Puberty and Microvascular Function in Healthy Children and Adolescents

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Objective To determine the role of pubertal status on microvascular function in healthy children and adolescents.

Study design Children and adolescents (n = 112; age 10-16 years) were investigated in 2 separate prospective cross-sectional studies. The main outcome measure was microvascular function, assessed by peripheral arterial tonometry to determine the reactive hyperemic index (RHI). Physical activity was assessed using 7-day recall in one study and accelerometry in the other study. Subjects were grouped based on their self-assessed pubertal status according to Tanner stage: group 1 (prepuberty, Tanner I), group 2 (mid-puberty, Tanner II/III), and group 3 (late puberty, Tanner IV/V). Stepwise multiple regression analysis was performed to identify independent predictors of the RHI.

Results Complete data were available for 94 subjects (55 females) with a median (IQR) age of 14 (3.0) years and a mean body mass index of 19.0 ± 3.63 kg·m⁻². Significant correlations with RHI were observed for Tanner stage (r = 0.569; P < .001), age (r = 0.567; P < .001), stature (r = 0.553; P < .001), systolic blood pressure (r = 0.494; P < .001), and body mass index (r = 0.309; P = .001), but not for sex and moderate-to-vigorous physical activity. In stepwise regression analysis, pubertal status was the only independent predictor of microvascular function (R² = 0.242; β = 0.492; P < .001). Prepubertal children (group 1) had a significantly lower RHI [1.14 (0.24)] compared with group 2 [1.65 (0.57)] and group 3 [1.70 (0.75)] (all P < .001).

Conclusion Pubertal status was the main predictor of microvascular function in healthy children and adolescents. Future studies investigating microvascular function in this age group should assess and control for pubertal maturation. (J Pediatr 2012;■:■ - ■).

Endothelial function testing has received growing interest for cardiovascular risk assessment in the pediatric population.1-3 Endothelial dysfunction, a precursor of atherosclerosis, begins early in life and can be studied by various techniques. Brachial artery flow-mediated dilation (FMD) is considered the gold standard for noninvasive assessment of endothelial function. Interest is growing in measuring endothelial microvascular function by peripheral arterial tonometry (PAT). Significant correlations between the 2 techniques have been reported in healthy adults4 and those with chest pain.5 Measurement of the microcirculation by PAT is reliable, operator-independent, and easily applicable in the pediatric age group.6 Excellent reproducibility and feasibility have been reported in healthy adolescents.6 PAT measures changes in digital pulse volumes at rest and after induction of reactive hyperemia. As a measure of reactive hyperemia, a computerized algorithm calculates the reactive hyperemic index (RHI), which is related to multiple cardiovascular disease risk factors in adults.7,8 Alterations in microvascular function have been demonstrated in even young persons with cardiovascular risk factors, such as type 1 diabetes and obesity.1,2,9

Previous studies have reported increasing RHI with age during childhood and adolescence,2,3,10,11 indicating that the development of microvascular function is not complete until late adolescence. Importantly, most of those studies did not control for pubertal status in their analysis. Bhangoo et al11 studied the effect of maturation on microvascular function using PAT and found a higher vasodilatory response with pubertal advancement, most likely due to an increase in steroid hormones. In that study, pubertal staging was assessed by ultrasensitive estrogen assays in venous blood. However, invasive techniques to assess pubertal maturation in otherwise healthy children can raise ethical concerns and are impracticable in research studies. Self-assessment of sexual maturation by children and adolescents has been shown to be a feasible technique with good to excellent agreement between the child and physical examiner.12,13 We thus aimed to determine the role of self-assessed pubertal status on microvascular function by PAT in healthy children and adolescents without cardiovascular disease risk factors.

BMI Body mass index
BP Blood pressure
FMD Flow-mediated dilation
FMD Flow-mediated dilation
MVPA Moderate-to-vigorous physical activity
PAT Peripheral arterial tonometry
RHI Reactive hyperemic index

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Peripheral microvascular function was examined in healthy children and adolescents in 2 separate prospective cross-sectional studies conducted at 2 schools in Switzerland. Both schools were located in the same county and were chosen to cover a large age range (10-16 years) of healthy Caucasian school children. We pooled the data from the 2 schools (school 1: n = 60, 34 females, aged 10-14 years and school 2: n = 52, 28 females, aged 13-16 years) to investigate the effect of pubertal maturation on microvascular function during childhood and adolescence. We excluded subjects with juvenile rheumatoid arthritis or vascular diseases (Reynaud or Kawasaki disease), hypertension, obesity, diabetes, or depression, and subjects who smoked or were taking any vasoactive medication.

