



# Coronary Artery Calcification in Obese Youth: What Are the Phenotypic and Metabolic Determinants?

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## OBJECTIVE

Obesity in adolescence has been associated with increased risk for coronary heart disease in adulthood. This study evaluated subclinical atherosclerosis in obese youth and the underlying risk factors.

## RESEARCH DESIGN AND METHODS

Ninety obese adolescents (37 normal glucose tolerant, 27 prediabetes, and 26 type 2 diabetes) underwent evaluation of coronary artery calcifications (CACs) by electron beam computed tomography, aortic pulse wave velocity (PWV), carotid intima-media thickness (IMT), lipids, leptin, inflammatory markers, and body composition (DEXA). A total of 68 underwent evaluation of insulin sensitivity (IS) (hyperinsulinemic-euglycemic clamp) and abdominal adiposity (computed tomography).

## RESULTS

A total of 50% had CACs (CAC+: Agatston CAC score  $\geq 1$ ). CAC+ youth had higher BMI, fat mass, and abdominal fat, with no difference in sex, race, IS per fat-free mass (IS<sub>FFM</sub>), glucose tolerance, PWV, or IMT compared with the CAC– group. PWV was inversely related to IS. In multiple regression analyses with age, race, sex, HbA<sub>1c</sub>, BMI (or waist circumference), IS<sub>FFM</sub>, diastolic blood pressure, non-HDL cholesterol, and leptin as independent variables, BMI (or waist) ( $R^2 = 0.41$ ;  $P = 0.001$ ) was the significant determinant of CAC; leptin ( $R^2 = 0.37$ ;  $P = 0.034$ ) for PWV; and HbA<sub>1c</sub>, race, and age ( $R^2 = 0.34$ ;  $P = 0.02$ ) for IMT.

## CONCLUSIONS

Early in the course of obesity, there is evidence of CAC independent of glycemia. The different biomarkers of subclinical atherosclerosis appear to be differentially modulated, adiposity being the major determinant of CAC, hyperglycemia, age, and race for IMT, and leptin and IS for arterial stiffness. These findings highlight the increased cardiovascular disease risk in obese youth and the need for early interventions to reverse obesity and atherosclerosis.

Longitudinal studies in adults demonstrate that obesity is a risk factor for coronary artery disease (CAD) independent of other risk factors (1). Autopsy studies show that atherosclerosis starts in childhood and is related to obesity (2). The Pathobiological Determinants of Atherosclerosis in Youth study (2) revealed a strong association between obesity and the extent and severity of early coronary

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atherosclerosis in adolescents and young adult men, age 15–34 years, dying from external causes.

In more recent prospective cohort studies, elevated BMI in adolescence was associated with increased risk for angiography-proven coronary heart disease, independent of BMI in adulthood (3). The detection of coronary artery calcifications (CACs) utilizing electron beam computed tomography (EBCT) is considered ~100% sensitive for the presence of atherosclerotic CAD (4) and a strong predictor of future cardiovascular (CV) events (5,6). Using this modality, studies indicated a link between early life risk factors and subclinical atherosclerosis in adults. In the Muscatine Study (7), coronary calcium measured in adulthood correlated with obesity in childhood and its associated coronary risk factors (increased blood pressure [BP] and low HDL cholesterol). In the Young Finns study (8), LDL cholesterol and systolic BP in adolescence predicted CAC in adulthood independent of the longitudinal changes in these risk factors.

The physiologic mechanisms that link obesity to atherosclerosis are still not very clear and not always explainable by traditional risk factors (2). Several studies implicate insulin resistance as a risk factor for atherosclerosis and CAD (9,10) and as a predictor of the progression of CAC independent of the traditional CAD risk factors (11). In addition, hyperglycemia is implicated in the risk for CAD. Individuals with diabetes have higher mean CAC values than patients without diabetes for all age groups (age categories from 0–39 years and up to >69 years, with an average age of 54.2 years in patients without diabetes and 57.3 years in patients with diabetes) and in both sexes (12). The relationship of insulin sensitivity (IS) and dysglycemia to the development of CAC in childhood has not been investigated. Given the increasing rates of obesity in youth and the rise of type 2 diabetes (T2DM), this study aimed to: 1) determine the prevalence of CAC in obese adolescents with and without abnormalities in glucose metabolism; and 2) investigate the risk factors for CAC in obese youth. We hypothesized that: 1) CAC in adolescents is associated with adiposity measures, particularly abdominal adiposity; 2) prevalence of CAC is

higher in obese youth with dysglycemia than normoglycemia; and 3) CAC is likely to be associated with other markers of subclinical atherosclerosis such as pulse wave velocity (PWV) and intima-media thickness (IMT) and with markers of inflammation.

