

# Endothelial Pulse Amplitude Testing: Feasibility and Reproducibility in Adolescents

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**Objectives** To test prospectively the reproducibility and feasibility of endothelial pulse amplitude testing (Endo-PAT), a novel Food and Drug Administration-approved technology, in healthy adolescents.

**Study design** We performed Endo-PAT testing on 2 different days separated by no more than 7 days in 30 healthy fasting adolescents, ages 13 to 19 years, to assess reproducibility and feasibility. The reported level of discomfort, as measured on a pain scale of 1 to 5, was documented.

**Results** The mean difference in paired Endo-PAT indices was 0.12 (95% CI, -0.09-0.33;  $P = .24$ ; intraclass correlation coefficient, 0.78), and the within-subject variation of Endo-PAT index was 0.16. The Endo-PAT index on test days 1 and 2 were  $1.91 \pm 0.57$  and  $1.78 \pm 0.51$  (mean plus or minus SD), respectively. All attempted studies (100%) were completed (95% CI, 88%-100%), and all completed studies (100%) could be analyzed (95% CI, 88%-100%). The median pain score was 1 on both days.

**Conclusion** In healthy adolescents, Endo-PAT is feasible and has excellent reproducibility. This technology may provide an easy and reliable means of assessing endothelial function in the pediatric population. (*J Pediatr* 2009;154:901-5)

Atherosclerosis begins early in life. The Bogalusa and the Pathological Determinants of Atherosclerosis in Youth studies demonstrated that pathological changes of atherosclerosis begin in some children in the first decade of life and that these changes correlate with cardiovascular risk factors, including high blood pressure, triglyceride level, insulin resistance, and body mass index.<sup>1,2</sup> Endothelial dysfunction has been shown in adults to be a precursor to atherosclerotic events,<sup>3,4</sup> and in children, it has been correlated with vascular risk factors and diseases associated with early atherosclerosis, such as chronic renal failure,<sup>5</sup> hyperlipidemia,<sup>6</sup> Kawasaki disease,<sup>6,7</sup> and diabetes mellitus.<sup>8</sup>

Although non-invasive testing of endothelial function has been used as a research methodology, there are significant technical difficulties with the application of current technologies such as brachial artery ultrasound testing, particularly in the pediatric age group.<sup>9</sup> Endothelial pulse amplitude testing (Endo-PAT) is a tool recently approved by the Food and Drug Administration that has been proposed as an alternative method to assess endothelial function. This non-invasive method assesses post-occlusive volume changes at the fingertip and has been shown to identify coronary artery dysfunction<sup>10</sup> and to correlate with brachial artery reactivity testing in adults.<sup>11</sup>

To date, 3 reports have been published on Endo-PAT testing in children or adolescents in referral populations<sup>12-14</sup>; none report reproducibility in healthy adolescents. Furthermore, quantitative data about temporal variation in Endo-PAT indices in adolescents is needed for endpoint selection, sample size calculation, and determination of study feasibility.

We sought to test prospectively the reproducibility and feasibility of Endo-PAT testing in adolescents.

## METHODS

Subjects were recruited primarily through an online community listing (Craig's List) between July 2006 and January 2008. To be eligible, subjects had to meet each of the

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This study was supported, in part, by the Higgins Family Noninvasive Cardiac Imaging Research Funds, by funding from the Maram and Carpenter families, the Ciaranello Family Fund, and the Children's Hospital Boston General Clinic Research Center through a grant (#MOI-RR02172) from the National Center for Research Resources, National Institutes of Health. Dr de Ferranti was supported by an Eleanor and Miles Shore scholarship and by a National Institutes of Health grant from National Heart, Lung, and Blood Institute (K23HL85308-2). The authors declare no conflicts of interest.

Submitted for publication Sep 4, 2008; last revision received Nov 26, 2008; accepted Dec 17, 2008.