In both schools, weight was measured with the subjects in light clothing, to the nearest 0.1 kg using a digital-balanced scale, and stature was measured to the nearest 0.5 cm using a wall-mounted stadiometer. Body mass index (BMI) was calculated as weight divided by stature squared (kg·m⁻²). Blood pressure (BP) was measured with an oscillometric device (Critikon Dinamap Vital Signs Monitor; GE Healthcare, Waukesha, Wisconsin), and resting heart rate was automatically calculated by the PAT device during the 15-minute testing period. The z-scores were derived from age- and sex-specific normal values for BMI and BP. Pubertal stage (Tanner I-V) was assessed using a validated self-assessment tool. Subjects were than categorized into 3 groups based on self-assessed Tanner stage: group 1 (puberty, Tanner stage I), group 2 (early puberty, Tanner stage II/III), and group 3 (late puberty, Tanner stage IV/V). Written informed consent was obtained from parents/caregivers in children aged ≤13 years or from subjects and parents/caregivers together in children aged ≥13 years. Ethical approval was obtained from the Cantonal Ethical Committee of Bern, Switzerland.

Peripheral microvascular function was measured by non-invasive PAT (EndoPAT2000; Itamar Medical, Caesaerea, Israel) with the subjects in a fasted state on a normal school day between 8 a.m. and 12 p.m. All subjects were tested while supine with the hands at heart level and fingers hanging freely. The standardized experimental protocol included baseline, occlusion, and postocclusion periods, each lasting 5 minutes. Pneumatic fingertip probes were placed on both index fingers to continuously record pulse wave amplitudes throughout the protocol. The measurement started once a stable pulse wave amplitude signal was obtained. The occlusion period was induced by cuff inflation to suprasystolic pressures (220 mm Hg) on the nondominant arm. After 5 minutes, the cuff was deflated rapidly to allow for reactive hyperemia. The RHI was automatically calculated by a computerized algorithm of the software program. An RHI cutoff of 1.35 has been used in adults to identify subjects with coronary endothelial dysfunction.

Physical activity was assessed with a 7-day recall in school 1. The questionnaire addressed activities of daily living and those that induce sweating and rises in heart rate, comparable with activities of moderate-to-vigorous intensity, and was tested in a large cohort of Swiss children. In school 2, physical activity was measured using an uniaxial accelerometer (MTI/CSA 7164; ActiGraph, Shalimar, Florida). The accelerometer monitor was attached at the right hip and worn for 8 consecutive days except for water activities and contact sports. The device was programmed to record data (ie, raw activity counts and steps) every 5 seconds; time periods with more than 20 minutes of continuous zero values were omitted. Data from the accelerometers were downloaded and checked for spurious counts; excessively high counts (≥20,000) were removed from the analysis. A minimum of 5 days including 1 weekend day with ≥9 hours of daily wear time were required for inclusion in the data analysis. Data are expressed as the total daily time spent in moderate-to-vigorous physical activity (MVPA) (min·day⁻¹).

Data Analysis
Statistical analyses were performed with SPSS for Windows version 17.0 (IBM, Armonk, New York). Kolmogorov-Smirnov tests were used to check data for normal distribution. The χ² test was used to compare groups on baseline categorical variables. Normally distributed data are presented as mean ± SD, and variables with skewed distribution are shown as median (IQR). The RHI data were logarithmically transformed to achieve a distribution closer to normal. Differences among the 3 Tanner groups were analyzed by ANOVA or the nonparametric Kruskal-Wallis signed-rank test, followed by the Mann-Whitney U test. Associations among variables were investigated using the Spearman correlation coefficient. Univariate models including pubertal status, age, sex, stature, BMI, systolic BP, and MVPA were applied. Variables with significant correlations with RHI were included into a backward stepwise multiple regression analysis. P < .05 was considered to indicate statistical significance.

Results
A total of 112 healthy children and adolescents [62 females; median age, 13.0 (3.0) years] were investigated. Of those, 18 had to be excluded for various reasons (ie, missing Tanner data, insufficient physical activity monitoring data, and technically inadequate EndoPAT tests), resulting in a complete dataset for 94 subjects. Anthropometric and clinical characteristics, summarized in the Table, were comparable in the 2 study schools. BMI and systolic and diastolic BP z-scores were not significantly different between the schools (all P > .25).

Weight, stature, BMI, and systolic and diastolic BP increased and resting heart rate decreased with increasing pubertal status (all P < .05). BMI and BP z-scores did not differ significantly among the 3 pubertal stage groups. Within the 3 groups, there was no difference by sex with regard to the aforementioned variables. Physical activity levels, expressed
as daily time spent in MVPA, decreased significantly with increasing age \((P < .001)\).