## RESEARCH DESIGN AND METHODS

### Study Population

Ninety obese adolescents: 37 with normal glucose tolerance (NGT), 26 with T2DM, 27 with prediabetes, including impaired fasting glucose (IFG) ( $n = 3$ ), impaired glucose tolerance ( $n = 22$ ), or both ( $n = 2$ ) based on 2-h oral glucose tolerance test (OGTT) (13) were studied. Impaired glucose regulation (IGR) was defined as having either prediabetes or T2DM. Twenty-three of the girls (13 NGT and 10 with prediabetes) had a clinical diagnosis of polycystic ovarian syndrome and were not receiving any pharmacotherapy. All of the rest had exogenous obesity with no clinical evidence of endocrinopathy or syndromes associated with obesity. They were not involved in any regular physical activity or weight-reduction programs. Except for treatment of T2DM with metformin and/or insulin, participants were not on any medications that affect glucose metabolism. None of the participants was known to have CV disease risk factors such as dyslipidemia or hypertension and were not on antihypertensive or lipid-lowering agents. Smoking history was obtained and categorized as present (any number of cigarettes smoked, current or past) or absent. A subset of the participants ( $n = 68$ ) had undergone a 3-h hyperinsulinemic (80 mU/m<sup>2</sup>/min)-euglycemic clamp to assess in vivo IS and abdominal CT at L4–L5 for abdominal fat distribution. In youth with T2DM, metformin and long-acting insulin were discontinued 48 h before the clamp studies. Most of the prediabetic and T2DM subjects have been reported in previous work related to  $\beta$ -cell function and IS (14,15). They were recruited to undergo the CV disease (CVD) risk-assessment investigation. The youth with T2DM included 11 males and 15 females with negative pancreatic autoantibodies (14), mean age of  $15.1 \pm 0.4$  years, duration of diabetes  $10.8 \pm 2.5$  months, and HbA<sub>1c</sub>  $6.8 \pm 0.3\%$ . All studies were approved by the Institutional Review Board of the University of Pittsburgh.

Informed consent was obtained. Clinical characteristics of the study subjects are summarized in Table 1.

### Fasting Measurements and OGTT

A fasting blood sample was obtained for determination of lipid profile, HbA<sub>1c</sub>, glucose, insulin, adiponectin, leptin, and markers of inflammation as detailed below under BIOCHEMICAL MEASUREMENTS. OGTT (1.75 g/kg, maximum 75 g Trutol) was performed with glucose, insulin, and C-peptide determination at –15, 0, 15, 30, 60, and 120 min.

### EBCT

CAC was measured by EBCT using an Imatron C-150 ultrafast computed tomography scanner using well-established protocol in the Cardiovascular Institute at the University of Pittsburgh by one of the investigators (D.E.) (16). In the supine position, while subjects held their breath, ~30 scans were obtained in 3-mm contiguous sections from the level of the aortic root to the apex of the heart. Scans are triggered by electrocardiogram signals at 80% of the R-R interval. A focus of CAC is considered to exist if there are  $\geq 3$  contiguous pixels (area 1.03 mm<sup>2</sup>) of  $\geq 130$  Hounsfield units in density (17). CAC score (Agatston CAC score) is determined according to the method of Agatston et al. (18) by multiplying the area of each lesion (in mm<sup>2</sup>) by a weighted CT attenuation score (18). Scores are measured for the left main, left anterior descending, left circumflex, and right coronary arteries. The intraclass correlation coefficient of the coronary score during the previous reproducibility studies in the Cardiovascular Institute was 0.99 (19).

### Arterial Stiffness, Carotid IMT, and BP

Aortic PWV was measured after 30 min of supine rest by Doppler ultrasound of the right carotid and femoral arteries at the University of Pittsburgh Ultrasound Research Laboratory (20). As arteries become stiff, the velocity of the pulse wave as it travels down the aorta becomes faster (higher PWV). Carotid IMT was measured by high-resolution B-mode ultrasonography. Briefly, B-mode images are obtained from the near and far wall of the distal common carotid artery, 1-cm proximal to the carotid bulb, electronically tracing the intima-media interface and the media-adventitia interface across a 1-cm segment. The

