Atherosclerosis 234 (2014) 410–414

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Younger age is associated with lower reactive hyperemic index but not lower flow-mediated dilation among children and adolescents

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ARTICLE INFO

Article history:
Received 7 October 2013
Received in revised form
24 March 2014
Accepted 25 March 2014
Available online 1 April 2014

Keywords:
Endothelial function
Reactive hyperemic index
Flow-mediated dilation
Children
Adolescents

ABSTRACT

Background: The use of digital reactive hyperemia as a measure of endothelial function among children and adolescents is becoming increasingly common. However, unexpected observations of low reactive hyperemic index values in younger children in our laboratory led us to conduct a study evaluating the influence of age, sex, height, weight, blood pressure, body mass index (BMI), and finger volume on RHI values.

Methods: Endothelial function, measured by digital reactive hyperemia (reactive hyperemic index: RHI) was assessed in 113 children and adolescents (mean age 12.4 $\pm$ 3.8 years; 64 males), with 102 also assessed for brachial artery flow-mediated dilation (FMD) using ultrasound imaging. Associations with age, sex, height, weight, systolic and diastolic blood pressure (SBP, DBP), BMI, and finger volume were evaluated.

Results: Using GLM regression, age ($\beta = 0.03$, $P = 0.014$) and SBP ($\beta = 0.015$, $P = 0.004$) were significantly associated with RHI. No measures were associated with FMD. In the subset of individuals with measured finger volume, age ($\beta = 0.025$, $P = 0.037$) was the only measure significantly associated with log RHI. Similarly, no measures were associated with FMD.

Conclusion: Younger age is associated with lower RHI but not lower FMD among children and adolescents. These findings call into question the validity and usefulness of digital reactive hyperemia as a method to quantify endothelial function among younger children.

1. Background

The pathological process of cardiovascular disease begins in the first two decades of life [1–4]. Therefore, identifying the initial signs of potential problems among children and adolescents may improve screening, risk stratification, and lead to earlier intervention. Endothelial activation is one of the seminal events of the atherosclerotic process, and various measures of endothelial dysfunction have been shown to predict subsequent atherosclerosis [5, 6] and future cardiovascular events [6–9]. Because of its non-invasive nature, the most commonly-employed technique used to measure endothelial function in children is ultrasound imaging of the brachial artery during flow-mediated dilation (FMD) [10]. However, new methods have emerged including the measurement of digital reactive hyperemia, which quantifies small changes in finger volume at rest and during reactive hyperemia. Endothelial function, as assessed by this technique, is nitric oxide-dependent [11] and has been demonstrated to be positively associated with coronary artery blood flow [12] and negatively with multiple cardiovascular risk factors [13]. Furthermore, lower values have been shown to independently predict future cardiovascular events in adults [14]. Recently, a number of pediatric studies have utilized this technique using the EndoPAT device (EndoPAT 2000, Itamar Medical, Caesarea, Israel) [15–19].

As our research group began using the EndoPAT device in some of our pediatric studies, we observed that younger participants often had very low reactive hyperemic index (RHI) values. Subsequently, two separate research groups reported interesting associations between pubertal development and RHI [15, 20] and between age, sex, and RHI [16] in children and adolescents. In an attempt to investigate these relationships further and compare them to another endothelial function technique, we conducted a

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http://dx.doi.org/10.1016/j.atherosclerosis.2014.03.031
0021-9150/

study in which we simultaneously measured RHI along with FMD to evaluate their respective associations with age, sex, height, weight, blood pressure, body mass index (BMI), and finger volume. We hypothesized that age and body size measures (including finger volume) would be significantly associated with RHI but not FMD, owing to the fact that the finger probes used with the digital reactive hyperemia technique were designed for adults (i.e., the "one size fits all" nature of the finger probe may not be valid for use in children). We believed this phenomenon might explain the low RHI values often observed among younger children.

2. Materials and methods

The study protocol was approved by the University of Minnesota Institutional Review Board (IRB). The study procedures adhered to the University of Minnesota's IRB and the Health Insurance Portability and Accountability Act (HIPAA) guidelines. All parents and participants provided written informed consent and assent, respectively.

2.1. Study design and participants

This cross-sectional study included 113 children and adolescents (64 males, 49 females) between 6 and 19 years of age (mean age 12.4 $\pm$ 3.8 years) who either participated in a longitudinal community-based study investigating the early development of obesity, insulin resistance, and other cardiovascular risk factors among families, or served as healthy controls in a study assessing the vascular effects of stimulant medication use in attention deficit hyperactivity disorder. Children and adolescents from the longitudinal cohort were healthy community-dwelling youth and were included if they were offspring of parents who had participated in the initial study (many years prior). No exclusion criteria were used. Brachial artery FMD measures were obtained on all but 11 of the subjects. Inability to obtain FMD measures was the result of too much movement by the participant leading to poor image quality. Finger volume was obtained on 48 of the subjects (finger volume measurements were initiated approximately halfway through the study).