Prepubertal children (group 1) had a significantly lower RHI compared with the mid-pubertal (group 2) and late-pubertal (group 3) (Figure 1). Significant correlations were detected between RHI and Tanner stage \((r = 0.569; P < .001)\), age \((r = 0.567; P < .001)\), stature \((r = 0.553; P < .001)\), systolic BP \((r = 0.494; P < .001)\), and BMI \((r = 0.309; P = .001)\). Spearman correlation analysis revealed no significant correlation between RHI and MVPA \((r = -0.196; P = .071)\) or between RHI and sex \((P = .581)\). In a backward stepwise multiple regression analysis, pubertal status was the sole independent predictor of microvascular function, explaining 24% of the variance in the model \((R^2 = 0.242; \beta = 0.492; P < .001)\). In group 1, 81% of subjects had a RHI of <1.35, compared with 20% in group 2 and 19% in group 3. Figure 2 (available at www.jpeds.com) displays 2 representative PAT recordings comparing the reactive hyperemic response in a prepubertal subject and a late-pubertal subject.

### Discussion

The present study has provided 2 clinically relevant findings. First, self-assessed pubertal status proved to be the major determinant of microvascular function in healthy children and adolescents. Second, using cutoff values for endothelial dysfunction established in adults, 81% of the prepubertal children and 19% of the adolescents in our study population would be classified as dysfunctional. Considering the healthy study sample with regard to BMI and BP, a low RHI in children and adolescents is more likely to reflect immature microvascular function rather than dysfunction. However, lacking knowledge of the underlying mechanisms, we suggest using the term “juvenile microvascular response” to describe this condition.

Measurement of vascular function is clinically relevant for identifying children at risk for early atherosclerotic disease, such as those with obesity and type 1 diabetes. Noninvasive PAT is a feasible and reliable method for assessing the microvascular function of the peripheral vascular beds. Excellent reproducibility was reported in 30 healthy adolescents (aged 13-19 years), with a mean RHI difference of 0.12 between 2 tests. With the growing interest in assessing peripheral microvascular function, knowledge of the determinants is crucial to the evaluation of vascular endothelial function during maturation. Previous studies suggested that microvascular function matures with age in healthy children. Chen et al concluded that microvascular maturation develops until late adolescence. However, because chronological age does not necessarily mirror the biological maturation of a young person, our data demonstrate the need to control for pubertal status when microvascular function is measured.

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**Table. Characteristics of the study population**

<table>
<thead>
<tr>
<th></th>
<th>All ((n = 94))</th>
<th>Group 1 ((Tanner I; n = 42))</th>
<th>Group 2 ((Tanner II-III; n = 10))</th>
<th>Group 3 ((Tanner IV-V; n = 42))</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, median (\text{IQR})</td>
<td>14.0 (3.0)</td>
<td>11.0 (1.0)</td>
<td>14.0 (1.0)</td>
<td>14.0 (1.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Female sex, n (%)</td>
<td>52 (55.3)</td>
<td>27 (64.3)</td>
<td>6 (60.0)</td>
<td>22 (52.4)</td>
<td>.539</td>
</tr>
<tr>
<td>Stature, m, median (\text{IQR})</td>
<td>1.63 (0.26)</td>
<td>1.44 (0.09)</td>
<td>1.66 (0.09)</td>
<td>1.70 (0.10)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Weight, kg, median (\text{IQR})</td>
<td>48.75 (23.0)</td>
<td>35.0 (10)</td>
<td>50.6 (15.0)</td>
<td>59.9 (9.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>BMI, kg m(^{-2}), mean (\pm SD)</td>
<td>19.0 ± 3.63</td>
<td>17.6 ± 3.0</td>
<td>18.8 ± 2.8</td>
<td>20.4 ± 2.4</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>BMI z-score, mean (\pm SD)</td>
<td>-0.071 ± 1.17</td>
<td>-0.072 ± 1.41</td>
<td>-0.618 ± 1.22</td>
<td>-0.590 ± 0.85</td>
<td>0.263</td>
</tr>
<tr>
<td>Systolic BP, mm Hg, median (\text{IQR})</td>
<td>110.0 (17.0)</td>
<td>100 (10.0)</td>
<td>116.5 (10.25)</td>
<td>114.3 (18.5)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Systolic z-score, median (\text{IQR})</td>
<td>-0.30 (0.70)</td>
<td>-0.30 (0.70)</td>
<td>0.19 (1.03)</td>
<td>-0.32 (0.88)</td>
<td>.176</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg, median (\text{IQR})</td>
<td>64.5 (10.0)</td>
<td>60 (10.0)</td>
<td>70.3 (9.63)</td>
<td>66.0 (9.13)</td>
<td>.026</td>
</tr>
<tr>
<td>Diastolic z-score, median (\text{IQR})</td>
<td>-0.10 (0.86)</td>
<td>-0.10 (0.70)</td>
<td>0.59 (0.93)</td>
<td>-0.21 (0.74)</td>
<td>.029</td>
</tr>
<tr>
<td>Resting heart rate, bpm, mean (\pm SD)</td>
<td>74.5 ± 15.0</td>
<td>82.5 ± 10.2</td>
<td>72.7 ± 10.7</td>
<td>68.0 ± 10.2</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>MVPA, min day(^{-1}), median (\text{IQR})</td>
<td>66.4 (39.2)</td>
<td>85.5 (49.9)</td>
<td>54.2 (19.63)</td>
<td>62.3 (15.30)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>RHI, median (\text{IQR})</td>
<td>1.44 (0.74)</td>
<td>1.14 (0.24)</td>
<td>1.65 (0.57)</td>
<td>1.70 (0.75)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Data were analyzed with ANOVA, the nonparametric Kruskal-Wallis signed-rank test, or the \(\chi^2\) test. A \(P\) value <.05 indicates statistical significance.
function is being examined during childhood and adolescence. Most previous studies that used PAT as an outcome measure in children and adolescents neither assessed nor controlled for pubertal status in their analysis. Bhangoo et al. studied 89 children and adolescents and found increased microvascular function by PAT with pubertal advancement. In their study, pubertal staging was based on ultrasensitive estrogen assays, and positive correlations between the RHI and steroid hormone levels were evident. We confirmed that observation in a population with a broader age range using self-assessment of sexual maturation. Self-assessed sexual maturation has proven to be a feasible method, reporting good to excellent agreement between child and clinician assessment. It is a cheap, easy, and rapidly applied tool that avoids possibly embarrassing adolescents with a clinical examination.