**Table 1—Phenotypic and metabolic characteristics of obese adolescents with (CAC+) vs. without CAC (CAC−)**

	CAC− (n = 45)	CAC+ (n = 45)	P value
<b>Physical characteristics</b>			
Male/female	12/33	14/31	ns
AA/white	23/22	21/24	ns
Smoking history (no/yes/not available)	35/1/9	34/1/10	ns
Age (years)	14.7 ± 0.3	15.3 ± 0.3	ns
BMI (kg/m <sup>2</sup> )	32.8 ± 0.6	37.6 ± 0.8	<0.001
Fat mass (kg)	37.8 ± 1.4	45.9 ± 1.7	<0.001
FFM (kg)	46.1 ± 1.3	54.6 ± 1.7	<0.001
Body fat (%)	43.6 ± 1.0	44.7 ± 0.8	ns
WC (cm)	99.2 ± 1.6	110.7 ± 2.0	<0.001
Total abdominal fat (cm <sup>2</sup> )	539.1 ± 27.9	671.1 ± 32.0	0.003
	(n = 33)	(n = 32)	
SAT (cm <sup>2</sup> )	470.5 ± 24.5	590.7 ± 27.8	0.002
VAT (cm <sup>2</sup> )	68.6 ± 5.5	80.3 ± 5.3	0.1
<b>Vascular markers</b>			
CAC score	0	6.0 ± 0.9	<0.001
CAC volume (mm <sup>3</sup> )	0	15.2 ± 2.1	<0.001
PWV (cm/s)	663.0 ± 38.6	713.0 ± 51.5	0.14
	(n = 40)	(n = 33)	
IMT (mm)	0.52 ± 0.01	0.52 ± 0.01	ns
	(n = 42)	(n = 43)	
<b>Metabolic parameters</b>			
NGT/prediabetes/T2DM	22/10/13	15/17/13	ns
HbA <sub>1c</sub> (%)	5.7 ± 0.1	5.8 ± 0.2	ns
HbA <sub>1c</sub> (mmol/mol)	39.9 ± 1.1	40 ± 2.2	ns
Fasting glucose (mg/dL)	94.7 ± 1.7	97.5 ± 2.9	ns
Fasting insulin (μU/mL)	34.7 ± 4.7	38.1 ± 3.8	ns
Leptin (ng/mL)	33.5 ± 2.5	39.0 ± 2.7	0.09
Total cholesterol (mg/dL)	159.8 ± 5.2	150.2 ± 4.1	ns
LDL cholesterol (mg/dL)	94.7 ± 4.2	91.3 ± 3.6	ns
HDL cholesterol (mg/dL)	40.7 ± 1.4	38.5 ± 1.5	ns
Non-HDL cholesterol (mg/dL)	119.1 ± 5.0	111.7 ± 4.0	ns
Triglycerides (mg/dL)	122.6 ± 10.3	101.8 ± 7.0	ns
SBP (mmHg)	124.6 ± 1.8	128.4 ± 2.2	ns
DBP (mmHg)	62.2 ± 0.9	66.3 ± 1.4	0.025

The  $\chi^2$  analysis revealed no significant differences between groups with respect to ethnicity, sex, and number of the NGT, prediabetic, and T2DM with vs. without CAC. Abdominal CT data available in a subset (n = 65). One additional individual had VAT data only as abdominal circumference was larger than the field of view. IMT was missing in three subjects in the CAC− group and two subjects in the CAC+ group secondary to technical difficulty; PWV data not available in five subjects in the CAC− group and 12 in the CAC+ group because of inadequate quality of the study secondary to obesity. ns, not significant. DBP, diastolic blood pressure; SBP systolic blood pressure.

mean of the average IMT readings obtained at the near and far walls was calculated (21). At the time of a PWV measurement, four supine BP measurements were taken with an automated BP cuff and averaged.

#### Clamp Studies and In Vivo Insulin Sensitivity

A representative sample (n = 68) of the participants with similar age, sex, ethnicity, presence or absence of CAC, BMI, percentage of body fat, and HbA<sub>1c</sub> to the total group, underwent a hyperinsulinemic-euglycemic clamp to evaluate in vivo IS, within a 1–3-week period from the CV evaluation (14). Intravenous crystalline insulin (Humulin; Eli Lilly

and Company, Indianapolis, IN) was infused at a constant rate of 80 mU/m<sup>2</sup>/min as before (14), and plasma glucose was clamped at 100 mg/dL (5.6 mmol/L) with a variable rate infusion of 20% dextrose (14).

#### Body Composition

Body composition was determined by DEXA, and subcutaneous abdominal adipose tissue (SAT) and visceral adipose tissue (VAT) by a single-slice CT scan at L4–L5 (22).

#### Biochemical Measurements

Plasma glucose was measured with a glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, OH), insulin, C-peptide, and adiponectin by radioimmunoassay as

before (23). HbA<sub>1c</sub> was measured by high-performance liquid chromatography (Tosoh Medics, Inc.) and lipids using the standards of the Centers for Disease Control and Prevention (23). Leptin concentrations were determined by radioimmunoassay (Linco Research Inc., St. Charles, MO). Interleukin-6, vascular cell adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1 (ICAM-1), and E-selectin levels were quantified using a double-sandwich enzyme-linked immunoassay (R&D Systems, Minneapolis, MN) (24). Plasminogen activator inhibition factor (PAI-1) and tumor necrosis factor- $\alpha$  were measured using a flow cytometry-based platform (Luminex MAP 200; Millipore, St. Charles, MO). High-sensitivity C-reactive protein levels were measured at Esoterix, Inc.

#### Calculations

Insulin-stimulated glucose disposal rate (Rd) was calculated during the last 30 min of the euglycemic clamp to be equal to the rate of exogenous glucose infusion and expressed per fat-free mass (FFM; mg/min/kg<sub>FFM</sub>). Peripheral IS was calculated by dividing the Rd by the steady-state insulin concentration and expressed per FFM (mg/min/kg<sub>FFM</sub>/μU/mL) (14).

