Impact of Pubertal Development on Endothelial Function and Arterial Elasticity

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Objectives Little is known about the relation of pubertal development on endothelial function and arterial elasticity in children and adolescents; therefore, we compared brachial artery flow-mediated dilation and carotid artery elasticity across Tanner (pubertal) stages in children and adolescents.

Study design Blood pressure, fasting lipids, glucose and insulin, body fat, insulin sensitivity adjusted for lean body mass, brachial flow-mediated dilation (percent dilation and area under the curve), endothelium-independent dilation (peak dilation and area under the curve), and carotid artery elasticity were evaluated across pubertal stages (Tanner I vs Tanner II-IV vs Tanner V) in 344 children and adolescents (184 males, 160 females; ages 6 to 21 years).

Results One hundred twenty-four subjects (mean age 8.23 ± 0.15 years; 52 females) were Tanner stage I; 105 subjects (mean age 13.19 ± 0.17 years; 47 females) were Tanner stages II-IV; and 115 subjects (mean age 17.19 ± 0.16 years; 61 females) were Tanner stage V. There were no significant differences for any of the measures of vascular structure and function across pubertal stages.

Conclusion Results of the current study indicate that smooth-muscle and endothelial function, as well as carotid artery elasticity, do not differ throughout pubertal development and that accounting for pubertal stage when reporting vascular data in children and adolescents may be unnecessary. *(J Pediatr 2013;163:1432-6)*.

Throughout puberty, transient changes in cardiometabolic risk factors occur as a normal part of development. The relationship between puberty and cardiometabolic factors, such as body fatness, body mass index (BMI), lipids, and insulin resistance have been well documented.[1-13] Specifically, increased insulin resistance is often associated with pubertal onset, with levels returning to near prepubertal status following maturation.[9] Although insulin resistance is associated with adiposity throughout childhood and adolescence,[9,11] differences in body fatness do not entirely explain the development of insulin resistance during puberty.[7,9,11] It is possible that insulin resistance influences vascular health during pubertal development in children and adolescents.

A number of studies among children and adolescents have reported that endothelial function, as measured by flow-mediated dilation (FMD), is associated with obesity,[14] dyslipidemia,[15] blood pressure,[16] insulin resistance,[14,17] and oxidative stress.[15] Studies also have measured arterial elasticity among children and adolescents.[18,19] Among adults, BMI and elements of metabolic syndrome have been reported to have an inverse relationship with large and small artery compliance.[20,21] Although it has been well-documented that cardiovascular and metabolic risk factors differ by pubertal stage, to our knowledge, no studies have examined whether measures of vascular function and stiffness differ across pubertal stages among children and adolescents. Therefore, the purpose of this study was to evaluate the influence of Tanner stage on endothelial function and arterial elasticity among children and adolescents. We hypothesized that vascular variables would differ by pubertal stage in accordance with associated changes in cardiometabolic risk factors. Specifically, we hypothesized that FMD and arterial elasticity would be lowest among Tanner stages II-IV compared with Tanner stages I and V.

Methods

The study protocol was approved by the University of Minnesota Institutional Review Board (IRB). The study procedures adhered to the University of Minne-

AUC	Area under the curve
BMI	Body mass index
DBP	Diastolic blood pressure
EID	Endothelium-independent dilation
FMD	Flow-mediated dilation
IRB	Institutional Review Board
NTG	Nitroglycerin
SBP	Systolic blood pressure

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sota's IRB and the Health Insurance Portability and Accountability Act guidelines. All parents and subjects provided informed consent and assent, respectively, for study participation.

Three hundred forty-four subjects (184 males, 160 females), who had participated in a study evaluating cardiovascular risk among families, were included in this cross-sectional study. Enrolled children and adolescents were between the ages of 6 and 21 years (mean age 12.7 ± 4.1 years). Subjects had been fasting for at least 8 hours prior to the vascular assessment and were asked to abstain from caffeine ingestion on the morning of testing and to avoid strenuous exercise or physical activity for 24 hours prior to the study visit. Subjects who were older than 18 years of age were excluded from BMI-percentile, systolic blood pressure (SBP)-percentile, and diastolic blood pressure (DBP)-percentile calculations.

