Early Biomarkers of Subclinical Atherosclerosis in Obese Adolescent Girls with Polycystic Ovary Syndrome

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Objectives Because in obese youth, pulse wave velocity (PWV), an early cardiovascular disease marker, is elevated, we tested if obese girls with polycystic ovary syndrome (OB-PCOS) have higher PWV and carotid intima-media thickness (cIMT) compared with obese girls without PCOS (OB-non-PCOS) and normal-weight girls without PCOS (NW-non-PCOS) and whether PWV and cIMT correlate with inflammatory and circulating endothelial function biomarkers.

Study design Cross-sectional study of PWV and cIMT in 91 OB-PCOS, 30 obese controls (OB-non-PCOS), and 19 normal-weight controls (NW-non-PCOS). Body composition, blood pressure, fasting glucose, insulin, lipid concentrations, and endothelial function biomarkers were measured. OB-non-PCOS and OB-PCOS underwent 2-hour oral glucose tolerance testing.

Results PWV was higher in OB-PCOS (664 ± 24 cm/s) and OB-non-PCOS (624 ± 37 cm/s) compared with NW-non-PCOS (468 ± 13 cm/s, $P < .001$), with no differences in cIMT. Systolic blood pressure, low-density lipoprotein, and non-high-density lipoprotein cholesterol were higher, and high-density lipoprotein cholesterol and indices of insulin sensitivity were lower in OB-PCOS and OB-non-PCOS compared with NW-non-PCOS. Vascular cell adhesion molecule-1 and high-sensitivity C-reactive protein were higher in OB-PCOS compared with NW-non-PCOS. PWV correlated with adiposity ($r_s = .46$), insulin sensitivity index (homeostatic model assessment-insulin resistance $r_s = .31$), systolic blood pressure ($r_s = .24$; $P \leq .003$ for all), and free testosterone ($r_s = .24$; $P = .03$). In multiple regression analysis with PWV as the dependent variable and age, race, body mass index, PCOS, and dysglycemia as independent variables, only body mass index was an independent contributor to the model ($r^2 = 0.068$, $P = .003$).

Conclusions In adolescent girls, obesity and not PCOS appears to be associated with heightened cardiovascular disease risk. Increased PWV, vascular cell adhesion molecule-1, and high-sensitivity C-reactive protein may be the earliest subclinical atherosclerosis biomarkers in OB-PCOS. *(J Pediatr 2016;168:104-11)*.

Polycystic ovary syndrome (PCOS) is believed to be the most common endocrine disorder with a prevalence of approximately 5%-10% among US women.[1] PCOS is frequently associated with obesity, insulin resistance (IR), diabetes, hypertension, and dyslipidemia, conditions conducive to increased cardiovascular disease (CVD) risk.[1] Indeed the prevalence of the metabolic syndrome is increased in both adult women and adolescents with PCOS.[2] Girls with PCOS are not only more overweight but are 4.5 times more likely to have metabolic syndrome than age-matched girls of the Third National Health and Nutrition Examination Survey after adjusting for body mass index (BMI).[2,3] Moreover, impaired glucose tolerance and type 2 diabetes mellitus are more prevalent in both adult women and adolescents with PCOS.[4,5]

In women with PCOS, evidence of subclinical CVD was shown by increased pulse wave velocity (PWV) and carotid intima-media thickness (cIMT).[6] However, controversy continues whether CVD is increased in PCOS. Epidemiologic

BMI	Body mass index	IR	Insulin resistance
cIMT	Carotid intima-media thickness	LDL	Low-density lipoprotein
		NW-non-PCOS	Normal-weight girls without PCOS
CRP	C-reactive protein		
CV	Cardiovascular	OB-non-PCOS	Obese girls without PCOS
CVD	Cardiovascular disease	OB-PCOS	Obese girls with PCOS
HbA1c	Hemoglobin A1c	OCP	Oral contraceptive pills
HDL	High-density lipoprotein	PCOS	Polycystic ovary syndrome
HOMA	Homeostatic model assessment	PWV	Pulse wave velocity
		SBP	Systolic blood pressure
hsCRP	High-sensitivity C-reactive protein	TG	Triglyceride
		VCAM-1	Vascular cell adhesion molecule-1
ICAM-1	Intercellular adhesion molecule-1		

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data demonstrate greater cardiovascular (CV) events and lower survival,[7] whereas other studies show no increased prevalence of CVD in women with PCOS compared with women without PCOS.[8,9]

Data are limited with respect to subclinical CVD using imaging or circulating endothelial function biomarkers in adolescent girls with PCOS. Based on our previous observations of increased PWV in obese adolescent boys and girls compared with their normal weight peers,[10] we hypothesized that adolescent obese girls with PCOS (OB-PCOS) will have evidence of subclinical CVD and increased inflammatory and circulating endothelial function biomarkers. Therefore, this study was undertaken to examine PWV and cIMT in adolescent OB-PCOS, in comparison with obese control peers (obese girls without PCOS [OB-non-PCOS]) and normal weight controls (normal-weight girls without PCOS [NW-non-PCOS]), and to assess the relationships between PWV, cIMT, insulin sensitivity, and traditional and nontraditional CVD markers.