#### Statistics

Statistical analyses were performed using t test or the Mann–Whitney U non-parametric equivalent for two-group comparisons. Analysis of variance or Kruskal–Wallis test was used for three-group comparisons. Pearson or Spearman correlation and multiple regression analyses were used to evaluate bivariate and multivariate relationships and  $\chi^2$  for categorical variables. CAC score was not normally distributed, and log (CAC score+1) was used in the regression analyses to be able to account for the CAC scores of 0. Similarly, log PWV was used as the dependent variable in regression models. The independent variables in the regression model included traditional CVD risk variables and those that showed a relationship to dependent variables in the simple bivariate model. Further model building included addition of inflammatory markers to the above model. Power analysis based on detecting differences in BMI between two groups of obese children with and without CAC indicated that a sample size of 34 subjects per group was needed to detect a difference

of five units of BMI with a power of 0.8 and  $\alpha = 0.05$  (25). Data are presented as mean  $\pm$  SEM. Two-tailed  $P \leq 0.05$  was considered statistically significant, except for one-tailed testing of our hypothesis that IS is lower in CAC+ compared with CAC- youth, based on literature reports of a relationship between CAC and insulin resistance (11,26). A  $P$  value of 0.06–0.09 was reported as tendency for significance.

## RESULTS

### Subject Characteristics

Any amount of CAC is unexpected in youth (25). Therefore, participants were divided in two groups depending on whether or not CAC was detected by EBCT scan. Those having an Agatston score  $\geq 1$  were considered CAC+. Table 1 depicts the characteristics of the two groups of obese adolescents: those with Agatston CAC score  $\geq 1$  (CAC+) and those with CAC score  $< 1$  (CAC-). The two groups of subjects included similar numbers of youth with NGT and IGR including prediabetes and T2DM. The Agatston CAC score ranged from 0 to 29.5 with a median of 0.5. Participants with IGR were more likely to have a CAC score  $\geq 1$ , 56% (30 of 53) compared with

40.5% (15 of 37) in the NGT group, but this was not statistically different.

There were no significant differences in age, sex, ethnic distribution, or smoking history between the two groups. All subjects were pubertal. There was no significant difference in the CAC score or in the prevalence of CAC between African American (AA) versus American White (AW) ( $\sim 30\%$  in both), between girls and boys ( $\sim 30\%$  in both), or between girls with versus without polycystic ovarian syndrome of similar BMI and body composition (data not shown). T2DM youth with and without CAC did not differ in age, sex, ethnicity, HbA<sub>1c</sub>, or diabetes duration.

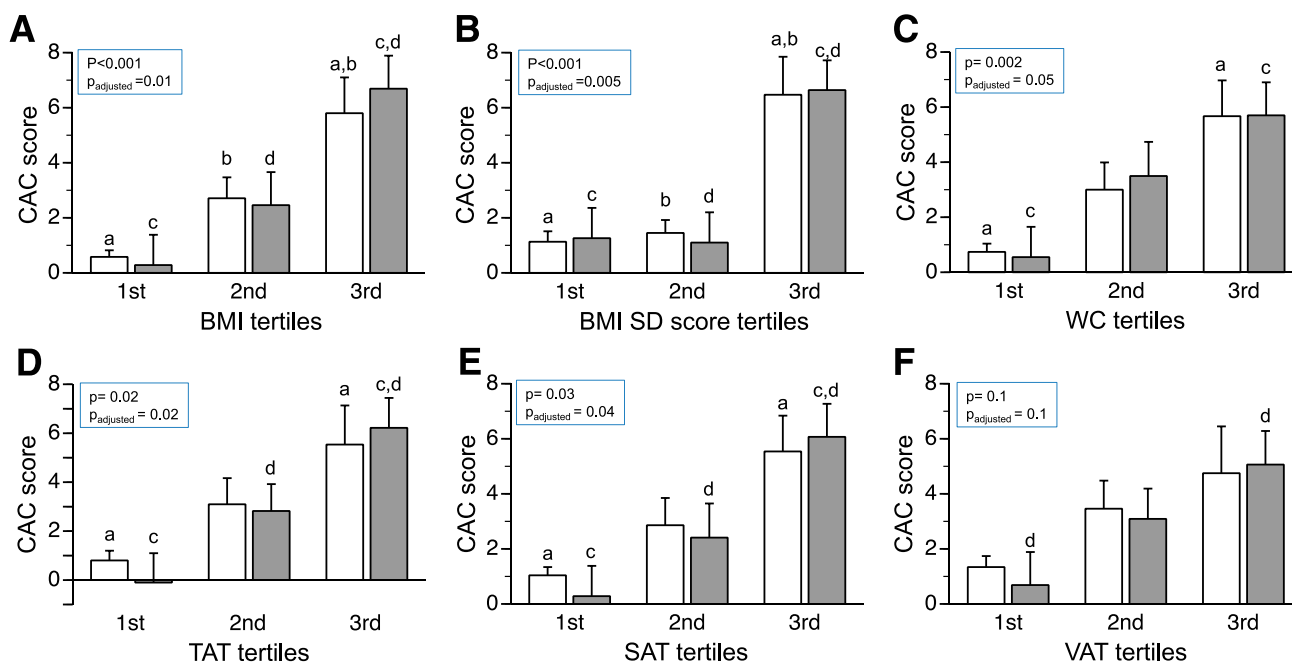
The CAC+ group had a significantly higher BMI, waist circumference (WC), fat mass, and FFM compared with the CAC- group with no differences in percentage of body fat. In the subset of participants who had abdominal CT scans ( $n = 65$ ), total abdominal adipose tissue (TAT), particularly SAT, was significantly higher in the CAC+ vs. CAC- group (Table 1). CAC score increased with increasing tertiles of BMI, BMI SD score, WC, TAT, SAT, and VAT (Fig. 1A–F).