Measurements for height and weight were obtained with a standard stadiometer (model S100; Ayrton, Prior Lake, Minnesota) and electronic scale (serial no. 5002-8893; ST Scale-Tronix, White Plains, New York), respectively. BMI was calculated as weight in kilograms divided by height in meters-squared. Body composition was obtained using dual energy X-ray absorptiometry (Lunar Prodigy, Software v. 10.5; GE Healthcare Lunar, Madison, Wisconsin). Blood pressure percentile data was classified based on The National Heart, Lung, and Blood Institute guidelines.

Seated blood pressure was measured by a random-zero sphygmomanometer in the right arm, and the average of two SBP measurements and fifth phase Korotkoff DBP measurements were analyzed. Tanner staging of pubertal development was performed by trained providers and was based on breast and pubic hair development in girls and pubic hair development in boys. Participants were classified as prepubertal (Tanner I), pubertal (Tanner II-IV), and postpubertal (Tanner V).

Insulin sensitivity, adjusted for lean body mass, was determined by euglycemic hyperinsulinemic clamp as previously described.[11] Fasting blood samples were obtained for serum insulin and lipids as well as plasma glucose. Insulin levels were determined using a chemoluminescence immunoassay (Immulite Insulin DPC, Los Angeles, California). Samples for serum lipids were analyzed with standard procedures at the Fairview-University Medical Center clinical laboratory.

Testing was performed in the Vascular Biology Laboratory in the University of Minnesota Clinical and Translational Science Institute. All the vascular studies were performed in a quiet, temperature-controlled environment (22-23°C). Vascular images were measured by a noninvasive ultrasound with subjects in the supine position. Images were digitized and stored on a personal computer for later off-line analysis with an electronic wall-tracking software program (Vascular Research Tools 5; Medical Imaging Application, LLC, Iowa City, Iowa).

Following 15 minutes of quiet rest in the supine position, vascular images of the brachial artery were obtained using a conventional ultrasound scanner (Acuson, Sequoia 512; Siemens Medical Solutions USA, Inc, Mountain View, California) with a 7.5 MHz linear array probe held at a constant distance from the skin and at a fixed point over the imaged artery. Assessment of FMD was performed by imaging the left brachial artery at the distal third of the upper arm using techniques previously described.[22,23] After a 15-minute break following FMD assessment, endothelium-independent dilation (EID) was assessed using 0.3 mg sublingual nitroglycerin (NTG), the dose considered appropriate by the University of Minnesota IRB for subjects <18 years old, and 0.4 mg sublingual NTG for subjects >18 years old. Brachial artery diameter was assessed continuously for 5 minutes post-NTG administration. Peak dilation during the study was defined as the greatest percent change from resting brachial artery diameter, and area under the curve (AUC) was defined as the total relaxation of the brachial artery from resting baseline following reactive hyperemia or sublingual NTG administration.

Carotid artery images, as well as supine SBP and DBP and pulse pressure, were concurrently measured by a noninvasive ultrasound with subjects in the supine position. Following 15 minutes of quiet rest in the supine position, luminal systolic and diastolic diameters were obtained at a fixed point over the left common carotid artery, approximately 1 cm proximal from the carotid bulb. Images were collected at 20 frames per second for 10 seconds (200 frames) to ensure the capture of full arterial diameter change during a cardiac cycle. SBP and DBP were recorded with an automated blood pressure sphygmomanometer during the 10-second carotid measurement. The mean diameter through the 10-second cycle was used to calculate measures of compliance and distensibility. The ultrasound scanning system was interfaced with a standard personal computer equipped with a data acquisition card for attainment of radio frequency ultrasound signals from the scanner. Digital image analysis was performed by the same trained reader blinded to group assignments.

Pulse pressure (ΔP) was calculated as the difference between SBP and DBP. Additionally, maxDiamM denotes maximum diameter measurement, and minDiamM denotes minimum diameter measurement.

Statistical Analyses

SAS Software Package (v. 9.2, 2009; SAS Inc, Cary, North Carolina) was used for statistical analyses. Our study data were segmented into Tanner stages I, II-IV, and V groupings because of the equal-size homogenous clusters within each stage of our study population. Results are expressed as mean ± SD, unless otherwise stated. A one-way ANOVA was used to compare demographic characteristics by pubertal stage groups, as well as to compare vascular measures between-groups of pubertal stage groups, adjusting for age, sex, race, and baseline brachial artery diameter. An alpha value of 0.05 was used to signify statistical significance.

Results

Tanner stage I included 124 children (mean age 8.23 ± 0.15 years; 52 females); Tanner stages II-IV included 105 children