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

Diabetes Care 2015;38:1551–1557 | DOI: 10.2337/dc15-0111

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 ± 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...
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 receptorDef 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 × 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 highsensitivity ELISA. HbA1c 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 autoanalyzer. Glomerular filtration rate (GFR) was then calculated using the Schwarz formula GFR (mL/min/1.73 m²) = (0.41 × height in cm)/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. AxI 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 AxI, a measure of arterial stiffness and pulse wave reflections (6). The tonometer was placed over the right radial artery and three measures of AxI were collected. A single value, calculated as an average of three AxI measurements, was used in the analysis. The pressure waves were calibrated using mean arterial BP and diastolic BP obtained using the SphygmoCor device (SphygmoCor SCOR-PVx System; Atcor Medical, Sydney, Australia). BrachD was measured using the DynaPulse Pathway instrument (Pulse Metric, Inc.).

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 from 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 HBA1c, glucose, HDL cholesterol, triglycerides (TGs), CRP, creatinine, and 25(OH)D. χ² 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 AxI), 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

| Characteristic                  | 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)                    | 165.7 ± 10.7  | 166.7 ± 10.4   | 169.4 ± 9.9   | <.001    |
| Weight (kg)                    | 58.4 ± 11.5   | 100.8 ± 19.7   | 103.5 ± 26    | <.001    |
| BMI (kg/m²)                    | 21.1 ± 2.5    | 36.2 ± 6.0     | 35.9 ± 7.8    | <.001    |
| Systolic BP (mmHg)             | 107.4 ± 10.2  | 117.1 ± 11.2   | 121.7 ± 12.3  | <.001    |
| Diastolic BP (mmHg)            | 59.6 ± 13.0   | 66.7 ± 12.1    | 67.1 ± 13.0   | <.001    |
| HBA1c (%)                      | 5.4 (5.1, 5.5)| 5.5 (5.2, 5.7)| 8.2 (5.9, 10.3)| <.001 |
| HBA1c (mmol/mol)               | 36 (32, 37)   | 37 (33, 39)    | 46 (41, 89)   | <.001    |
| Glucose (mg/dL)                | 89.5 (85.5, 94.2)| 92.6 (88.3, 96.5) | 155.6 (94.3, 204) | <.001 |
| Insulin (μU/mL)                | 11.3 (8.3, 13.2)| 22.8 (13.4, 27.3) | 26.3 (13.6, 32.7) | <.001 |
| LDL cholesterol (mg/dL)        | 90.0 (72, 105) | 103.5 (84, 121)| 107.7 (83, 132)| <.001 |
| HDL cholesterol (mg/dL)        | 57.5 (49, 63) | 46.3 (41, 52)  | 44.1 (36, 50) | <.001 |
| TGs (mg/dL)                    | 71.3 (48, 84) | 106.1 (63, 123)| 141.3 (77, 169)| <.001 |
| CRP (μg/L)                     | 0.9 (0.2, 0.9) | 4.1 (1.2, 6.3) | 4.6 (1.3, 6.9) | <.001 |
| Calcium (mg/dL)                | 9.8 ± 0.8     | 9.7 ± 1.6      | 9.8 ± 0.9     | n/s      |
| Creatinine (mg/dL)             | 0.7 ± 0.2     | 0.73 ± 0.2     | 0.64 ± 0.2    | <.001    |
| 25(OH)D (ng/mL)                | 21.3 (14, 26.7) | 14.3 (8.8, 18.2) | 14.1 (8.3, 18.7) | <.001 |

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: *T2DM > lean and obese. †T2DM and obese > lean. ‡Lean > T2DM and obese. §Lean and obese > T2DM. ¶T2DM > 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 AIx 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 AIx 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 AIx 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 AIx 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 AIx. Other independent determinants of AIx 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 AIx in the group with T2DM.

The GLM analysis for PWV showed that serum 25(OH)D level was an independent 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 HbA1c 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 AIx, 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 AIx of 1%. Considering that AIx in the group with T2DM was 5.7 ± 12.0% (mean ± 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...
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)2 vitamin D (7). 25(OH)D has been shown to have antiproliferative 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 AIx 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 AIx was associated with season (higher AIx 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 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

| Table 2—Independent determinants of AIx and PWV |
|-----------------------------------------------|
| 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 |
| R² | 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).
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.