Metabolic parameters including HbA<sub>1c</sub>, fasting glucose, fasting insulin,

lipid profile, and systolic BP were not different between the two groups, but diastolic BP was significantly higher in the CAC+ group (Table 1). Aortic PWV and carotid IMT were not significantly different between the groups with and without CAC (Table 1).

### Relationship of IS to CAC

In vivo IS was evaluated in 35 youth with no CAC (12 male, 23 female; 17 AA and 18 AW; and 12 NGT and 23 with IGR) and 33 CAC+ (11 male, 22 female; 14 AA and 19 AW; and 10 NGT and 23 IGR). There were no significant differences in age, sex, ethnicity, HbA<sub>1c</sub>, or glucose tolerance status between the two groups. BMI was significantly higher in the CAC+ group compared with the CAC- group ( $38.2 \pm 1.0$  kg/m<sup>2</sup> vs.  $33.0 \pm 0.7$  kg/m<sup>2</sup>;  $P < 0.01$ ). Consistent with our hypothesis, parameters of in vivo glucose metabolism trended to be lower in CAC+ compared with CAC- youth, including insulin-stimulated glucose disposal (expressed per FFM [Rd/FFM]) ( $9.8 \pm 0.7$  mg/kg<sub>FFM</sub>/min in CAC+ vs.  $11.2 \pm 0.7$  mg/kg<sub>FFM</sub>/min in CAC-;  $P = 0.08$ ), IS/kg body weight ( $1.9 \pm 0.2$  in CAC+ vs.  $2.4 \pm 0.3$  mg/kg/min/ $\mu$ U/mL in CAC-;  $P = 0.055$ ), and IS/kg FFM ( $3.6 \pm 0.4$  in CAC+



**Figure 1**—CAC score across tertiles of BMI ( $n = 90$ ) (A), BMI SD scores ( $n = 90$ ) (B), WC ( $n = 85$ ) (panel C), TAT ( $n = 65$ ) (D), SAT ( $n = 65$ ) (E), and VAT ( $n = 66$ ) (F).  $P$  values for ANOVA, unadjusted and adjusted for sex, race, IS<sub>FFM</sub>, and glucose tolerance status. White bars represent unadjusted means, and dark bars represent adjusted means. Pairs of similar letters indicate two groups' post hoc significant differences (a and b for unadjusted and c and d for adjusted model). In the adjusted model for SAT and TAT, glucose tolerance status (NGT vs. IGR) contributed to the variance of the model ( $P < 0.05$ ) in addition to the main effect of SAT and TAT.

vs.  $4.6 \pm 0.5$  mg/kg<sub>FFM</sub>/min/ $\mu$ U/mL in CAC<sup>-</sup>,  $P = 0.06$ ) using one-tailed  $t$  test.

#### Relationship of Inflammatory and Endothelial Smooth Muscle Biomarkers to CAC

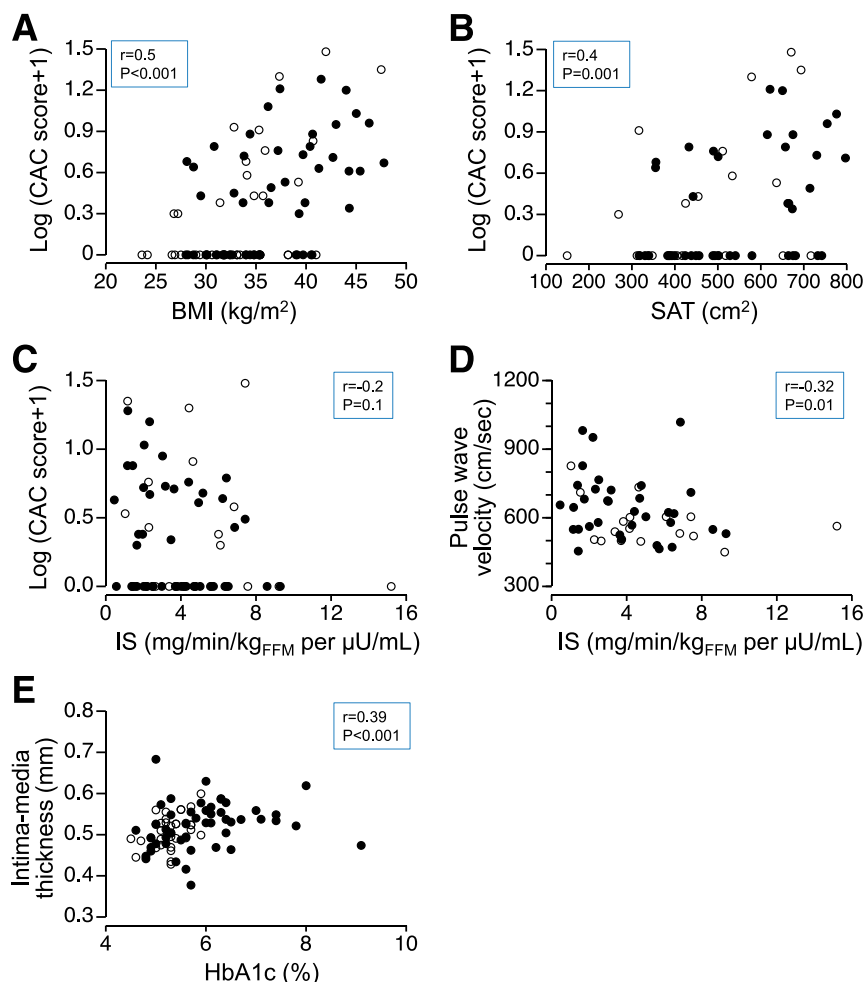
Leptin level tended to be higher in the CAC<sup>+</sup> group ( $39.0 \pm 2.7$  vs.  $33.5 \pm 2.5$  ng/mL;  $P = 0.09$ ), but after adjusting for BMI or adiposity differences, this significance disappeared. There were no significant differences in adiponectin ( $6.5 \pm 0.5$  vs.  $7.4 \pm 0.6$   $\mu$ g/mL), high-sensitivity C-reactive protein ( $2.6 \pm 0.4$  vs.  $1.9 \pm 0.3$  mg/L), PAI-1 ( $45.4 \pm 3.9$  vs.  $41.1 \pm 5.0$  pg/mL), interleukin-6 ( $8.7 \pm 0.8$  vs.  $14.0 \pm 4.0$  pg/mL), tumor necrosis factor- $\alpha$  ( $4.0 \pm 0.3$  vs.  $5.8 \pm 1.3$  pg/mL), E-selectin ( $31.5 \pm 2.6$  vs.  $36.3 \pm 2.6$  ng/mL), ICAM-1 ( $145.2 \pm 6.5$  vs.  $161.7 \pm 14.6$  ng/mL), or VCAM-1 ( $145.2 \pm 6.5$  vs.  $161.7 \pm 14.6$ ) in CAC<sup>+</sup> vs. CAC<sup>-</sup> groups, respectively.