In contrast to the findings of Bhangoo et al., we found significant positive correlations between microvascular function and stature and between microvascular function and BMI. One important difference in our study was the exclusion of overweight and obese subjects. Mean BMI z-scores did not differ among our 3 groups and were negative in all groups, reflecting the relatively low body weight and healthy status of our subjects. An abnormally high BMI has been inversely correlated with RHI in adults and adolescents, demonstrating the potentially negative impact of obesity on endothelial function. In healthy normal-weight children, the parallel and age-dependent increase in BMI and RHI may represent the normal process of maturation. Obesity may attenuate this positive association.

Interestingly, we found no relationships between physical activity level and microvascular function. In children and adolescents, positive associations between endothelial function and physical activity were reported using the FMD technique. Physical exercise is known to increase the vasodilatory response of conduit arteries through an up-regulation of endothelial nitric oxide synthase activity, leading to increased bioavailability of nitric oxide. Importantly, PAT and FMD are positively related and are both largely nitric oxide–dependent. Nohria et al. found that the inhibition of endothelial nitric oxide synthase with intra-arterial infusion of NG-nitro-L-arginine methyl ester blunted the reactive hyperemic response by 46% ± 21% as assessed by PAT. In contrast, an exclusively nitric oxide–mediated vasodilation of the brachial artery has been detected using FMD. Obvi-ously, different physiological mechanisms contribute to the varying vascular responses between measures of microcirculation and macrocirculation. In addition, physiological differences based on vessel size have been suggested to underlie the divergent reactivity of brachial and digital vasculature.

This study should be viewed in the light of several limitations. First, our data were collected in 2 different schools at different times, and a recruiting and measurement bias is possible. However, we included only healthy subjects from the same county, and anthropometric and BP measurements revealed no differences between the 2 schools. Moreover, the main study outcome was microvascular function assessed by PAT. This technique is operator-independent, and standardized protocols were used in both studies. Second, our study subjects were classified based on Tanner stage, resulting in an unequal distribution of study subjects among the 3 groups, in particular affecting the sample size of group 2 (n = 10). This might limit the generalizability of our data on microvascular function during mid-puberty. However, our aim was to determine differences in microvascular function between prepuberty and late puberty. In these 2 groups, the sample size was equally distributed. Although physical activity was assessed using 2 different methods (questionnaire and accelerometry), our data confirm the typical decline in physical activity levels seen during the transition from childhood to adolescence, supporting the accuracy of our data. In addition, physical activity was not a primary outcome measure in this study, and moderately high validity has been reported for the assessment of MVPA using a 7-day recall compared with Actigraph accelerometry. Further-more, the vasodilatory response of vessels is known to vary during the menstrual cycle. We did not control for the girls’ menstrual cycles, because many of them were premenstrual and others did not exactly remember the date of their last menstrual cycle. Finally, our data were obtained from a healthy Caucasian population, which limits the generalizability to other ethnicities.

The observed low RHI in the prepubertal age group indicates a juvenile microvascular response and does not reflect endothelial dysfunction. Therefore, maturity-adjusted normal values must be established. Future studies investigating microvascular function in children and adolescence should assess and control for pubertal maturation.


Figure 2. Comparison of the reactive hyperemic response on PAT recordings in a prepubertal subject and a late-pubertal subject.