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0022-3476/\$ - see front matter

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10.1016/j.jpeds.2008.12.028

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Endo-PAT	Endothelial pulse amplitude testing	ICC	Intraclass correlation
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following eligibility criteria: 1) age 13 to 19 years; 2) absence of known cardiovascular risk factors, including obesity (defined as body mass index  $\geq 85\%$  for age and sex), hyperlipidemia, diabetes mellitus, smoking, and vascular disease such as Raynaud's disease, Kawasaki's disease, or juvenile rheumatoid arthritis, and 3) absence of other significant medical conditions. Subjects were excluded when they: 1) had latex allergy; 2) had artificial or long fingernails they did not want to cut for testing; 3) were taking medications regularly that might affect endothelial function, including oral contraceptive pills; 4) were smokers; or 5) were unable to comply with the requirements of the study, including fasting overnight. Consent was obtained from a parent or guardian for participants  $<18$  years old or from the participant when  $\geq 18$  years old. Participants  $<18$  years old gave assent. The study was approved by Children's Hospital Boston Institutional Review Board.

Endo-PAT is a noninvasive technology that captures a beat-to-beat plethysmographic recording of the finger arterial pulse-wave amplitude with pneumatic probes. The Endo-PAT finger probe consists of a thimble-shaped sensor cap that imparts a uniform pressure field and exhibits a clamp-like effect on the entire surface of the distal phalanx and measures pulsatile volume changes. Endo-PAT applies a significant counterpressure on the digit and avoids distal venous distention, thereby inhibiting venous pooling and blood stasis. Flexible tubing connects the finger probe to isolated volume reservoirs that buffer pressure changes within the probes. The pressure change signals are then filtered, amplified, displayed, and stored for further analysis.

Endo-PAT testing was performed on 2 separate days separated by no more than 7 days, with the same physiologic and environmental conditions to the greatest degree possible. The subject's weight and height were measured on test day 1. On both testing dates, blood pressure was measured on the dominant arm in a sitting position with the oscillometric method (Dinamap) after at least 5 minutes of resting. The testing room was arranged to provide a quiet, restful environment with a comfortable temperature of 72 to 75° F. Before testing, subjects were asked to fast overnight for 12 hours, except for the consumption of water. Unless they were taking a daily vitamin, they were asked to refrain from taking vitamin pills and over-the-counter medications; in the case that an over-the-counter medication was used, it was documented.

The Endo-PAT testing protocol, as instructed and described by the company (Itamar Medical Ltd, Caesarea, Israel),<sup>15</sup> was performed on both testing dates in the morning (starting time between 8 and 11:00 am; protocol completed within an hour) by E.S.S.T. or S.deF.

Non-invasive pneumatic probes were placed on the index fingers of both hands. The pulse wave amplitude was recorded continuously from both index fingers. A reactive hyperemia procedure was performed by occluding the brachial artery of one arm with a blood pressure cuff for 5 minutes (to 200-220 mm Hg). The tracing in the non-occluded arm served as a control for changes in overall physiologic state. The Endo-PAT data were analyzed with the proprietary

software package, without any input from the examiner. The Endo-PAT index was defined as the ratio of the average pulse amplitude during the 1 minute period beginning after exactly 90 seconds of reactive hyperemia compared with the average pulse amplitude during the 210-second pre-occlusion baseline period.<sup>11</sup> We also calculated F\_RHI (Framingham Reactive Hyperemia Index or Endoscore), the log transformation of the hyperemia ratio that does not contain baseline correction factor.

Patients were asked to grade discomfort/pain immediately after the test was completed by using the Wong-Baker Faces Pain Scale, ranging 1 to 5.<sup>16</sup> A nutritionist performed a 24-hour dietary recall on each testing day and analyzed the data with ESHA software, version 10.1.

We used the paired *t* test to determine whether the mean Endo-PAT index differed significantly on the 2 test days; a 95% CI for the mean difference was estimated. We calculated the intraclass correlation (ICC) coefficient as a measure of reproducibility. The within-subject variance was also determined.

The proportions of completed studies among all studies attempted, and of analyzable studies among all those completed, were estimated, as was 95% exact binomial confidence intervals. For patient characteristics, including clinical and nutrition data, comparisons were made between the 2 test days by using either the paired *t* test or the Wilcoxon signed-rank test for continuous variables and the McNemar test for dichotomous variables. *P* values  $<.10$  are reported; *P*  $<.05$  was used to determine statistical significance. Analyses were performed with Stata software version 10.0 (College Station, Texas).