#### Determinants of CACs, IMT, and PWV

Adiposity measures showed significant correlations with Agatston CAC score (and Ca volume), BMI ( $r = 0.51$ ;  $P < 0.001$ ), WC ( $r = 0.51$ ;  $P < 0.001$ ), total body fat ( $r = 0.4$ ;  $P < 0.001$ ), total abdominal fat ( $r = 0.4$ ;  $P < 0.001$ ), SAT ( $r = 0.41$ ;  $P = 0.001$ ), and VAT ( $r = 0.26$ ;  $P = 0.04$ ). Leptin correlated with the CAC score ( $r = 0.22$ ;  $P = 0.06$ ), Ca volume ( $r = 0.24$ ;  $P = 0.04$ ), and PWV ( $r = 0.26$ ;  $P = 0.045$ ). Diastolic BP correlated with CAC ( $r = 0.22$ ;  $P = 0.04$ ), PWV ( $r = 0.21$ ;  $P = 0.08$ ), and IMT ( $r = 0.25$ ,  $P = 0.02$ ). There were no significant correlations between lipids and CAC, PWV, or IMT except for triglycerides and CAC ( $r = -0.23$ ;  $P = 0.03$ ). Only IMT correlated with HbA<sub>1c</sub> ( $r = 0.39$ ;  $P < 0.001$ ), and only PWV showed correlations with IS ( $r = -0.32$ ;  $P = 0.01$ ) (Fig. 2) and smooth muscle biomarkers, ICAM ( $r = 0.24$ ;  $P = 0.07$ ), VCAM ( $r = 0.3$ ;  $P = 0.03$ ), E-selectin

( $r = 0.3$ ;  $P = 0.03$ ), and PAI-1 ( $r = 0.3$ ;  $P = 0.05$ ). Analysis of the CAC<sup>+</sup> group separately showed that CAC score tended to correlate with IMT ( $r = 0.28$ ;  $P = 0.07$ ) but not with PWV.

In multiple regression analyses (Table 2) with age, sex, race, HbA<sub>1c</sub>, BMI, IS<sub>FFM</sub>, diastolic BP, and non-HDL cholesterol as independent variables and log (CAC score + 1) as the dependent variable, BMI ( $\beta = 0.52$ ;  $P < 0.001$ ) was the only independent contributor to the variance in CAC score ( $R^2 = 0.31$ ;  $P = 0.005$ ). In the same regression model with IMT as the dependent variable, HbA<sub>1c</sub> ( $\beta = 0.26$ ;  $P = 0.045$ ), race ( $\beta = -0.36$ ;  $P = 0.005$ , higher in blacks), and age ( $\beta = 0.31$ ;  $P = 0.015$ ) together and independently contributed to the variance in IMT ( $R^2 = 0.36$ ;  $P = 0.002$ ). With log PWV as the dependent variable, the above model was not significant. The addition of inflammatory markers to the above



**Figure 2**—Relationship of Log (CAC score + 1) to BMI ( $n = 90$ ) (A), SAT ( $n = 65$ ) (B), and IS<sub>FFM</sub> ( $n = 67$ ) (C). Relationship of PWV to IS<sub>FFM</sub> ( $n = 52$ ) (D) and of IMT to HbA<sub>1c</sub> ( $n = 85$ ) (E) in subjects with NGT (open circles) and IGR (filled circles).

