Relations among Adiposity and Insulin Resistance with Flow-Mediated Dilation, Carotid Intima-Media Thickness, and Arterial Stiffness in Children

Justin R. Ryder, PhD[1,2], Donald R. Dengel, PhD[1,3], David R. Jacobs, Jr., PhD[2], Alan R. Sinaiko, MD[1], Aaron S. Kelly, PhD[1,4], and Julia Steinberger, MD, MS[1]

Objective To determine the associations of adiposity and insulin resistance with measures of vascular structure and function in children.

Study design A cross-sectional study included 252 children (age 15.1 ± 2.4 years; body mass index percentile 68.2 ± 26.5%; Tanner 2-5). Measurements of body fat percentage were obtained with dual-energy X-ray absorptiometry and visceral adipose tissue (VAT) with computed tomography. Insulin resistance was measured with hyperinsulinemic euglycemic clamp. Vascular measurements for endothelial function (brachial artery flow-mediated dilation [FMD]), vascular structure (carotid intima-media thickness [cIMT]), vascular stiffness (carotid incremental elastic modulus), and pulse wave velocity were analyzed by tertiles of adiposity and insulin resistance. Additional analyses with ANCOVA and linear regression were adjusted for Tanner, sex, race, and family relationship; FMD was also adjusted for baseline artery diameter.

Results FMD was positively associated with high adiposity (body mass index, body fat percentage, and VAT) ($P < .01$ all). Insulin resistance was not associated with FMD. cIMT was significantly, positively related to obesity, VAT, and insulin resistance ($P < .05$ all). No differences in carotid incremental elastic modulus and pulse wave velocity were observed in relation to adiposity or insulin resistance.

Conclusions The findings suggest that adiposity is associated with higher FMD, and insulin resistance and VAT are associated with higher cIMT in children. Further research is needed to clarify the progression of these relations. *(J Pediatr 2016;168:205-11)*.

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Studies in adults have shown adiposity and insulin resistance to be associated with vascular dysfunction and adverse thickening of the vascular wall, measured by carotid intima-media thickness (cIMT).[1-5] Although these relations are less clear in children, several studies have reported the same adverse relations for adiposity with vascular measures,[6-12] and others observed no significant relations.[13-15] However, a recent population-based cohort of prepubertal children provided data suggesting that adiposity tends to be associated with both an increase in brachial artery flow-mediated dilation (FMD) and decrease in arterial stiffness as measured by pulse wave velocity (PWV).[15] Thus, the relationship between adiposity with FMD, cIMT, and arterial stiffness (PWV) remains to be fully defined.

Insulin resistance is associated with obesity and cardiovascular risk factors, beginning in childhood.[16] Studies are needed to examine the relation between insulin resistance and FMD, cIMT, and arterial stiffness in children. Given that total body and visceral adipose tissue (VAT) play significant, and potentially different, roles in relation to insulin resistance and the pathophysiology of cardiovascular disease,[17] body fat measurements will improve our understanding of the role it plays in the early changes in FMD, cIMT, and arterial stiffness. Furthermore, studying the associations of insulin resistance and adiposity with measures of vascular structure and function, while controlling for pubertal maturation, may yield information toward the understanding of

AUC	Area under the curve
BF%	Body fat percentage
BMI	Body mass index
cIEM	Carotid incremental elastic modulus
cIMT	Carotid intima-media thickness
DXA	Dual-energy X-ray absorptiometry
FMD	Flow-mediated dilation
HOMA-IR	Homeostasis model assessment index of insulin resistance
PWV	Pulse wave velocity
VAT	Visceral adipose tissue

From the [1]Department of Pediatrics, University of Minnesota Medical School; [2]Division of Epidemiology and Community Health, University of Minnesota School of Public Health; [3]School of Kinesiology, University of Minnesota; and [4]Department of Medicine, University of Minnesota Medical School, Minneapolis, MN

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the complex relations between obesity and vascular function and structure in children.

The purpose of this study was to examine the relationship of FMD, cIMT, and arterial stiffness with multiple measures of adiposity and insulin resistance measured by hyperinsulinemic euglycemic clamp in a cohort of healthy children.

Methods

The study protocol was approved by the University of Minnesota Institutional Review Board, and consent/assent was obtained from parents/participants. Data for this study were collected from participants recruited from the Minneapolis-St. Paul Metro area, for 2 longitudinal studies conducted at the University of Minnesota: (1) a community-based study evaluating cardiometabolic risk in healthy children (2006-2011; n = 141; age 9-18 years); and (2) healthy siblings serving as a control group for a cohort of childhood cancer survivors (2007-2012; n = 111; age 8-20 years). Participants were included if they were Tanner stage 2-5, normotensive, nondiabetic, free from chronic diseases, and were not taking medications known to influence vascular function and/or glucose metabolism. Both studies were conducted in the Clinical Research Center using similar personnel, equipment, and protocols.