Assuming that the standard deviation of the differences in Endo-PAT indices for the 2 test days would be 0.5 and that we would want to detect a true difference in means  $>0.3$  with a paired *t* test conducted at the 0.05 level of significance, we determined that a sample of 30 subjects would provide 90% power.

## RESULTS

We enrolled 30 healthy adolescents in a period of 18 months (Table I). We compared patient characteristics on testing days 1 and 2 (Table II). Testing was separated by a median of 2 days (range, 1-7 days). There were no differences in the systolic or diastolic blood pressures, hours since last meal, the frequency of exercise in the preceding 24 hours, second-hand smoking exposure in the 24 hours before testing, or medications. Despite instructions, 3 patients took a medication on test day 2 but not on test day 1; the medications included Tylenol Cold, antibiotics, cough drops, and ibuprofen.

The total calorie intake, percent calories from fat, protein and carbohydrates, sources of calories from fat (saturated, monosaturated, trans fat, and polyunsaturated fats, grams of omega-3 and omega-6 fatty acids), and antioxidant intake including vitamins A, E, C, folate, and beta-carotene were not significantly different on testing day 1 compared with day 2.

**Table I. Demographic characteristics of the subjects\***

Characteristic	
Male sex	17 (57%)
Median age in years at first test (range)	17.3 (13.3-19.7)
Height (cm), mean (SD)	169.8 (9.1)
Weight (kg), mean (SD)	61.4 (10.1)
BMI, mean (SD)	21.2 (2.5)
BMI percentile (n = 24)†, mean (SD)	45.2 (25.0)
Race	
Caucasian	21 (70%)
African American	2 (7%)
Asian	3 (10%)
Native American or Pacific Islander	3 (10%)
More than one race	1 (3%)
Hispanic/Latino	2 (7%)
Recent cold	4 (13%)
Recent fever	0 (0%)
If female, days from last menstrual period to first test (median, range)	21 (1 to 42)
Family history	
Diabetes mellitus	11 (37%)
Hypertension	13 (43%)
High cholesterol	10 (33%)
Coronary artery disease	8 (27%)
Stroke	9 (30%)
Rheumatologic disease	3 (10%)

BMI, Body mass index.

\*Reported as number (%) unless otherwise indicated.

†BMI percentiles not available for adults (>18 years).

Dietary cholesterol intake was less and vegetable intake was more on test day 2 ( $P = .05$  and  $P = .01$ , respectively).

The mean difference in paired Endo-PAT indices was 0.12 (95% CI, -0.09-0.33;  $P = .24$ ; ICC coefficient, 0.78), and the within-subject variance of Endo-PAT index was 0.16. The median absolute difference was 0.3, with a mean absolute difference of 0.43 units between paired Endo-PAT indices.

The Endo-PAT index on test days 1 and 2 were  $1.91 \pm 0.57$  and  $1.78 \pm 0.51$  (mean  $\pm$  SD), respectively, similar to published data in children.<sup>12,13</sup> All attempted studies (100%) were completed (95% CI, 88%-100%), and all completed studies (100%) could be analyzed (95% CI, 88%-100%).

The mean difference between paired F\_RHI indices was 0.07 (95% CI, -0.10-0.25;  $P = .39$ ; ICC coefficient, 0.83), and the within subject variance of F\_RHI was 0.11. The median absolute difference was 0.26, with a mean absolute difference of 0.34 units between paired F\_RHI indices.

Most of the participants (23/30, 77%) were evaluated by the same operator on both days. There was no significant difference in the mean difference of 2 paired Endo-PAT test values comparing subjects tested by the same operator versus different operators on the 2 testing dates. Age, sex, blood pressure, body mass index, and time of the menstrual cycle did not have any effect on within-subject variance (Table III).