**Table 2—Determinants of CAC, PWV, and IMT in linear regression analyses**

Regression model independent variables	$R^2$ and determinants of the variance of dependent variables		
	Log (CAC +1) ( $n = 65$ )	Log (PWV) ( $n = 52$ )	IMT ( $n = 61$ )
Model 1 Age, race, sex, BMI, IS <sub>FFM</sub> , HbA <sub>1c</sub> , DBP, non-HDL cholesterol	$R^2 = 0.31$ ; $P = 0.005$  BMI $\beta = 0.5$ ; $P < 0.001$	ns	$R^2 = 0.36$ ; $P = 0.002$  Race $\beta = -0.4$ ; $P = 0.005$ Age $\beta = 0.3$ ; $P = 0.02$ HbA <sub>1c</sub> $\beta = 0.3$ ; $P = 0.045$ Sex $\beta = -0.2$ ; $P = 0.09$
Model 2 Variables in model 1 + leptin	$R^2 = 0.41$ ; $P = 0.001$  BMI $\beta = 0.6$ ; $P = 0.001$	$R^2 = 0.37$ ; $P = 0.034$  Leptin $\beta = 0.7$ ; $P = 0.005$ Age $\beta = 0.3$ ; $P = 0.06$	$R^2 = 0.34$ ; $P = 0.02$  Race $\beta = -0.3$ ; $P = 0.03$ Age $\beta = 0.3$ ; $P = 0.04$ HbA <sub>1c</sub> $\beta = 0.3$ ; $P = 0.08$
Model 3 Variables in model 2 + PAI-1	$R^2 = 0.46$ ; $P = 0.008$  BMI $\beta = 0.6$ ; $P = 0.003$	$R^2 = 0.48$ ; $P = 0.03$  PAI-1 $\beta = 0.4$ ; $P = 0.02$	$R^2 = 0.4$ ; $P = 0.04$  Race $\beta = -0.3$ ; $P = 0.045$ Age $\beta = 0.3$ ; $P = 0.05$

DBP, diastolic BP; ns, not significant.

models showed no independent contribution to the variance in CAC, IMT, or PWV.

## CONCLUSIONS

The current study demonstrates that CAC, a risk marker for future coronary events in adults, could be an early biomarker of subclinical atherosclerosis in obese youth. Importantly, adiposity parameters appear to be the main determinant of CAC in youth, and PWV and IMT do not seem to differ between obese youth with versus without CAC. This suggests that early in the course of obesity, CAC might be an important marker of future CVD. In addition, PWV is modulated by IS, while IMT is modulated by glycemia, both comorbidities of obesity.

Studies utilizing EBCT to evaluate CAD in youth are limited. Our findings of increased prevalence of CAC in obese youth is consistent with the findings of Gidding et al. (25), who found that 19 of 29 (66%) 11–23-year-old patients with heterozygous familial hypercholesterolemia had some evidence of CAC. Similar to our findings, BMI was the only significant independent predictor of CAC in these subjects with severe cholesterol elevation (25).

The calcifications in the vascular system are believed to be part of an organized process akin to the calcium hydroxyapatite deposition in bone and unlikely to occur in the absence of atheroma formation (27). Therefore, any evidence of CAC in these obese youth is highly alarming, as it indicates an atherosclerotic process not usually expected in this young age group. The similar prevalence of CAC in normoglycemic and dysglycemic youth in our study indicates an overriding and primary role of obesity in the pathogenesis of these calcifications, independent of abnormalities in glycemic regulation.

In our study, the relationship of CAC to IS was modest. This is unlike the findings in adults with type 1 diabetes and BMI matched control subjects, whereby CACs were more clearly related to IS (28). This may be due to the fact that our subjects were all obese and insulin resistant, thus not allowing us to discern the effect contributed by a wider spectrum of IS to this process. Consistent with this, when the individuals with type 1 diabetes (more insulin-resistant than control subjects) were evaluated separately from the controls in the study by Schauer et al. (28), the relationship of CAC to IS was no longer

significant. The somewhat higher prevalence of CAC and higher CAC score in our youth with dysglycemia compared with NGT youth is also consistent with the higher prevalence and higher grade of vulnerable angiographic plaques in adults with prediabetes and T2DM (29) and higher mean and median CAC values in adults with diabetes compared with patients without diabetes (12). The absence of a more marked difference in CAC prevalence in our youth with T2DM compared with patients without diabetes is likely due to the younger age of our subjects, relatively short duration of diabetes, and perhaps a tighter range of glycemic control. Moreover, the amount of calcification observed in our younger age group is relatively small and thus may not allow us to evaluate the full spectrum of these relationships. In the Framingham Heart Study (30), the relationship of CAC to IFG was no longer significant after adjusting for BMI, further supporting our findings of a stronger role of obesity rather than glycemia in the pathogenesis of CAC.