2.2. Assessment of anthropometrics, pubertal development, and other clinical variables

Height and weight were obtained using a standard stadiometer and electronic scale, respectively. BMI was calculated as weight in kilograms divided by height in meters-squared, and BMI percentile was determined based on population data from the United States. Left finger volume was measured by water displacement and reported to the nearest 0.25 mL. Tanner stage was determined by trained pediatricians. A single blood pressure measurement was obtained on the right arm with an appropriate cuff size using an automatic sphygmomanometer after 5 min of quiet rest in the supine position. Fasting ($\geq$8 h) blood samples were analyzed for glucose, insulin, and lipids using standard procedures.

2.3. Measurement of endothelial function

The vascular testing was performed in the morning after the participants had been fasting for at least 8 h. Following 15 min of quiet rest in the supine position, RHI (EndoPAT 2000, Itamar Medical, Caesarea, Israel) and brachial artery FMD using a conventional ultrasound scanner (Siemens, Sequoia 512, New York, NY, USA) were assessed simultaneously. A 15-8 MHz linear array probe held at a constant distance from the skin with a stereotactic arm and at a fixed point over the imaged brachial artery was utilized during FMD assessment. Following baseline measurements in the index fingers and brachial artery, a blood pressure cuff was placed on the upper forearm (immediately distal to the elbow) and inflated to a supra-systolic level for 5 min using techniques previously described [21, 22]. RHI was calculated with an automated algorithm using the EndoPAT device. The algorithm derives RHI values by calculating the ratio of pulse amplitude for 60 s beginning 1 min after cuff release (an average of the 60- to 90-s and 90- to 120-s intervals) to the baseline pulse amplitude divided by the corresponding ratio in the control finger. Brachial artery FMD images were digitized and stored on a personal computer for later off-line analysis with an electronic wall-tracking software program (Medical Imaging Applications, Coralville, IA, USA). FMD was calculated as the maximal percent change diameter in relation to the baseline diameter, regardless of when the maximal diameter change occurred after cuff release (i.e., no set time point post-cuff release was used). Reproducibility of FMD [23] and RHI [24] has been previously reported.

2.4. Statistical analysis

Statistical analyses were performed with Stata/SE 12.0 (StataCorp, College Station, TX, USA). Kolmogorov–Smirnov tests were used to test measures for normal distribution. Independent measurements included age, sex, height, weight, systolic blood pressure (SBP), diastolic blood pressure (DBP), BMI, and BMI percentile. Dependent measures included RHI and FMD; RHI was distributed non-normally and FMD was distributed normally. Demographic results are expressed as mean $\pm$ standard deviation (SD) for normally distributed data, and median $\pm$ interquartile range (IQR) for non-normal data. An alpha value of 0.05 was used to signify statistical significance.

Due to RHI non-normality within the main analysis, a generalized linear model (GLM) with a statistically determined 'family-link' was applied. A Modified Park Test was used to identify the appropriate 'family' as 'inverse-Gaussian (i.e., Wald),' and a Pregibon Link Test was used to identify the 'identity' link for the model. Moreover, an inverse-Gaussian GLM with an identity link was used to evaluate possible associations between RHI and the other measures. Conversely, given the normal distribution of FMD within the main analysis, an ordinary least squares (OLS) regression was used to evaluate possible associations between FMD and these measures as well.

Within a smaller subset of individuals with available finger volume data, RHI data were logarithmically transformed to generate a close-to-normal distribution. FMD data were normally distributed. Therefore, an OLS regression was used to evaluate possible associations between both RHI and FMD with the other measures. A cubic transformation was applied to DBP within the smaller subset due to its non-normal distribution.

Within all analyses, height, weight, BMI percentile, and right finger volume were not included within the regression analysis due to strong correlations ($\rho > 0.70$, multi-collinearity) concerns between age–height and weight, height–weight, BMI–weight, BMI–BMI percentile, and right–left finger volume. Additionally, Spearman's rank-order correlation analyses were used to assess potential associations between RHI and FMD with body size measures (including finger volume).

Age groups 6–9 years, 10–14 years, and 15–19 years were further assessed for differences in RHI and FMD. A Kruskal–Wallis one-way analysis of variance (ANOVA) and Mann–Whitney rank sum tests were used to assess potential age group differences in RHI, and a one-way ANOVA with a Bonferroni correction was performed to assess differences in FMD and peak shear stress between age groups. A separate analysis of possible associations between