tree. Second, since 25[OH]D is known to limit the release of inflammatory cytokines (8) and suppress the renin aldosterone axis (28), it is possible that the lower 25[OH]D concentrations observed in T2DM have a larger effect on the vasculature. Confirmatory studies are needed in a larger cohort of adolescents with T2DM.

A recent study in T1DM youth found an association between serum 25[OH]D levels and PWV after adjusting for age, sex, race, and season. However, the relationship was no longer significant after adjusting for BMI z score, lipids, and BP (36). In contrast, we found that 25[OH]D was independently associated with arterial stiffness in lean individuals and individuals with T2DM after adjusting for adiposity (waist to height), lipids, and BP. Our study and the T1DM study have some important differences to explain the discrepant results. First, 25[OH]D levels were much lower in this group with T2DM compared with the previously published group with T1DM. Second, in contrast to the T1DM study, where PWV alone was reported as a marker of arterial stiffness, we studied the relationship between 25[OH]D levels

and three measurements of arterial stiffness. Finally, there are likely inherent differences in the relationship between 25[OH]D levels and arterial stiffness in youth with T1DM and T2DM because of the degree of obesity in T2DM.

This study has limitations. First, it is cross-sectional and thus represents serum 25[OH]D and arterial stiffness at one point in time. Second, it should be noted that the 25[OH]D measurements were performed on frozen samples. However, previous work has shown no difference in 25[OH]D levels with frozen storage (20).

Arterial stiffness is an important predictor of increased cardiovascular risk. Prior studies have shown that youth with obesity and T2DM have increased arterial stiffness, suggesting that they are at higher risk for early CVD (6). However, conventional cardiovascular risk factors do not fully explain the observed differences in arterial stiffness among lean and obese individuals and individuals with T2DM (6). The data presented here suggest that 25[OH]D may be one of the nontraditional cardiovascular risk factors that contribute to arterial stiffness. Further randomized controlled trials in youth are needed to establish the causation and mechanism by which 25[OH]D affects arterial stiffness and to establish whether supplementation in this group may reduce, or slow, the process of arterial stiffness to some extent.

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