Endothelial Function in Youth: A Biomarker Modulated by Adiposity-Related Insulin Resistance

Anca Tomsa, MD[1,2], Sara Klinepeter Bartz, MD[1,2], Rajesh Krishnamurthy, MD[3], Ramkumar Krishnamurthy, PhD[3], and Fida Bacha, MD[1,2]

Objective To investigate the physical and metabolic determinants of endothelial dysfunction, an early marker of subclinical atherosclerosis, in normal weight and overweight adolescents with and without type 2 diabetes mellitus.

Study design A cross-sectional study of 81 adolescents: 21 normal weight, 25 overweight with normal glucose tolerance, 19 overweight with impaired glucose regulation, and 16 with type 2 diabetes mellitus underwent evaluation of reactive hyperemia index (RHI) and augmentation index (AIx) at heart rate 75 bpm by peripheral arterial tonometry; oral glucose tolerance test, lipid profile, and hyperinsulinemic-euglycemic clamp to measure insulin sensitivity; and dual energy X-ray absorptiometry scan and abdominal magnetic resonance imaging for percentage of body fat and abdominal fat partitioning.

Results Participants across tertiles of RHI (1.2 ± 0.02, 1.5 ± 0.02, and 2.0 ± 0.05, $P < .001$) had similar age, sex, race, lipid profile, and blood pressure. Body mass index z-score, percentage body fat, abdominal fat, and hemoglobin A1c decreased, and insulin sensitivity increased from the first to third tertile. RHI was inversely related to percentage body fat (r = –0.29, $P = .008$), total (r = –0.37, $P = .004$), subcutaneous (r = –0.39, $P = .003$), and visceral (r = –0.26, $P = .04$) abdominal fat. AIx at heart rate 75 bpm was higher (worse) in the lower RHI tertiles ($P = .04$), was positively related to percentage body fat (r = 0.26, $P = .021$), and inversely related to age, insulin sensitivity, and inflammatory markers (tumor necrosis factor-α and plasminogen activator inhibition-1).

Conclusions Childhood obesity, particularly abdominal adiposity, is associated with endothelial dysfunction manifested by worse reactive hyperemia and higher AIx. Insulin resistance appears to mediate this relationship. *(J Pediatr 2016;178:171-7).*

The process of atherosclerosis occurs along a continuum and has its origins in the adolescent years as documented by several cohort studies.[1-3] There is a relationship between obesity and central arterial stiffness in youth with and without type 2 diabetes mellitus (T2DM).[4-6] Aortic pulse wave velocity (PWV) was inversely related to insulin sensitivity and inflammatory markers in these obese adolescents. Intima media thickness was found to be higher in youth with T2DM compared with lean and obese peers[7] and was related to hemoglobin (Hb) A1c measure of glycemia.[4] These findings support the importance of identifying early biomarkers of vascular function that are easily obtainable and applicable in large-scale studies to detect children at higher risk for cardiovascular abnormalities. Endothelial dysfunction is an early manifestation of subclinical atherosclerosis and results in abnormal regulation of the vessel tone.[5,8] Evaluation of the change in pulse wave amplitude during reactive hyperemia using peripheral arterial tonometry (PAT) is a noninvasive validated method to evaluate endothelial function in the microvasculature.[9] The reactive hyperemia index (RHI) derived from this measurement correlates well with brachial artery ultrasound measured flow mediated dilation[9] and with the coronary blood flow in response to acetylcholine, a direct measure of coronary endothelial function.[10] RHI predicts cardiovascular events in prospective studies in adults.[11,12] In addition, the augmentation index (AIx), a measure of peripheral arterial stiffness, can be derived from PAT.[13] The determinants of RHI and AIx and the effect of obesity related insulin resistance vs hyperglycemia on these measures of endothelial function in youth have not been comprehensively evaluated.

In this study, we hypothesized that obese youth have evidence of endothelial dysfunction compared with normal weight peers, and that this endothelial

AIx	Augmentation index	LDL	Low-density lipoprotein
AIx-75	AIx at heart rate 75 bpm	NGT	Normal glucose tolerance
BMI	Body mass index	PAI	Plasminogen activator inhibition
BP	Blood pressure	PAT	Peripheral arterial tonometry
FFM	Fat-free mass	PWV	Pulse wave velocity
Hb	Hemoglobin	RHI	Reactive hyperemia index
HDL	High-density lipoprotein	T2DM	Type 2 diabetes mellitus
IGR	Impaired glucose regulation	TNF	Tumor necrosis factor

From the [1]US Department of Agriculture/Agricultural Research Service Children's Nutrition Research Center, Texas Children's Hospital, Baylor College of Medicine, Houston, TX; [2]Division of Pediatric Endocrinology and Diabetes, Texas Children's Hospital, Baylor College of Medicine, Houston, TX; and [3]Department of Radiology, Texas Children's Hospital, Baylor College of Medicine, Houston, TX

Supported by the US Department of Agriculture-Agricultural Research Service Award (6250-51000-057) and Thrasher Research Fund. The authors declare no conflicts to disclose.

Portions of the study were presented at the meeting of the Endocrine Society, San Diego, CA, March 2015.

http://dx.doi.org10.1016/j.jpeds.2016.07.025

dysfunction is mediated by lower insulin sensitivity and/or inflammation and is exacerbated by hyperglycemia. Therefore, we investigated the relationship of endothelial function (RHI and AIx) to body composition, insulin sensitivity, glycemia, and circulating inflammatory markers in normal weight and overweight adolescents with and without abnormalities in glucose metabolism.