All testing was performed in the morning after an overnight fast (including no caffeine consumption) of at least 8 hours. Height and weight were measured using a wall-mounted stadiometer and an electronic scale, respectively. Body mass index (BMI) was calculated as body weight in kilograms divided by the height in meters squared. BMI percentiles were determined using age and sex based Centers for Disease Control definitions.[18] Normal weight was defined as >5th to <85th percentile, overweight ≥85th to <95th percentile, and obesity ≥95th percentile. Tanner staging for pubertal maturation was performed by a trained nurse or physician.[19,20] Blood pressure was measured twice on the right arm using a digital blood pressure monitor after participants were sitting in a quiet room for at least 5 minutes, and the average of the 2 values was reported for systolic blood pressure and diastolic blood pressure. A fasting lipid profile (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides), and fasting insulin were measured using standard procedures at the Fairview Diagnostic Laboratories at the Fairview-University Medical Center (Minneapolis, Minnesota), a Centers for Disease Control and Prevention-certified laboratory.

Insulin resistance was measured by hyperinsulinemic euglycemic clamp, as previously described.[21] Insulin was infused at a constant rate of 1 μU/kg/min for 3 hours, and glucose was infused at a variable rate to maintain euglycemia (100 mg/dL). Insulin resistance (M) was expressed as the amount of glucose required to maintain euglycemia in the last 40 minutes of the clamp (mg/kg/min of glucose) with adjustment for lean body mass (M_{lbm}). Lower M_{lbm} represents greater insulin resistance.

Body fat percentage (BF%) was measured using dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy, GE Medical Systems, Waukesha, Wisconsin) and analyzed using its enCore software (platform ver. 13.6; GE Healthcare, Madison, Wisconsin). Estimates of abdominal VAT were obtained by computed tomography using a Siemens Sensation 16 (Siemens Medical Solutions, Malvern, Pennsylvania) with 2 separate 10 mm slices obtained at the L4-L5 interspace. The 2 images were subdivided into 5 mm slices and the first and third 5 mm slices were combined and analyzed for VAT. The upper limit of adipose tissue density was −30 Hounsfield units and the lower limit was −190 Hounsfield units. Image slices were individually analyzed by 1 trained technician using a computer program (Fat Scan ver. 3.0; N2 System, Osaka, Japan).

Endothelial function was evaluated using brachial artery FMD measured with standard ultrasound using a 8-15 MHz linear array transducer held at a constant pressure on the skin and at a fixed point over the imaged artery by a stereotactic arm to obtain B-mode images (Siemens, Sequoia 512; New York, New York) following current guidelines.[22] An electronic wall-tracking software program (Medical Imaging Applications, Coralville, Iowa) was used for the measurement of brachial artery diameter and blood flow. Following baseline measurements, a blood pressure cuff was placed on the forearm (distal to the imaged area) and inflated to a suprasystolic level (>200 mmHg) for 5 minutes. After 5 minutes, cuff occlusion was released and B-mode ultrasound images were captured for approximately 3 minutes after release. The maximum diameter recorded following reactive hyperemia was reported relative to baseline vessel diameter (FMD = peak diameter − baseline diameter/baseline diameter). An area under the curve (AUC) for FMD was calculated using the trapezoidal method. All measurements were conducted by the same group of sonographers, under the supervision of the same laboratory director. Our laboratory has previously documented satisfactory FMD reproducibility with the same individual tested 1 week apart having a mean difference of 0.53% ± 0.28%.[23]

Vascular structure was evaluated using cIMT. Images for determining cIMT were obtained at end-diastole (gated by R wave on electrocardiography) using B-mode images of the far wall of the left common carotid artery. Measurements were obtained at the distal 10 mm of the common carotid artery as recommended by pediatric guidelines.[24] An electronic wall-tracking software program was used for the analysis of cIMT. Our laboratory has previously documented satisfactory reproducibility for measurement of cIMT, with the mean difference for repeated measurements on separate days in the same subjects of 0.02 ± 0.03 mm.[25]

Carotid arteries also were imaged to capture the left common carotid artery diastolic and systolic lumen diameters to determine carotid incremental elastic modulus (cIEM), mmHg, a measure of carotid artery stiffness. Systolic and diastolic blood pressures were recorded with an automated blood pressure sphygmomanometer (Colin Medical Instruments Corp, San Antonio, Texas) during the 10-s