**Funding.** This study was supported by the Cincinnati Diabetes and Obesity Center Pilot Grant Award, 2012. This study was also supported by National Institutes of Health grant R01-HL-07629 (T2CVD).

**Duality of Interest.** No potential conflicts of interest relevant to this article were reported.

**Author Contributions.** P.J. researched data and wrote the manuscript. L.M.D., E.M.U., and T.R.K. designed the study and reviewed and edited the manuscript. P.R.K. conducted the statistical analyses. A.S.S. designed the study and wrote the manuscript. A.S.S. is the guarantor of the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Prior Presentation.** This study was presented as a late-breaking abstract at the 96th Annual Meeting of the Endocrine Society, Chicago, IL, 21–24 June 2014.

**References**

1. Lloyd-Jones D, Adams RJ, Brown TM, et al.; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Executive summary: heart disease and stroke statistics—2010 update: a report from the American Heart Association. Circulation 2010;121:948–954
2. Grundy SM, Benjamin IJ, Burke GL, et al. Diabetes and cardiovascular disease: a statement for healthcare professionals from the American Heart Association. Circulation 1999;100:1134–1146
3. Grundy SM, Pasternak R, Greenlaw P, Smith S Jr, Fuster V. Assessment of cardiovascular risk by use of multiple-risk-factor assessment equations: a statement for healthcare professionals from the American Heart Association and the American College of Cardiology. Circulation 1999;100:1481–1492
4. Urbina EM, Brinton TJ, Elkasabany A, Berenson GS. Brachial artery distensibility and relation to cardiovascular risk factors in healthy young adults (The Bogalusa Heart Study). Am J Cardiol 2002;89:946–951
5. Williams B, Lacy PS, Thom SM, et al.; CAFE Investigators; Anglo-Scandinavian Cardiac Outcomes Trial Investigators; CAFE Steering Committee and Writing Committee. Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study. Circulation 2006;113:1213–1225
6. Urbina EM, Kimball TR, Khoury PR, Daniels SR, Dolan LM. Increased arterial stiffness is found in adolescents with obesity or obesity-related type 2 diabetes mellitus. J Hypertens 2010;28:1692–1698
7. O’Connell TD, Simpson RU. Immunoochemical identification of the 1,25-dihydroxyvitamin D3 receptor protein in human heart. Cell Biol Int 1996;20:621–624
8. Oh J, Weng S, Felton SK, et al. 1,25(OH)2 vitamin D inhibits foam cell formation and suppresses macrophage cholesterol uptake in patients with type 2 diabetes mellitus. Circulation 2009;120:687–698
9. Szeto FL, Reardon CA, Yoon D, et al. Vitamin D receptor signaling inhibits atherosclerosis in mice. Mol Endocrinol 2012;26:1091–1101
10. Al-Mheid I, Patel R, Murrow J, et al. Vitamin D status is associated with arterial stiffness and vascular dysfunction in healthy humans. J Am Coll Cardiol 2011;58:186–192
11. Giallauria F, Milaneschi Y, Tanaka T, et al. Arterial stiffness and vitamin D levels: the Baltimore-longitudinal study of aging. J Clin Endocrinol Metab 2012;97:3717–3723
12. Lee JI, Oh SJ, Ha WC, et al. Serum 25-hydroxyvitamin D concentration and arterial stiffness among type 2 diabetes. Diabetes Res Clin Pract 2012;95:42–47
13. Mayer O Jr, Filipovsky J, Seidlervö J, et al. The association between low 25-hydroxyvitamin D and increased aortic stiffness. J Hum Hypertens 2012;26:650–655
14. Liel Y, Ulmer E, Shary J, Hollis BW, Bell NH. Low circulating vitamin D in obesity. Calcif Tissue Int 1988;43:199–201
15. Shah AS, Dolan LM, Kimball TR, et al. Influence of duration of diabetes, glycemic control, and traditional cardiovascular risk factors on early atherosclerotic vascular changes in adolescents and young adults with type 2 diabetes mellitus. J Clin Endocrinol Metab 2009;94:3740–3745