Methods

Pubertal adolescents (n = 81), 12-19 years of age (mean: 15.5 ± 0.2 years), 35 males and 46 females were recruited via advertisements in the community and from Texas Children's Hospital clinics. The study population consisted of 21 normal weight controls (body mass index [BMI]: <85th percentile for age and sex) including 12 males and 9 females, and 60 overweight adolescents (BMI ≥85th percentile for age and sex). The overweight adolescents included 25 (7 males, 18 females) with normal glucose tolerance (NGT), 19 (9 males, 10 females) with impaired glucose regulation (IGR), and 16 (7 males, 9 females) with T2DM. IGR and T2DM were diagnosed according to the American Diabetes Association criteria.[14] Participants were not involved in any regular physical activity or weight reduction programs. Youth with T2DM were required to be in adequate glycemic control (HbA1c < 8%) on metformin (n = 14) and/or lifestyle therapy (n = 2) and to discontinue metformin 24 hours prior to PAT testing and 48 hours prior to clamp studies, as before.[4,15] Participants with T2DM had an average HbA1c of 6.2 ± 0.16% (range 5.2%-7.5%), and a mean duration of diabetes 25.8 ± 6.2 months (range 0-89 months). Exclusion criteria included presence of disease other than T2DM or medication that would interfere with glucose metabolism, positive pregnancy test in females, history of smoking, and diagnosis of dyslipidemia or hypertension. Some data from 53 of the participants were included in a previous article.[15] All studies were approved by the institutional review board of Baylor College of Medicine and were performed at the Children's Nutrition Research Center, Texas Children's Hospital. Informed consent/assent was obtained from all participants and their parents prior to any study-related procedures after a detailed explanation of the study.

Anthropometrics, Body Composition, and Abdominal Fat Distribution

Height was measured on a fixed-wall stadiometer (Holtain Limited, Crymych, Dyfed, United Kingdom) to the nearest centimeter 3 times and then averaged. Weight was measured in light clothing to the nearest 0.1 kg on a balance scale. Puberty stage was assessed in all participants by a pediatric endocrinologist using Tanner staging criteria. Blood pressure (BP) was measured using a Welch Allyn Spot Vital Signs LXi (Welch Allyn Inc, Skaneateles, New York). Body composition including percentage body fat, fat mass, and fat-free mass (FFM), was determined by a dual-energy X-ray absorptiometry scan (Delphi-A V12.1; Hologic, Bedford, Massachusetts). Abdominal fat distribution was obtained by magnetic resonance imaging scan at L4-L5 intervertebral space.

Fasting Measurements and Oral Glucose Tolerance Test

Fasting blood sample was obtained for determination of total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, HbA1c, and circulating inflammatory markers as detailed below. Each participant underwent a 2-hour oral glucose tolerance test (1.75 g/kg of dextrose [max 75 g]) after an overnight fast (minimum 8 hours) to verify glucose tolerance status.

Hyperinsulinemic-Euglycemic Clamp

In vivo insulin sensitivity was evaluated during a hyperinsulinemic-euglycemic clamp, after 10-12 hours overnight fast as before.[4,15] Briefly, intravenous insulin (Humulin; Lilly, Indianapolis, Indiana) was infused at a rate of 40 mU/m^2/min in lean individuals and 80 mU/m^2/min in obese individuals to suppress hepatic glucose production.[16] Plasma glucose was clamped at 100 mg/dL (5.5 mmol/L) with a variable-rate infusion of 20% dextrose based on plasma glucose determinations every 5 minutes.

Endothelial Function Studies

Assessment of endothelial function was performed by PAT using EndoPAT (Itamar Medical, Atlanta, Georgia)[15,17] in fasting state, in a quiet, temperature-controlled room. Briefly, the index fingers are placed into pneumatic probes, and pulse wave is recorded. After 5 minutes equilibration period, a BP cuff placed on the nondominant test arm is inflated to suprasystolic pressure for 5 minutes (the nonoccluded arm serving as a control). Thereafter, the cuff is rapidly deflated to allow for reactive flow-mediated hyperemia. The RHI is calculated by an automated algorithm as the ratio of the pulse wave amplitude during reactive hyperemia relative to baseline.[17] The AIx is an estimate of pulse wave reflection and a measure of arterial stiffness. AIx is calculated as the difference between the early (P1) and late (P2) systolic peaks of the pulse wave relative to the early peak wave (P2–P1/P1) expressed as percentage.[13] Because AIx is affected by the heart rate, values are adjusted to a standard heart rate of 75 beats per minute (AIx-75).[18]

Biochemical Measurements

Plasma glucose was measured with a glucose analyzer (YSI, Yellow Springs, Ohio); insulin levels were measured by electrochemiluminescence immunoassay (Elecsys 2010; Roche Diagnostics, Indianapolis, Indiana). HbA1c was measured using Tina-quant HbA1c immunoassay from Roche and lipids were measured using the standards of the Centers for Disease Control and Prevention, via Labcorp, Inc (Burlington, North Carolina). Leptin, tumor necrosis factor (TNF)-α, and plasminogen activator inhibition (PAI)-1 concentrations were determined by MAGPIX (milliplex MAP) immunoassay (EMD milipore Corporation; Billerica, Massachusetts).