Methods

Overweight/obese adolescent girls (n = 91) with a diagnosis of PCOS[11,12] were recruited from the PCOS Center at Children's Hospital of Pittsburgh, 30 otherwise healthy OB-non-PCOS were recruited from the Weight Management and Wellness Center at Children's Hospital of Pittsburgh, and 19 healthy NW-non-PCOS (BMI <85th percentile) were recruited through newspaper and hospital advertisements. OB-non-PCOS and NW-non-PCOS had regular menses and no clinical evidence of hyperandrogenism. The diagnosis of PCOS was made based on the presence of clinical signs and symptoms of hyperandrogenism and/or biochemical hyperandrogenemia, consistent with the Endocrine Society Clinical Practice Guidelines and our publications.[11-14] Many girls with PCOS were recruited shortly after their diagnosis in our PCOS Center and before pharmacologic therapy was initiated. Inclusion criteria were age 10-20 years, postmenarche, and Tanner stage III-V. Exclusion criteria were: (1) pregnancy; (2) preexisting diabetes; (3) use of medications that impact carbohydrate or lipid metabolism (oral contraceptive pills [OCP], metformin, anti-epileptics, antipsychotics, statins, fish oil); and (4) smoking history. The investigation was approved by the Institutional Review Board and performed in the Pediatric Clinical and Translational Research Center of Children's Hospital of Pittsburgh and The Department of Epidemiology Ultrasound Research Laboratory at the University of Pittsburgh. Parental informed consent and child assent were obtained from all participants before participation in accordance with the ethical guidelines of Children's Hospital of Pittsburgh.

Each participant underwent a physical examination, as well as height, weight, waist and hip circumference measurements. Fasting blood was obtained for lipid profile, glucose, insulin and hemoglobin A1c (HbA1c), adipokines (leptin, adiponectin), inflammatory marker (high-sensitivity C-reactive protein [hsCRP]) and circulating soluble cell adhesion molecule biomarkers (intercellular adhesion molecule-1 [ICAM-1], vascular cell adhesion molecule-1 [VCAM-1], and E-selectin). Dual-energy X-ray absorptiometry was performed in all participants to assess body composition. Obese participants with and without PCOS had a 2-hour oral glucose tolerance test to assess glucose tolerance, and a free testosterone panel to assess for hyperandrogenemia. Dysglycemia was defined as a fasting plasma glucose ≥100 mg/dL, 2-hour glucose ≥140 mg/dL, or both. Age-specific BMI z-scores were calculated using Epi Info (v 3.3.2; Centers for Disease Control and Prevention, Atlanta, Georgia).

cIMT and aortic PWV were measured at the Ultrasound Research Laboratory of the Department of Epidemiology at the University of Pittsburgh, a key laboratory in multiple adult and pediatric trials.[10,15-17] Using a Toshiba SSA-270A scanner (Toshiba American Medical Systems, Tustin, California) equipped with a linear 5 MHz transducer, the right and left carotid artery was interrogated. Although this transducer is a lower frequency than recommended in published standards,[18] lower frequency probes are used in obese populations where the vessel may be deeper. Detailed B-mode images of the near and far walls of the distal common carotid artery (1 cm proximal to the carotid bulb), far wall of the bulb, and first centimeter of the far wall of the internal carotid artery were obtained in end-diastole from each side for a total of 8 images. These images were digitized for later reading using a semi-automated edge detection software.[19] The scanning and reading protocols that were used are well established.[10,17] Intima-media thickness measures were obtained by electronically tracing the lumen-intima interface and the media-adventitia interface across a 1-cm segment; 1 measurement was generated for each pixel over the area, for a total of approximately 140 measures for each segment. For analyses, the mean and maximum values of the average readings at all 8 locations were used. The Ultrasound Research Laboratory requires certification of sonographers and readers, and monitors quality control with several ongoing quality control systems. Monthly repeat scans were performed by 2 separate sonographers on the same day, and several of the scans were reviewed to monitor the intersonographer (scanning) reproducibility. Quality control was also performed between readers on a regular basis. Quarterly, scans were read by 2 separate readers to monitor interreader reproducibility. Reproducibility of cIMT measures was excellent with an intraclass correlation coefficient between sonographers of 0.82-0.97 and within reader of 0.87-0.99 across the study period.

To measure aortic PWV, 2 unidirectional transcutaneous Doppler flow probes (model 810-a, 10 MHz; Parks Medical Electronics, Aloha, Oregon) were used; one to detect the pulse wave as it reaches the right carotid artery and one to detect the pulse wave as it reaches the right femoral artery. The time required for the pulse wave to travel from 1 probe to the other, combined with the distance between the 2 probes, allowed for the calculation of central PWV and was performed several times until waveforms were clear. Heart