**Table II. Clinical summary**

Endo-PAT study (n = 30)	Test day 1	Test day 2	P value
Systolic BP (mm Hg), mean (SD)	116 $\pm$ 10	113 $\pm$ 10	NS
Diastolic BP (mm Hg), mean (SD)	71 $\pm$ 7	71 $\pm$ 8	NS
Endo-PAT index, mean (SD)	1.91 $\pm$ 0.57	1.78 $\pm$ 0.51	NS
F_RHI*, mean (SD)	0.57 $\pm$ 0.46	0.49 $\pm$ 0.41	NS
Pain score, median (range)	1 (0-3)	1 (0-2)	NS
Hours since last meal, mean (SD)	13.3 $\pm$ 1.4	12.9 $\pm$ 0.9	NS
Number of subjects taking medications in last 24 hours (%)	1 (3%)	4 (13%)	NS
Multivitamins†	5 (16%)	4 (13%)	NS
Coffee in the last 24 hours	0 (0%)	1 (3%)	NS
Tea in the last 24 hours	2 (7%)	1 (3%)	NS
Soft drinks in the last 24 hours	3 (10%)	3 (10%)	NS
Alcohol in the last 24 hours	0 (0%)	1 (3%)	NS
Number of subjects who exercised last 24 hours (%)	10 (33%)	10 (33%)	NS
Second-hand smoking exposure last 24 hours	2 (7%)	2 (7%)	NS

BP, Blood pressure; NS, not significant.

\*F\_RHI is the log transformation of the hyperemia ratio that does not contain baseline correction factor.

†Although these 5 subjects were taking daily multivitamins, 1 forgot to take his vitamin on testing day 2.

The median pain scale score was 1 on both days. In 21 subjects, the reported pain score remained the same on the testing days, whereas it increased in 3 subjects and decreased in 4 subjects. Only 2 participants reported a pain score of 3 on test day 1. A large number of subjects (n = 12) reported a pain score of 0 on test day 2.

## DISCUSSION

Several lines of evidence support the concept that the physiologic perturbation described as “endothelial dysfunction” is a precursor of anatomic changes of atherosclerosis.<sup>17-19</sup> Endothelial dysfunction has been demonstrated in non-atherosclerotic blood vessels of adults with established atherosclerotic disease. Furthermore, both adults and children with risk factors for atherosclerosis, such as smoking, hypercholesterolemia, and diabetes mellitus have impaired endothelial function compared with control subjects.<sup>6,20-23</sup>

Non-invasive testing of endothelial function by brachial artery ultrasound scanning is an established research tool used to detect endothelial dysfunction<sup>6,9</sup>; however, there are significant technical difficulties with the application of this technology, particularly in the pediatric age group. Ultrasound scanning measurement of brachial artery reactivity requires substantial training and expertise; it is technically challenging and has a significant learning curve.<sup>9</sup> A non-invasive tool to

**Table III. Differences in endothelial pulse amplitude testing (day 1 versus day 2) by using patient characteristics**

	Difference in EndoPAT	P value
Sex		
Male	0.36 ± 0.25	.34
Female	0.51 ± 0.51	
Age		
<17 years	0.49 ± 0.36	.39
≥17 years	0.37 ± 0.40	
Race		
White	0.37 ± 0.42	.20
Non-white	0.54 ± 0.27	
BMI		
<20	0.32 ± 0.28	.26
≥20	0.47 ± 0.42	
PATographer*		
Same	0.42 ± 0.34	.87
Different	0.45 ± 0.52	
Systolic BP (median)		
<114 mm Hg	0.54 ± 0.45	.10
≥114 mm Hg	0.31 ± 0.26	
Diastolic BP (median)		
<70 mm Hg	0.29 ± 0.30	.07
≥70 mm Hg	0.54 ± 0.42	

Values shown are mean plus or minus SD.

\*Person performing the EndoPAT testing.

assess endothelial function in adolescents that is easy to operate would improve our ability to evaluate pediatric patients with traditional cardiovascular risk factors and children with other medical conditions that might put them at vascular risk, including cardiac transplantation, cardiopulmonary bypass, cyanosis, and inflammatory disorders.