16. Urbina EM, Kimball TR, McCoy CE, Khoury PR, Daniels SR, Dolan LM. Youth with obesity and obesity-related type 2 diabetes mellitus demonstrate abnormalities in carotid structure and function. Circulation 2009;119:2913–2919
17. American Diabetes Association. Executive summary: standards of medical care in diabetes—2014. Diabetes Care 2014;37(Suppl. 1):S5–S13
18. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics 2004;114(Suppl. 4th Report):555–576
19. Daniels SR, Morrison JA, Sprecher DL, Khoury P, Kimball TR. Association of body fat distribution and cardiovascular risk factors in children and adolescents. Circulation 1999;99:541–545
20. Antoniucci DM, Black DM, Sellmeyer DE. Serum 25-hydroxyvitamin D is unaffected by multiple freeze-thaw cycles. Clin Chem 2005;51:258–261
21. Laurent S, Cockcroft J, Van Bortel L, et al.; European Network for Non-invasive Investigation of Large Arteries. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J 2006;27:2588–2605
22. Brinton TJ, Kotter B, Kalsam MT, et al. Development and validation of a noninvasive method to determine arterial pressure and vascular compliance. Am J Cardiol 1997;80:323–330
23. Browning LM, Hsieh SD, Ashwell M. A systematic review of waist-to-height ratio as a screening tool for the prediction of cardiovascular disease and diabetes: 0.5 could be a suitable global boundary value. Nutr Res Rev 2010;23:247–269
24. Lee CM, Huxley RR, Wildman RP, Woodward M. Indices of abdominal obesity are better discriminators of cardiovascular risk factors than BMI: a meta-analysis. J Clin Epide- miol 2008;61:646–653
25. Urbina, EM, Wada WA, Davis C, et al. Prevalence of increased arterial stiffness in children with type 1 diabetes mellitus differs by measurement site and sex: the SEARCH for Diabetes in Youth Study. J Pediatr 2010;156:731–737, 737.e1
26. Chen S, Law CS, Grigby CL, et al. Cardiomycocyte-specific deletion of the vitamin D receptor gene results in cardiac hypertrophy. Circulation 2011;124:1838–1847
27. Salum E, Kampus P, Zilmer M, et al. Effect of vitamin D on aortic remodeling in streptozotocin-induced diabetes. Cardiovasc Diabetol 2012;11:58
28. Xiang W, Kong J, Chen S, et al. Cardiac hyper- trophy in vitamin D receptor knockout mice: role of the systemic and cardiac renin-angiotensin systems. Am J Physiol Endocrinol Metab 2005;288:E125–E132
29. Ross AC, Manson JE, Abrams SA, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab 2011;96:53–58
30. Olson ML, Maalouf NM, Oden JD, White PC, Hutchison MR. Vitamin D deficiency in obese
children and its relationship to glucose homeostasis. J Clin Endocrinol Metab 2012;97:279–285
31. Scragg R, Sowers M, Bell C; Third National Health and Nutrition Examination Survey. Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. Diabetes Care 2004;27:2813–2818
32. Mancia G, De Backer G, Dominiczak A, et al.; Management of Arterial Hypertension of the European Society of Hypertension; European Society of Cardiology. 2007 Guidelines for the Management of Arterial Hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens 2007;25:1105–1187
33. Urbina EM, Williams RV, Alpert BS, et al.; American Heart Association Atherosclerosis, Hypertension, and Obesity in Youth Committee of the Council on Cardiovascular Disease in the Young. 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;54:919–950
34. London GM, Blacher J, Pannier B, Guérin AP, Marchais SJ, Safar ME. Arterial wave reflections and survival in end-stage renal failure. Hypertension 2001;38:434–438
35. Wadwa RP, Urbina EM, Anderson AM, et al.; SEARCH Study Group. Measures of arterial stiffness in youth with type 1 and type 2 diabetes: the SEARCH for diabetes in youth study. Diabetes Care 2010;33:881–886
36. Lieberman R, Wadwa RP, Nguyen N, et al. The association between vitamin D and vascular stiffness in adolescents with and without type 1 diabetes. PLoS ONE 2013;8:e77272