In addition to adiposity measures, CAC was related to diastolic BP (important traditional CVD risk factor) but not to cholesterol, LDL, HDL, triglycerides, age, or sex in our study. This is

consistent with the findings from Young Finns study (8) in which the odds ratio for CAC in young adulthood was 1.38 for a 1-SD increase in adolescent BP and with the Epidemiology of Diabetes Interventions and Complications study (31) showing a relationship between CAC score and waist-to-hip ratio and systolic blood pressure but not cholesterol in adults with type 1 diabetes. However, triglycerides ( $r = 0.08$ ,  $P = 0.005$ ) and a diagnosis of hypercholesterolemia were significantly related to CAC in the Epidemiology of Diabetes Interventions and Complications study. This may be related to the age (obese adolescents vs. adults with type 1 diabetes) and different ethnic mix of our population with a larger proportion of AAs in our study. We did not find ethnic differences in the prevalence of CAC. This is in contrast to some studies (32) that reported lower CAC prevalence in blacks compared with whites despite higher CVD risk in blacks. However, our results are consistent with the findings in 443 black and white young adults (age 28–40 years) in the Coronary Artery Risk Development In Young Adults (CARDIA) study (33) and the population-based Dallas Heart study (34) of middle-age (~50-year-old) men and women.

Obesity-associated adipocytokines and inflammation have been postulated to be the culprit for increased atherosclerosis. In our study, leptin but not adiponectin levels correlated with CAC and PWV. This is consistent with *in vitro* findings of a role of leptin to promote pro-osteogenic differentiation and calcification of vascular cells (35). This is also consistent with findings from the Study of Inherited Risk of Coronary Atherosclerosis (26), which reported similar relationship of leptin but not adiponectin to CAC in Caucasian adults with family history of premature atherosclerosis. In youth, leptin, independent of fat mass, contributed significantly to the variance in arterial distensibility in 13–16-year-old adolescents of different adiposity levels (36). Taken together, these findings and our current results suggest that leptin may be an important mediator of the effect of obesity on the vascular system. Other inflammatory and endothelial function vascular markers were not significantly different in our youth with CAC compared with those without. This is consistent with a recent meta-analysis

that showed that almost all adult studies that explored the relationship between inflammatory markers and CAC found a weak relationship that was lost after correction for obesity and BMI (37).

In this study, we examined other measures of subclinical atherosclerosis including PWV and IMT. *In vivo* IS emerged as a significant determinant of arterial stiffness, whereas race, age, and HbA<sub>1c</sub> were significant determinants of IMT. Similar to our findings, PWV was related to fasting indices of insulin resistance in normal weight and obese adolescents and young adults with T2DM (38). This is also consistent with earlier reports from our group in which HOMA-IS was an independent predictor of PWV in youth with obesity, youth with T2DM, and normal-weight control subjects (39). The relationship of HbA<sub>1c</sub> to IMT in our study is consistent with the findings of Urbina et al. (40) of higher carotid IMT in children with T2DM compared with lean and obese peers. Racial differences in IMT were reported in studies of young adults in the Bogalusa Heart Study (41). Our findings suggest that race is a modulator of IMT starting in childhood and an important determinant of CVD risk in addition to hyperglycemia.

Overall, our findings suggest that the proatherogenic environment related to obesity might differentially impact various pathological processes of vascular structure and function. CAC can be detected early in obese youth, is related to obesity, and not completely explained by traditional CVD risk factors (related to BP but not to lipids). Arterial stiffness appears to be modulated by IS and inflammatory markers, whereas IMT is associated with hyperglycemia and race.

The strengths of this study include evaluation of CAC in children, studying high-risk obese youth with normoglycemia and dysglycemia, and using three important risk-predictive biomarkers of CVD and inflammatory markers. Our study is limited by studying only obese insulin-resistant adolescents, which may have prevented the appreciation of a potential relationship of a wider spectrum of IS with CAC. Another limitation in imaging obese individuals is the potential for artifact related to obesity. However, this is a common challenge in studies of individuals with elevated BMI. The evaluation of CAC involves a

carefully monitored, focused, low-dose radiation of 0.5 to a maximum of 4 mSv, and the amount of CAC in youth is modest compared with that described in adults. Therefore, until there is better understanding of the CVD risk burden in adolescents, evaluation of CAC in youth should be restricted to the research setting. Given the cross-sectional study design, risk prediction analyses are not possible. Studies are needed to prospectively assess the long-term significance of early subclinical atherosclerosis in obese youth.

In conclusion, our study demonstrates that the vascular calcification process begins in childhood in obese youth and is primarily determined by obesity. Different aspects of subclinical atherosclerosis appear to be differentially regulated by obesity, IS, and hyperglycemia. Our findings support the need for intervention trials that aim to reverse the early atherosclerotic process in these high-risk obese youth in addition to primary prevention efforts to combat childhood obesity and its comorbidities.

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**Author Contributions.** F.B. conducted the study, obtained funding, performed data analysis and interpretation, and wrote the manuscript. D.E. performed evaluation of the EBCT scan and reviewed the manuscript. K.S.-T. supervised performance of the PWV and IMT evaluations. S.L. and H.T. contributed data and reviewed the manuscript. S.A.A. contributed to

study design, funding, data acquisition and interpretation, and critically reviewed and edited the manuscript. F.B. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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