Endo-PAT is a technology that has been validated for detecting adult patients with impaired coronary microvascular endothelial function.<sup>10</sup> In this study, a group of 94 adults underwent both angiography and Endo-PAT examinations. None of these patients had coronary artery disease with angiography. Abnormal coronary endothelial function, defined as an increase in coronary blood flow <50% in response to acetylcholine, was found in 39 of these 94 patients. The Endo-PAT index was lower in this group and yielded a sensitivity rate of 82% and a specificity rate of 77% for detecting coronary endothelial dysfunction.<sup>10</sup> Endo-PAT has been demonstrated to be reproducible in adults.<sup>24</sup> There are few data on Endo-PAT testing in the pediatric age range.

Our study prospectively tested healthy adolescents with Endo-PAT. We demonstrated excellent reproducibility of this technology, finding a small, non-significant mean difference between the first and second Endo-PAT readings and only small within-subject variability. Our participants found the testing easy to tolerate. Testing did not appear to be operator dependent or substantially affected by recent nutritional intake. The results also provide a normative dataset,

because the only reported published reference range pediatric values thus far have been for “control” groups.

Reports of the use of Endo-PAT in adolescents are less than a handful, mainly those testing children with type I diabetes mellitus, a group known to have a significant risk of microvascular and macrovascular disease. Haller et al reported lower mean Endo-PAT indices in patients with diabetes mellitus compared with healthy control subjects ( $1.63 \pm 0.5$  versus  $1.95 \pm 0.3$ ;  $P = .01$ ), suggesting the presence of endothelial dysfunction.<sup>12</sup> Mahmud et al reported a similar result in male adolescents with diabetes mellitus, whereas the female adolescents with diabetes mellitus did not have significantly different Endo-PAT values when compared with control subjects.<sup>14</sup> Another study showed that HbA1C levels were significantly higher in children with type I diabetes mellitus and endothelial dysfunction, defined as an Endo-PAT threshold of 1.67 on the basis of adult data, compared with children with type 1 diabetes mellitus but without endothelial dysfunction.<sup>13</sup> Only the aforementioned report by Haller et al measured intra-patient variability (mean intra-patient SD, 0.261; mean coefficient variation, 14.8) by repeating the Endo-PAT testing in the patients with diabetes mellitus in 4 weeks.<sup>12</sup> Although the age range of that study was similar to this one, our sample size was larger, and we found greater variability in our healthy adolescents.

Our study also explored the use of the F\_RHI, a novel index calculated with Endo-PAT, in adolescents. Recent data from the Framingham cohort ( $n = 1957$ ; age,  $40 \pm 9$  years) demonstrated a correlation of the mean signal amplitude in the baseline section with cardiovascular risk factors.<sup>25</sup> Thus, baseline correction can mask information on pathological state. The authors concluded that the log-transformed score is more highly associated with cardiovascular risk factors than the Endo-PAT index. When we applied this new score to our dataset, we demonstrated even better reproducibility than with the Endo-PAT index.

This study should be viewed in light of its limitations. Non-invasive vascular testing as a measure of atherosclerotic risk may be influenced by small changes in physiologic state. We conducted all tests in the fasting state, in a controlled environment (comfortable, quiet, and darkened room), and obtained pulse amplitude tracings in both arms to provide an internal control. Dietary intake in the previous 24 hours also has been shown to influence vascular function.<sup>26,27</sup> The only differences in dietary intake on the 2 testing days were that dietary cholesterol was lower and vegetable serving intake was higher on day 2 compared with day 1. We did not measure Tanner stage, lipid profiles, or serum cotinine levels, but expect that these would not have varied significantly within the short time between Endo-PAT studies. We did not perform simultaneous brachial ultrasound scanning testing, which might have provided further comparison of these 2 methods in this population. Also, the study represents a predominantly Caucasian adolescent population. Finally, the finger probes only come in one size, which at this point precludes its use in younger children.

In conclusion, in healthy adolescents, the mean difference between paired Endo-PAT indices and the within-subject variation of Endo-PAT index is small, and Endo-PAT is a well-tolerated test with good feasibility and excellent reproducibility. Future research should explore the relationship between Endo-PAT and vascular risk factors in adolescents.

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